Biological soil crusts in Alberta's grasslands: increasing our knowledge of their taxonomy, diversity, and sensitivity to drought and defoliation

by

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Abstract

Biological soil crusts (BSCs) play a central role in the biodiversity, health, resilience, and function of drylands like Alberta's grasslands. In Alberta, much of the biocrust cover consists of lichens, and many crusts are dominated by species of *Cladonia*. Despite the drought-resistant nature of biocrusts, they have been found to be sensitive to changes in precipitation and disturbance in other regions. This finding leads to questions of if, and how, land-management strategies should change to retain rangeland function as drought becomes more frequent. To answer these questions we need monitoring and experiments investigating biocrust response to change. However, for a group of lichens in Alberta's grassland biocrusts, the *Cladonia cariosa* group, taxonomic boundaries are imprecise, potentially obscuring changes in biocrust communities. To clarify boundaries in the *Cladonia cariosa* group, I implemented an integrative taxonomic study using morphology, secondary chemistry, a multi-locus molecular dataset, and a genotype-by-sequencing (GBS) dataset. While the multi-locus study using the internal transcribed spacer region of nuclear rDNA (ITS) and DNA-dependent RNA polymerase 2 (*rpb2*) provided low resolution, the GBS dataset generated a highly-supported phylogeny. Three lineages corresponded to previously described species in the group, *Cladonia cariosa, Cladonia symphycarpa,* and *Cladonia acuminata,* and two lineages correspond to undescribed species. While *Cladonia cariosa, Cladonia symphycarpa,* and *Cladonia acuminata* could clearly be differentiated by morphology and chemistry, the two undescribed species had few distinguishing traits from *Cladonia symphycarpa,* and are thought to be semi-cryptic. This work results in the addition of two putative species to the *Cladonia cariosa* group. One of the new putative species, for now referred to as Clade E, occurs in Alberta along with *Cladonia cariosa, Cladonia symphycarpa, Cladonia acuminata,* and *Cladonia decorticata;* while the second new putative

species, Clade F, appears to have a distribution limited to Europe. Following clarification of species boundaries, I assessed the effects of simulated drought and grazing on grassland BSC using a manipulative experiment conducted over four years at seven North American temperate grassland sites. A 45% reduction in precipitation and four defoliation treatments simulating common grazing management systems were applied to study responses of biological soil crust cover and community composition, as well as environmental variables. Effects differed by site, with BSC lichen and moss experiencing an increase in cover with defoliation at some sites, but seemed largely resistant to drought treatments. *Selaginella densa* was sensitive to combined effects of drought and defoliation at some sites, experiencing the largest cover decrease in drought and spring+fall defoliated plots. Both moss and lichen cover were found to have a negative relationship with litter. Lichen had a positive relationship with light at a site with high annual net primary production (ANPP) and a negative relationship with light at a site with low ANPP. Beta-diversity in BSC communities was found to be significantly higher in undefoliated treatments, and lower in spring+fall defoliated treatments. The varying effects of drought and defoliation on BSC by site highlights the importance of using ecological knowledge to make landscape management decisions, and that one-size-fits-all management approaches for grasslands in the Great Plains region are likely innapropriate.

This research has greatly expanded our understanding of the phylogenetics of the *Cladonia cariosa* group, which is ubiquitous in our area of the Great Plains, as well as the influence of drought and defoliation on BSC composition and cover.

Preface

This thesis is an original work by myself, Megan Lewis, with research conducted in collaboration with multiple partners. Contributions by the thesis author and others are detailed below.

The design for Chapter 2 was determined by myself, Dr. Diane Haughland, and Dr. Raquel Pino-Bodas. PCR and library preparation of molecular samples were completed by Dr. Raquel Pino-Bodas at the Kew Royal Botanic Gardens. NGS sequencing was completed by the University of Wisconsin Biotechnology Center (Madison, USA). Cryo-sectioning was completed by Lynette Elder at the Alberta Diabetes Institute Histocore Lab. The experimental design for Chapter 3 was determined by Batbaatar Amgaa, Dr. Cameron Carlyle, and Dr. Diane Haughland. Field site selection and initial setup and treatment of sites was conducted in 2016 by Batbaatar Amgaa. Data collection in 2016 was completed by Dr. Diane Haughland. Laboratory procedures were conducted at the Royal Alberta Museum, the Kew Royal Botanic Gardens, the University of Alberta, or as indicated.

Data analysis, interpretation and conclusions in Chapters 2 and 3 are my original work, as well as the literature review in Chapter 1 and conclusions in Chapter 4. The laboratory work, data collection, data analysis and the manuscript composition were completed by me. Dr. Diane Haughland, Dr. Raquel Pino-Bodas and Dr. Cameron Carlyle were the supervisory authors; all were involved with data analysis, manuscript composition, and providing substantive feedback on manuscript drafts.

No part of this thesis has been previously published.

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List of Abbreviations

ABGD	Automatic barcode gap discovery
ABMI	Alberta Biodiversity Monitoring Institute
AGRASID	Agricultural Region of Alberta Soil Inventory Database
AM	Ambient
ANOVA	Analysis of Variance
ANPP	Annual Net Primary Productivity
API	Application Programming Interface
BEAGLE	Broad-platform Evolutionary Analysis General Likelihood Evaluator
BEAST	Bayesian Evolutionary Analysis Sampling Trees
BEAUti	Bayesian Evolutionary Analysis Utility
BED	Browser Extensible Data
BSC	Biological Soil Crust
BPP	Bayesian Phylogenetics and Phylogeography
bPTP	Bayesian Poisson Tree Processes
BWA	Burrows-Wheeler Alignment
°C	Degrees Celsius
cm	Centimetre
CO_2	Carbon dioxide
CTAB	Cetyltrimethylammonium bromide
DNA	Deoxyribonucleic acid
DPX	Distyrene dibutyl phthalate xylene
ef1a	Eukaryotic translation elongation factor 1
GBS	Genotyping-by-sequencing
GDP	Gross domestic product

GMYC	General Mixed Yule Coalescent
GTR	General Time Reversible
GLS	Generalized Least Squares
HH	High-High (spring and fall defoliated)
HN	High-None (spring-defoliated)
HPC	High-performance cloud computing
ILS	Incomplete Lineage Sorting
ITS	Internal transcribed spacer
JC	Jukes-Cantor
-LnL	Negative log-likelihood
MAFFT	Multiple alignment using fast Fourier transform
masl	Metres above sea level
MAT	Mean Annual Temperature
MAP	Mean Annual Precipitation
MCMC	Markov Chain Monte Carlo
ML	Maximum likelihood
m	Metre
mm	Millimetre
MUSCLE	MUltiple Sequence Comparison by Log-Expectation
NE	North East
NGS	Next-generation sequencing
NH	None-High (fall-defoliated)
nlme	Nonlinear Mixed Effects
NMDS	Non-metric multidimensional scaling
NN	None-None (undefoliated)

NW	North West
PAUP	Phylogenetic Analysis Using Parsimony
PCR	Polymerase chain reaction
perMANOVA	Permutational Analysis of Variance
pH	Potential of Hydrogen
PPT	Precipitation
PTP	Poisson Tree Processes
RAD-seq	Restriction-site associated deoxyribonucleic acid sequencing
RAxML	Randomized Axelerated Maximum Likelihood
rbp2	RNA polymerase subunit II
rDNA	Ribosomal deoxyribonucleic acid
RS	Rainout-shelter
s	Second
SE	South East
SEM	Scanning electron microscope
SNP	Single Nucleotide Polymorphism
STemp	Soil Temperature
SVDquartets	Singular Value Decomposition Scores for Species Quartets
SW	South West
TLC	Thin-layer chromatography
µL	Microlitre
µm	Micrometre
µmol	Micromole
USA	United States of America
UV	Ultraviolet

Chapter 1.

An Introduction to Biological Soil Crusts in the Northern Great Plains

1.1 Introduction

Grasslands are a threatened ecosystem (Samson et al., 2004) that are being degraded through land-use intensification and the effects of climate change. Biological soil crusts (BSC) are an important, yet often overlooked, component of grassland communities that perform key ecological functions and are major drivers of grassland function, health, and resilience (Ferrenberg et al., 2017).

Previous studies have proposed that biocrust cover and community composition could be altered dramatically by anthropogenic-induced climate change and land-use intensification (Ferrenberg et al., 2015a; Rodriguez-Caballero et al., 2018) resulting in decreased net CO_2 uptake and fixation (Maestre et al., 2013), suggesting that degradation of these communities could have detrimental potential global impacts on biogeochemical pools and fluxes (Elbert et al., 2012; Porada et al., 2014; Reed et al., 2016). Despite the global significance of this problem (Ferrenberg et al., 2017), little study on this topic has been completed in Alberta's grassland BSCs (Pyle, 2018).

Taxonomic clarity is important in understanding and predicting BSC response to land-use change and effects on ecosystem function, and has important implications for conservation and land management approaches (Edwards et al., 2004; Haughland et al., 2018), but currently there is insufficient taxonomic resolution in biocrust lichen communities to determine if genetic diversity is lost under different grassland disturbance regimes. Approximately 20% of BSC lichen species

documented in Alberta's grasslands and adjacent parklands are within the genus *Cladonia* (Haughland, unpublished). The *Cladonia cariosa* group is particularly abundant in Alberta's grasslands, dominating BSCs at many sites but it is unknown if the species described within this group represent different evolutionary lineages, phenotypic plasticity within one species, or something in-between. Field identification of species in this group of *Cladonia* has proven a challenging task for lichenologists as identifying characters are limited and species are highly variable (Ahti, 2000).

This thesis will address these challenges by clarifying the taxonomy of the *Cladonia cariosa* group and studying the response of BSC to changes in climate and land use in the Great Plains.

1.2 Grasslands

Native grasslands are among the most threatened ecosystems in North America (Samson et al., 2004). Globally, temperate grasslands were identified as the most endangered ecosystem, with over 40% of temperate grasslands being converted from their indigenous state (IUCN, 2008), and were found to be the global biome with the greatest biodiversity impacts from land-use pressures (Newbold et al., 2016). Grasslands provide and support critical ecosystem goods and services, such as biodiversity, water cycling, nutrient cycling, pollination, and carbon sequestration (Havstad et al., 2007).

Alberta's grasslands are characterized by a continental climate, sub-humid to semi-arid with short hot summers, long cold winters, low precipitation, and high evaporation. Mean annual temperatures range from 2.7°C to 4.4°C. Mean winter temperatures range from -14°C to -2°C and mean summer temperatures from 4°C to 18°C. Mean annual precipitation is highly variable, ranging from 333 mm in the Dry Mixedgrass Subregion, to 470 mm in the Foothills Fescue

Subregion, and mean elevation ranges from 550 to over 1500 masl. Geological deposits consist of glacial till, lacustrine, and some eolian and fluvial materials. Soils tend to be calcareous, and mainly consist of Chernozems, Solonetzic soils over saline parent material, Gleysols in wetlands, and bedrock exposures in badlands. The vegetation ranges from grass-dominated with blue-grama and needle and thread grass in the Dry Mixedgrass; needle and thread (*Hesperostipa comata)*, western porcupine grass (*Hesperostipa curtiseta)*, northern and western wheatgrass (*Agropyron cristatum, Pascopyrum smithii)* in the Mixedgrass; plains rough fescue (*Festuca hallii)*, western porcupine grass (*Hesperostipa curtiseta)*, buckbrush (*Symphoricarpos occidentalis)* and rose (*Rosa* spp). shrublands in the Northern Fescue; mountain rough fescue (*Festuca campestris)* and western wheatgrass (*Pascopyrum smithii)* in the Foothills Fescue regions. The Central Parkland consists of aspen (*Populus tremuloides*) groves interspersed with grasslands dominated by plains rough fescue (*Festuca hallii)* (NRC, 2006).

1.3 Grazing

The grasslands in Alberta are known to have a long evolutionary history of grazing by herbivores (Milchunas and Lauenroth, 1993; Morgan, 1980), and today supports extensive cattle ranching and cultivation. Large herds of herbivores including bison, elk, deer, and pronghorn historically migrated across North America's prairies in the millions (Samson et al., 2004). Herbivore grazing is a keystone ecological process in the Great Plains (Knapp et al., 1999; Milchunas et al., 1988), with grazers shaping the land by creating landscape heterogeneity, altering vegetation composition, soils, nutrient cycles, fire regimes, and promoting grassland species that associate with open, intensively-grazed habitat (Fuhlendorf & Engle, 2001). In turn, removing grazing has been shown to be particularly disruptive in this system, resulting in landscape homogenization in

the vascular community and loss of biodiversity (Augustine et al., 2021). Today, livestock provide the ecological functions that historical wild ungulates once did, although at a constrained spatial scale (Allred et al., 2011).

1.4 Drought and Climate Change

Alberta's grasslands have a long history of experiencing short and long-term periods of multi-year drought (Bonsal et al., 2011); however, climate change is expected to have a substantial impact on drought frequency, duration and severity in the near future. Climate predictions in the Canadian Prairies for the next 50 years forecast drying and increased climatic variability, with increases in extreme wet and dry years and more frequent extreme weather events (Kharin et al., 2007; Kharin & Zwiers, 2000; Mladjic et al., 2010; Tam et al., 2019). Prolonged drought can have major impacts on economies and ecosystems, including decreased agricultural production, reduced water quality, degraded soil and erosion, depleted soil moisture, and diminished groundwater supplies (Bonsal et al., 2011), which have potential to be exaserbated by the interaction of climate change with other processes, like grazing.

1.5 Biocrust Function and Ecology

Biological Soil Crusts (BSCs) are major components of dryland areas like the grasslands of the Canadian Prairies. Biocrusts are communities of organisms that live at the soil surface, and consist of an assortment of drought-tolerant organisms, including cyanobacteria, algae, fungi, bacteria, lichens, bryophytes, and microarthropods (Belnap et al., 2016). Biological soil crusts can constitute up to 75% ground cover in drylands (Ferrenberg et al., 2017) and have important roles in these ecosystems, including soil formation and stabilization (Belnap and Budel, 2016);

influencing the hydrologic cycle (Chamizo et al., 2016); regulating seed establishment and germination (Li et al., 2005: Pyle, 2018), and enhancement of soil fertility (Belnap, 2003). Biocrusts make major contributions to global biogeochemical carbon and nitrogen cycles (Poulter et al., 2014; Porada et al., 2014), and some models suggest that up to 49% of global terrestrial nitrogen fixation and 7% of global carbon fixation are carried out by biocrust communities (Elbert et al., 2012). Biocrust cover and community composition have the potential to be altered dramatically by anthropogenically-induced climate change and land-use intensification (Rodriguez-Cabellerro et al., 2015; Ferrenberg et al., 2015) resulting in decreased net CO_2 uptake and fixation (Maestre et al., 2015), suggesting that degradation of these communities could have detrimental global impacts (Elbert et al., 2012; Porada et al., 2014; Reed et al., 2016; Ferrenberg et al., 2017).

Biocrust communities in other regions of the world have been found to be highly sensitive to climate change and physical disturbance. Grazing, and more specifically, trampling, can reduce biocrust cover and alter species composition (Eldridge et al., 2013; Marble and Harper, 1989; Muscha and Hild, 2006), triggering decreased diversity, decreased biomass, and impaired nutrient cycling (Dettweiler-Robinson et al., 2013). Some biocrust organisms possess adaptations that allow them to survive harsh UV radiation and extended precipitation-free periods. Biocrust organisms like mosses and lichen are poikilohydric, meaning that their internal water balance equilibrates with their surrounding environment. After prolonged periods of dehydration, they are able to take advantage of small water pulses to recover photosynthetic activity. However, despite desiccation tolerance of BSC organisms, prolonged drought has been shown to significantly slow reactivation of photosynthesis during re-hydration (Munzi et al., 2019). In addition, small water pulses cause individuals to break dormancy more often, making them prone

to resource exhaustion and carbon starvation (Coe et al., 2012). Coupled with increased evaporation rates from warmer temperatures, periods of hydration are shortened, thus shortening active photosynthesis, decreasing survivorship, and having impacts on biocrust function – namely hydrology, soil stability, and nutrient cycling (Coe et al., 2012). Modelling efforts have proposed that within the next 65 years, biocrust cover could decrease by 25-40% globally due to anthropogenically-induced climate change and land use intensification (Rodriguez-Caballero et al., 2018), despite the drought-tolerant adaptations of biocrust organisms.

Detailed information on biocrust community ecology is limited to a handful of locations and community states (Ferrenberg et al., 2017). In general, biological soil crust communities are shaped by disturbance, soil texture, soil chemistry, climate, and site history (Rosentreter et al., 2016). Studies in the semi-arid steppe of the inter-montane Columbia and Great Basins, and Idaho have found 80 to 99 biocust lichen taxa (Root et al., 2011; Root and McCune, 2012; Root et al., 2018), some species of which are also found in Alberta's grasslands (Haughland et al., 2018; ABMI 2018b). Calcareous and gypsiferous substrates were found to support unique lichen communities (Root et al., 2011; Ponzetti and McCune, 2001). Sandy sites tended to support different biocrust lichen communities than loamy sites, and species richness negatively correlated with shrubs of disturbed areas (*Gutierrezia* and *Chrysothamnus*), and heavily-grazed sandy sites (Root and McCune, 2012). While Alberta's grasslands are also considered semi-arid steppe, and have some ecological similarity to these areas, different geological and soil forming processes have shaped the prairies, resulting in unique biocrust communities worthy of study.

Locally, little is understood about how biocrust communities in the Great Plains are affected by climate change and physical disturbances such as grazing. Biocrust communities studied in the

Dry Mixedgrass Prairie subregion of Alberta were found to be highly sensitive to pipeline disturbance; communities were significantly reduced and were slow to regenerate following disturbance (Pyle, 2018). Biocrusts in areas impacted by physical disturbance consisted of *Nostoc, Cladonia pyxidata, Selaginella densa,* and *Phaeophyscia constipata*. Later seral crust communities farthest from disturbance consisted of a high cover of large, fragile fruticose and foliose lichens, such as *Cetraria aculeata, Cladonia cariosa,* and *Xanthoparmelia wyomingica* (Pyle, 2018). Surveys completed in Grasslands National Park in Saskatchewan found a rich diversity of lichen, finding a total of 194 lichen species, 15 rare lichen species to the park, and six species which may be rare nationally (Freebury, 2014), hinting at the potential richness of lichen species that may await discovery in the northern Great Plains. An early study of biocrust lichen in the Great Plains found lichen and bryophyte community associations with vascular plant communities (Looman, 1964a, 1964b). These findings have been supported by contemporary studies of *Cladonia rei* associations (Haughland et al., 2018) and data collected through the Alberta Biodiversity Monitoring Institute (ABMI), a large-scale, non-targeted monitoring initiative that has been monitoring human disturbance, habitat metrics, and the occurrence and relative abundance of birds, mammals, orbatid soil mites, vascular plants, bryophytes, and macrolichens in a systematic grid of permanent sites across Alberta (ABMI, 2010).

1.6 BSC Lichen

Lichens are a major component of BSC in the Great Plains. Lichens were traditionally considered a partnership between at least two organisms, a fungus (the mycobiont; typically providing the thallus structure) and an alga or cyanobacterium (the photobiont; providing carbohydrates to both partners through photosynthesis, or nitrogen through nitrogen fixation).

However, lichens are currently understood to be a self-sustaining ecosystem formed by the interaction of an exhabitant fungus and an extracellular arrangement of one or more photopynthetic partners and an indeterminate number of other microscopic organisms (Hawksworth & Grube, 2020). The vast majority of lichenized fungi are Ascomycetes (sac fungi) with a green algal photobiont, although other combinations of photobiont and mycobiont are possible. Basidiomycete fungi are capable of lichenization, and it was recently discovered that basidiomycete yeasts are a third member present in the lichenized thallus of many Parmeliaceae lichens (Spribille et al., 2016). In addition; host-specific bacterial microbiomes, as well as protists and viruses have been discovered in association with lichen (Grube et al., 2009, Wilkinson et al., 2015, Petrzik et al., 2019). Lichens are a paraphyletic group, and phylogenetic evidence indicates that the lichenized lifestyle has evolved independently several times in Ascomycota and Basidiomycota (Hodkinson et al., 2014; Lawrey et al., 2009; Lumbsch and Rikkinen, 2016; Nelsen et al., 2009, 2011; Prieto et al., 2013; Schoch et al., 2009). Molecular clock studies have indicated that the first modern lichens appeared approximately 200-300 million years ago, however, it has been postulated that lichen-like associations are far more ancient and may have existed as far back as the Precambrian (Hallbauer et al., 1977; Retallack, 1994, 1995, 2007, 2012).

The lichen ecosystem is complex and is greater than the sum of its parts: it has emergent properties (Goward, 2011). The mycobiont allows the alga to live in environments where it may not be able to exist as a free-living organism, provides shade or optimizes light reception in the form of protective cortical pigments and other structures, regulates moisture, and increases surface area for photosynthesis. The alga, possessing photosynthetic ability, produces carbohydrates in the form of sugar alcohols for the relationship. Lichens have been found to form

non-exclusive symbiotic relationships, that is, mycobionts have been found to pair with multiple different algal species in different environments within their range (Friedl, 1987; Blaha et al., 2006; Peska and Skaloud, 2011). It is hypothesized that different algal species or strains may confer an environmental advantage when lichenized under specific conditions. Likewise, a single species of photobiont may be found in a lichenized state with various species of mycobionts (Piercey-Normore and DePriest, 2001; Beck et al., 2002; Yahr et al., 2004; Yahr et al., 2006). Reproductive methods of lichens may have important implications on the specificity of the algal-fungal relationship, as sexual reproduction requires re-lichenization and asexual reproduction allows symbiotic relationships to be maintained – although there is an incomplete understanding of the importance of recombination and viability of spores in lichen fungi (Piercey-Normore, 2003).

1.7 Taxonomy and Molecular Genetics of Lichen

Taxonomic clarity is critical for assessing biocrust lichen community composition, and to accurately measure and understand changes in biocrust communities. Taxonomy for some lichens within biological soil crusts is uncertain, which hampers our ability to evaluate community-level responses or calculate commonly-used comparative metrics such as species richness or diversity. Clarifying biocrust lichen species concepts is critical to understanding, assessing, and predicting biocrust resilience, adaptability, importance and function in the northern Great Plains, as well as our ability to infer that knowledge to other regions and systems.

1.8 The *Cladonia cariosa* Group in Alberta

The *Cladonia cariosa* group is particularly abundant in Alberta's grasslands, dominating BSCs at many sites by forming mats of small, leaf-like structures called squamules. Out of 384 sites surveyed by the ABMI in the Grasslands and Aspen Parkland between 2005 and 2014, members of the *Cladonia cariosa* group were detected at 208 sites (ABMI, 2018b). It is unknown if the species described within this group represent different evolutionary lineages, phenotypic plasticity within one species, or something in-between. Members of the group include *Cladonia cariosa*, *Cladonia symphycarpa*, *Cladonia acuminata*, *Cladonia decorticata*, and *Cladonia scotteri* (Stenroos et al., 2019). Field identification of species in this group of *Cladonia* has proven a challenging task for lichenologists as identifying characters are limited and species are highly variable (Thomson, 1969; Ahti, 2000).

ABMI surveys have indicated that members of the group can be found throughout Alberta, except for *Cladonia scotteri* which has no reported occurrences in Alberta (ACIMS, 2017). *Cladonia symphycarpa* occurrences were recorded mainly in Grassland and Parkland regions, with some occurrences in the Boreal and Foothills, and rarely found in the Canadian Shield and Rocky Mountain regions (ABMI, 2018c). *Cladonia cariosa* had few occurrences in Grassland, Shield, Rocky Mountain, and Parkland, and was mostly detected in Boreal and Foothills regions (ABMI, 2018d). *Cladonia dahliana*'s distribution was found to be almost exclusively in Grasslands, and was very common in this region, with some drift into the Parkland, and trace detection in the Boreal (ABMI, 2018f). *Cladonia decorticata* and *Cladonia acuminata* have been recorded infrequently. *Cladonia decorticata* observations were limited to the Boreal and Shield regions, and *Cladonia acuminata* was detected mainly in Grasslands and the Boreal, with trace presence in the Foothills and Shield regions (ABMI, 2018a, 2018b).

1.9 Genetic studies of the *C. cariosa* group

Recent genetic studies place *Cladonia cariosa*, *Cladonia symphycarpa*, *Cladonia acuminata*, *Cladonia decorticata*, and *Cladonia scotteri* in subclade Helopodium (Stenroos et al., 2019). Members of Helopodium are characterized by fissured or striate podetia, a persistent primary thallus, and numerous chemotypes (Stenroos et al., 2019; Osyczka and Skubala, 2011). More molecular species than are currently described exist within the clade (Pino-Bodas et al., 2012), and more molecular work with a wider range of specimens is necessary (Stenroos et al., 2019).

1.10 Lichen Chemistry

Extracellular secondary metabolites are used as a taxonomic character for lichens, and for *Cladonia* in particular. More than 850 secondary metabolites currently are known from lichens (Huneck and Yoshimura, 1996). These secondary metabolites have an origin in the fungus, are deposited on the surface of the hyphae, and are typically water-insoluble (Elix and Stocker-Wörgötter, 2008). Secondary metabolite production is driven by genetic processes: sequentially arranged genes for secondary metabolites are encoded in clusters and are highly conserved (Keller and Hohn, 1997). Functions of secondary metabolites in lichens are diverse, and include defence against herbivory (McKey, 1974; Rhoades, 1979; Asplund et al., 2010), allelopathy, pollution tolerance, antioxidant activity, antimicrobial/anti-fungal properties (Molnár and Farkas, 2010), photoprotection (McEvoy et al., 2006; Armaleo et al., 2008) and have demonstrated correlations with humidity (Culberson and Armaleo, 1992; Stocker-Worgotter, 2001), pH (Fox and Howlett, 2008; Timsina et al., 2013; Zraik et al., 2017), as well as organic matter and grain-size characteristics (Zraik et al., 2017). These varied findings send the message that secondary metabolites probably play different roles in different lichens under differing ecologies (Culberson and Culberson, 2001).

Secondary metabolites known to occur in the *Cladonia cariosa* group include atranorin, norstictic acid, psoromic acid, fumarprotocetaric acid, rangiformic acid, bourgeanic acid, stictic acid, perlatolic acid, and homosekikaic acid (Goward, 1999; Hansen and Ahti, 2011; Pino-Bodas et al., 2012; Osyczka and Skubala, 2011), with other possible accessory compounds (Appendix A.3). Secondary chemistry in the *C. cariosa* group may be more valuable as an indicator of ecological condition rather than a definitive character of taxonomic value, as previous studies have found that a number of phylogenetic lineages include different chemotypes, and the chemotypes are largely shared among lineages (Pino-Bodas et al., 2012).

1.11 Morphological Characteristics

Recent research in diverse genera such as *Cladonia* (Pino-Bodas et al., 2012) and *Xanthoparmelia* (Leavitt et al., 2011) has shown that morphological traits such as the structure of the outer cortex or 'skin' using a scanning electron microscope (SEM) may be a relatively novel character that could inform accurate field identification (Appendix A.3). Previous species delimitations in the *C. cariosa* group have used length, breadth, and thickness of the primary squamules to distinguish *C. cariosa* from *C. symphycarpa* (Ahti, 2000), and papillose-maculate mature squamules on *C. symphycarpa* (Ahti and Hammer, 2002), all of which have been found to be subtle, yet reliable traits that align with recent genetic work (Pino-Bodas et al., 2012).

1.12 Research Objectives

1.12.1 Study 1: Taxonomic study of the C. cariosa group

The primary goal of this study was to clarify the taxonomy of the *Cladonia cariosa* group using an integrative taxonomic approach. The main objectives were as follows:

1. Study the variation of morphologic characters for phenotypic characterization of *C. cariosa* group lineages.
2. Study the variation of secondary metabolites in *C. cariosa* group lineages using thin layer chromatography.
3. Use a multi-locus (ITS rDNA and *rpb2*) study and GBS methodology to establish species phylogeny and look for reciprocally-monophyletic genetic lineages which may represent existing or undescribed species.
4. Determine whether significant differences in phenotype exist between genetically-identified groups.

1.12.2 Study 2: Biocrust response to drought and grazing

The goal of this study was to assess biocrust response to drought and different grazing management systems in grasslands across a climate gradient in the northern Great Plains in Alberta, Canada. I collected biocrust data as part of a multi-year, manipulative field experiment. My main objectives were as follows:

1. Assess BSC community responses to drought and defoliation through comparisons to community composition documented at the beginning of the experiment.
2. Assess whether the effects of drought and defoliation on BSC were consistent across the environmental gradients represented.

1.13 Implications of Research

Understanding of biocrust community composition and achieving precise taxonomic resolution also has important implications for conservation management approaches. Temperate grasslands

are endangered on a global scale with over 40% having been converted from their indigenous state (IUCN, 2008), and were the biome with the greatest biodiversity impacts from land-use pressures (Newbold et al., 2016). A fundamental challenge of land management in these areas is how to allow for a variety of land uses while balancing the conservation of biodiversity and other factors important to long-term sustainability of the landscape. An inherent component of the successful management of land for biodiversity outcomes is the awareness of rare species, knowledge of their distribution across the landscape, whether associations with ecotype or community exist, how these associations are related to population viability and persistence, and if these populations are afforded sufficient protection given land-use practices and disturbance regimes (Edwards et al., 2004). Incorrect taxonomic information can lead to land mismanagement, and ultimately, underestimation of biodiversity loss. Taxonomic clarity of biocrust lichen is critical in understanding and predicting biocrust resilience, adaptability, importance and function in Alberta, as well as our ability to infer that knowledge to other regions and systems.

Grasslands in Alberta are used extensively for beef production, and managed for forage production with little consideration for BSC. Beef production is an integral part of the Canadian economy, contributing $17 billion a year to Canada's GDP annually (Canadian Cattleman's Association, 2017), with 41.6% of Canadian beef cows raised in Alberta (Statistics Canada, 2017). Grass and pasturelands provide 80% of the feed used in Canadian beef production (Perry and Cecava, 1995), and the value of forage produced by native prairie in Alberta is approximately $200 million (ABMI, 2018), highlighting the economic importance of productive and functional rangeland systems. Maintaining the resiliency and productive capability of rangelands is critical for producers who rely on rangelands for their livelihoods, and for global

societies that rely on the wide range of ecosystem services that rangelands provide (Teague et al., 2013). Biocrust communities are known to be drivers of ecosystem function, health and resilience in drylands like Alberta's rangelands, but have been shown to be sensitive to disturbance and changes in climate in other ecosystems. Our understanding of Alberta's biocrust community composition and functional response to disturbance regimes and climate change is critical for land management planning into the future.

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Chapter 2.

Species delimitation in the Cladonia cariosa *group based on integrative taxonomy including genotype by sequencing data, morphology, and chemistry*

2.1 Abstract

Lichen forming fungi in the genus *Cladonia* exhibit morphological and chemical variation that make species identification and delimitation a challenging endeavor. We focused on the delimitation of the *Cladonia cariosa* group, currently consisting of *Cladonia cariosa, Cladonia symphycarpa* (and its chemotype, *Cladonia dahliana*), *Cladonia acuminata*, *Cladonia decorticata*, and *Cladonia scotteri,* using an integrative taxonomic approach. While multi-locus studies based on ITS rDNA and *rpb2* provided low resolution to establish the species boundaries, a genotype by sequencing (GBS) method generated a species hypothesis supported by a robust phylogeny. Additionally, the study of 28 morphological characters and secondary metabolites allowed us to phenotypically characterize each lineage. Three lineages correspond with the species previously described *Cladonia cariosa, Cladonia symphycarpa,* and *Cladonia acuminata*, and two lineages correspond to undescribed species. *Cladonia cariosa*, *C. symphycarpa*, and *C. acuminata* could be clearly and reliably differentiated by morphology and chemistry, but the two undescribed species overlap in multivariate space of morphological characters with *C. symphycarpa*, and are thought to be near-cryptic with few distinguishing traits. Our results demonstrate that GBS data improve the resolution of phylogenies over multi-locus methods and are useful in species delimitation in lichen.

2.2 Introduction

Species delimitation in lichen forming fungi historically has been achieved using morphological and chemical characteristics, with contemporary phylogenetic methods providing seemingly continuous fine-tuning to previously-held concepts of species circumscription (Devitt et al., 2019; Druzhinina et al., 2012; Dupuis et al., 2018; Eaton & Ree, 2013; Grewe et al., 2018; Massatti et al., 2016; Pino-Bodas et al., 2018; Prado et al., 2021). For lichen forming fungi, biological species concepts are challenging to test with classic genetic crossing experiments, due to difficulty inducing reproductive states in laboratory conditions (Honneger & Scherrer, 2008). As such, lichen forming fungi species in the pre-molecular era were classified a phenotypic species concept (Lücking et al., 2021). While useful, using phenotype alone to delimit species can have lower accuracy, as it may overlook cryptic species or misinterpret intra- and inter-specific variation. In some cases, genetic data have revealed greater diversity, such as in Parmeliaceae (Corsie et al., 2019; Leavitt et al., 2016), Teloschistaceae (Vondrák et al., 2009), and Cladoniaceae (Pino-Bodas et al., 2012), but have amalgamated species concepts in other cases (Cubero et al., 2004; Widhelm et al., 2016). Integrative taxonomy uses multiple lines of evidence, such as phylogenetic and phenotypic data, to build reliable species hypotheses (Padial et al., 2010).

Species delimitation in the genus *Cladonia* is challenging due to high within-species phenotypic variation, sometimes leading to conflicting species delimitation inferences when using chemical and morphological traits (Ahti, 2000; Stenroos et al., 2019). This is further complicated by homoplasy of taxonomic features (Osyczka & Rola, 2013; Pino-Bodas et al., 2015) and low

phylogenetic resolution of standardized gene regions used to distinguish species in fungi, such as ITS rDNA (Kelly et al., 2011; Pino-Bodas et al., 2013). Recently, numerous species delimitation studies have been attempted with mixed results (Pino-Bodas et al., 2010, 2013, 2015, 2018; Steinová et al., 2013; Stenroos et al., 2015), corroborating the complexity of establishing species boundaries within the genus.

The *Cladonia cariosa* group (Culberson, 1969; Culberson et al., 1993; Harris, 1975) is an example of a lichen forming fungi group with phenotypic disparity. Phylogenetic studies have shown that the *C. cariosa* group is monophyletic (Stenroos et al., 2002, 2019) and belongs to the subclade *Helopodium*, in the clade *Cladonia* (Stenroos et al., 2019). The group is comprised of *C. acuminata*, *C. cariosa*, *C. decorticata*, *C. symphycarpa*, *C. scotteri* and *C. dahliana.* However, *C. dahliana* (Kristinsson, 1974) - originally recognized based on the presence of a particular secondary metabolite - generally is no longer recognized as a distinct species and instead is considered a chemotype of *C. symphycarpa* s.l., a conclusion supported by recent phylogenetic studies (Ahti & Hammer, 2002; Burgaz et al., 2020; Pino-Bodas et al., 2012; Pino-Bodas & Stenroos, 2021). While *C. acuminata* and *C. decorticata* are easily distinguishable by podetium morphology, the rest of the species are morphologically very similar. Size of squamules and podetia, podetial fissuring patterns, and secondary metabolites have been historically used to distinguish *C. cariosa*, *C. scotteri* and *C. symphycarpa* (Ahti, 2000; Hansen & Ahti, 2011; Kristinsson, 1974; Thomson, 1984). However, species identification is challenging as members contain similar secondary metabolites and often present as primary squamules without podetia, resulting in few traits to differentiate species (Ahti & Stenroos, 2013; Bültmann & Lünterbusch, 2008; Culberson, 1969; Kristinsson, 1974; Osyczka & Skubała, 2011).

Additionally, the species of the *C. cariosa* group have overlapping distributions, and are reported from Asia, Europe, North America and South America, often growing on calcareous substrata (Ahti, 2000; Ahti & Stenroos, 2013; Burgaz et al., 2020; Hansen & Ahti, 2011).

A species delimitation study carried out for the *C. cariosa* group (Pino-Bodas et al., 2012) found four phylogenetic lineages, three of which corresponded to previously described species, *C. cariosa*, *C. symphycarpa* and *C. acuminata*, and one putative undescribed species. The study found significant differences in squamule length, width, and thickness between the lineages, confirming characters used for identification of species within the group historically. Additionally, subtle traits associated with the anatomy of the squamule cortex, namely, fissure characteristics studied under a scanning electron microscope (SEM) and the presence of an epinecral layer, were correlated with the lineages (Pino-Bodas et al., 2012). However, another study of the genus *Cladonia* suggested that the presence of an epinecral layer may be more reflective of habitat conditions and may not be an informative taxonomic character (Osyczka & Rola, 2013). Due to the ambiguity still present within the group, previous authors (Burgaz et al., 2020; Stenroos et al., 2019) have suggested a new species delimitation study is necessary to determine the number of species in this group and the characters useful to distinguish them.

High throughput next-generation sequencing (NGS) has drastically changed the scale of molecular datasets, making a vast amount of data across the genome available to establish phylogenetic relationships and species boundaries (Anderson et al., 2017; Eaton & Ree, 2013; Elshire et al., 2011; Massatti et al., 2016). Incomplete lineage sorting, horizontal gene transfer, gene duplication/loss, and hybridization present challenges to phylogenetic inference of closely

related species regardless of sequencing method, but can be particularly problematic for Sanger datasets with limited data and that have low power to resolve nodes (Massatti et al., 2016). One such NGS method, a modified RAD-seq protocol called Genotyping By Sequencing (GBS), targets genomic subsets using restriction enzymes to fragment DNA, generating a reduced representation of the whole genome. Different samples are tagged with barcodes, but with more straightforward restriction fragment generation, reduced sample handling, and less DNA purification steps than RAD-seq (Elshire et al., 2011). Reference-guided NGS has been successfully implemented in other lichen forming fungi to clarify phylogenetic relationships and species boundaries that could not be resolved with single or multi-locus studies, such as in *Rhizoplaca* (Grewe et al., 2017) and *Usnea* (Grewe et al., 2018). GBS could allow the study of thousands of loci across the genomes of the *C. cariosa* group, potentially expanding the phylogenetic resolution of past studies based on a few loci (nuclear ITS rDNA, *rpb2*, *ef1a*). Aligning species identified through phylogenetic analysis with differences in morphology in an integrative taxonomic approach will allow for greater confidence in species delimitation (Bateman et al., 2018; Dupuis et al., 2018).

Here we present the first study to use GBS to study the *C. cariosa* group. We revisit the species delimitation of the *C. cariosa* group using an integrative taxonomic approach with several sources of molecular data, an extensive suite of morphological characters, and an assessment of secondary metabolites. We anticipate that the *C. cariosa* group comprises of more species than currently described, and that the extended set of molecular data and morphological characters will help to propose robust species hypotheses and clarify the taxonomy of this group.

2.3 Materials & Methods

2.3.1 Specimen Sampling

Specimens of the following species were selected from different geographical origins in North America, Europe and Asia: *Cladonia cariosa* (38), *C. symphycarpa* (30), *C. acuminata* (10), *C. decorticata* (1), and *C. scotteri* (4). We endeavored to include specimens representing the breadth of geographic and ecologic ranges of each species. Specimen ecologic ranges were classified using the Koppen-Geiger climate classification (Kottek et al., 2006). The specimens are held at herbaria at the Royal Alberta Museum (PMAE), Royal Botanic Gardens, KEW (K-M), Complutense University of Madrid (MACB), and the University of Helsinki (H). The species were identified using morphological (squamule size, podetia characteristics), and chemical characters, according to Ahti, (2000); Goward, (1999); Hansen and Ahti, (2011); Kristinsson, (1974). Specimen details are available in Table 2.1, and a summary of previous work in the group is available in Appendix A.3.

2.3.2 Morphological and Anatomical Study

Three representative basal squamules and a podetium (if available) were selected from each specimen. Squamules and podetia without discoloration and without lichenicolous fungi or other types of parasitization were selected for measurements and molecular study. Prior to measurement, squamules and podetia were cleaned on a glass slide by soaking in de-ionized water and removing debris with a razor blade and dissecting needle. A set of two measurements were completed on specimens: dry and wet. For wet measurements, samples were re-constituted with de-ionized water until fully saturated and pliant to the touch.

Morphological measurements of specimens consisted of a series of morphological measurements as well as anatomical measurements. Morphological measurements included the length, width and thickness of basal squamules, the length of the basal squamule incision (the lobe split from the tip of the split squamule lobe to the trough of the lobe, if present), the height of the podetium from the base to the tip of the apothecium of the tallest branch, width of the podetial stalk measured midway between the base and the beginning of branching or midway if no branching was present, apothecium diameter at widest location, and squamule pycnidium length and width (if present). In addition, the presence of podetia, podetial squamules, primary podetial branching, secondary podetial branching, longitudinal podetial ribbing, soredia, apothecia, pruina on apothecia, pycnidia, and an epinecral layer, as well as location of pycnidia, axil description, and an assessment of whether podetial branching was narrow or wide. Branches were considered to be narrowly-branched if branches were parallel, or close to parallel, and were considered widely-branched if the outermost branch formed a 30-degree angle or greater with the central axis of the podetium. Measurement of macro-morphological features were completed using an ocular micrometer on a Leica MZ6 stereoscope at up to 64x magnification.

Anatomical measurements were completed on hand cut cross-sections of apothecia as well as on cryosectioned and stained cross-sections of squamules. Apothecia sections were hand-cut after rehydrating apothecia to saturation with de-ionized water. From cross-sections of apothecia, spore length, spore width, hymenium height from the base of the asci and paraphases to the tip of paraphases, and ascus height were measured. Other paraphase traits, in addition to their inclusion in hymenium height, were not assessed. Squamule cross-sections were cryosectioned with a Leica CM3050 cryostat by the Histocore lab at the Alberta Diabetes Institute at the University

of Alberta. Sections were cut to 16 µm thickness. Squamules were embedded in agar prior to cryosectioning to preserve orientation and to improve handling. Transversal sections of squamules were placed on supercharged slides and washed with deionized water, stained with lactophenol cotton blue, and placed through an ethanol-toluene bath series prior to mounting (deionized water, 70 % ethanol, 90% ethanol, 100% ethanol, 100% ethanol, toluene, toluene). Slides were mounted using DPX medium and measurements were taken from images of slides under compound microscopy. Squamule anatomical measurements included the following: basal squamule cortex thickness, medullary layer thickness, algal layer thickness, and epinecral layer thickness. All anatomical measurements of cryosectioned and stained squamules and hand-cut apothecia were completed using a compound microscope at 40x. When present, three spores per specimen were measured, with measurements completed at 100x magnification.

2.3.3 Thin Layer Chromatography and DNA Extraction

Thin layer chromatography (TLC) was used to identify secondary metabolites in each specimen. Where possible, podetia were selected for extractions. For specimens that did not have podetia, squamules were used. Methods followed Orange et al. (2001) using 20 x 10 cm silica gel 60 F_{254} coated glass plates (MilliporeSigma) with solvent systems A, B′, and C. Solvent A consisted of 90 mL toluene, 22.5 mL dioxan, 2.5 mL acetic acid; Solvent B′ of 70 mL hexane, 36 mL methyl *tert*-butyl-ether, 9 mL formic acid; and Solvent C of 85 mL toluene, 15 mL acetic acid. Plates for Solvent C were primed before running by placing in a sealed tank above 60% glacial acetic acid for 10 minutes. Specimen fragments were soaked in acetone for two hours. A warm water bath was used to heat the acetone solutions until boiling three times prior to spotting on silica gel plates (as per I. Brodo, pers. comm.). Plates were spotted with 19 specimens. A mix of *Cladonia*

ecmocyna and *Lobaria pulmonaria* were run in lanes 1, 9 and 19 for atranorin, the norstictic acid group, and fumarprotocetraric acid for Rf values. Additional lichens were run in other lanes on each plate for anticipated and Rf-similar metabolites, including: *Cladonia rangiformis* for atranorin and rangiformic acid; *Hypogymnia physodes* for protocetraric acid; *Imshaugia aleurites* for thamnolic acid; *Cladonia cenotea* for squamatic acid; *Stereocaulon* sp. previously analyzed via TLC for atranorin and stictic acid; *Cladonia rei* for homosekikaic acid; *Rhizoplaca chyrsoleuca* for psoromic acid; *Cladonia polycarpia* for norstictic and barbatic acid; and *Cladonia portentosa* ssp. *pacifica* for perlatolic acid and usnic acid. Prior to acid charring the plates with 10% sulfuric acid and heating on a hotplate, a water spray was used to determine the presence of fatty acids. Ambiguous results were re-run in all three solvents. The acetone-soaked specimens were air-dried and used for DNA extraction.

Genomic DNA was extracted from ca. 10 mg of lichen thallus using a modified cetyltrimethylammonium bromide (CTAB) protocol (Cullings, 1992; Doyle & Doyle, 1987). The DNA was dissolved in 50 μl of 10 mM Tris-Cl. The concentration of the DNA extractions was quantified using Qubit 2.0 fluorometer (Invitrogen, CA, USA) and the DNA quality was evaluated by 1% agarose gel electrophoresis. The single extraction was used for ITS and *rpb2* amplification, as well as library preparation for GBS.

2.3.4 Sanger Dataset

2.3.4.1 PCR and Sequencing

PCRs were carried out with DreamTaq Green PCR Master Mix (2X) (Thermo Fisher Scientific, Waltham, MA USA). The volume of reaction was 25 µL, containing: 12.5 µl of DreamTaq

Green PCR Master Mix, 1 µL of each primer (10 µm/µL), 1 µL of BSA (1 µm/µL), and 1 µL of extracted DNA. Two loci were amplified, ITS rDNA and *rpb2*, according to Pino-Bodas et al. (2013). The primers used to amplify ITS rDNA region were ITS1F and ITS4 (Gardes & Bruns, 1993; White et al., 1990). The primers used to amplify *rpb2* were RPB2dRaq/RPB2rRaq (Pino-Bodas et al., 2010) or CLRPB2-5F/CLRPB2-7R (Yahr et al., 2006). The PCR programs are described in Pino-Bodas et al. (2012). PCR products were cleaned with ExoProStar TM 1-step (GE Healthcare). The sequencing reactions were done at Macrogen Spain service (www.macrogen.com), with the same primers used for the PCR.

2.3.4.2 Sequence Assembly and Analyses

Sequencher (Gene Codes Corporation, 2016) was used to assemble the consensus sequences. BLAST searches (Altschul et al., 1990) were done to confirm that sequences were from target organisms. In addition, 34 verified *rpb2* and ITS sequences from *C. cariosa*, *C. symphycarpa*, and *C. acuminata* from GenBank were included in the datasets, as well as one *Cladonia latiloba* and two *Cladonia subcariosa* sequences for use as outgroups (Table 2.1). Alignments were completed in MAFFT v7.487 (Katoh & Standley, 2013) on the online server (Katoh et al., 2019) for each locus separately using default parameters. Subsequently, the initial alignment was visually and manually improved in BIOEDIT v7.2 (Hall, 1999). As the ITS region lacks a barcode gap in *Cladonia* (Kelly et al., 2011) and has provided poor resolution for species separation in *Cladonia* in other studies (Pino-Bodas et al., 2011; Kotelko and Piercey-Normore, 2010) we chose to retain two ITS alignments in downstream analysis: one with ambiguous positions removed (ITS Gblocks), and one with ambiguous positions retained (ITS Mafft). Ambiguous positions were delimited and removed in ITS alignments using Gblocks (Castresana,

2000). Single gene Maximum likelihood (ML) analysis of RBP2 and ITS rDNA were completed to check congruence between loci (available in Appendix A.1.).

No incongruences were detected and *rpb2* and ITS alignments were combined into a concatenated dataset, which was then analyzed by ML and Bayesian approaches. Analyses were executed through the Cipres Science Gateway HPC2 Workflow on XSEDE (Miller et al., 2010). The combined dataset was analysed considering four partitions: ITS rDNA, and each of three codon positions of *rpb2*, respectively. ML analysis was implemented using RAxML v8.2.12 (Stamatakis, 2014). RAxML achieves tree optimization through subtree rearrangements and branch length optimizations. Trees are constructed in a stepwise manner, and likelihood under the model is assessed through nucleotide substitutions observed. The best tree is iteratively updated with topology that improves likelihood, with possible subtrees re-arranged within the new best tree until the best solution is found. The program was run with 1000 rapid bootstrap iterations, with different start trees every 5 trees, to compute a set of 10 distinct final trees which were used to build a consensus tree and augment confidence into final results. The RAxML analysis was executed assuming a GTR+Gamma model.

Bayesian analysis was carried out using MrBayes v3.2.7a (Huelsenbeck & Ronquist, 2001). The analysis was run using four partitions (ITS, and each codon position of *rpb2*). The substitution model for each locus was selected with jModelTest (Posada, 2008). The SYM+I+gamma model was applied to the ITS partition, and the SYM+gamma model was applied to the *rpb2* codons. The posterior probabilities were approximated by sampling trees using Markov Chain Monte Carlo (MCMC). The posterior probabilities of each branch were calculated by counting the

frequency of trees visited during MCMC analysis. Two simultaneous runs with 20 000 000 generations, each starting with a random tree and employing 4 simultaneous chains, were executed. Every 1000th tree was saved into a file. The first 10 000 samples were deleted as the 'burn in' of the chain. Tracer 1.7 (Rambaut et al., 2018) was used to determine when the chains reached the stationary stage. The 50% majority-rule consensus tree was calculated using the 'sumt' command of MrBayes.

Three "discovery" species delimitation methods were used to infer the species limits using ITS rDNA and *rpb2* datasets: the General Mixed Yule Coalescent (GMYC) approach (Fujisawa & Barraclough, 2013), the Poisson Tree Processes (PTP) method (J. Zhang et al., 2013), and the automatic barcode gap discovery (ABGD) method (Puillandre et al., 2012). GMYC is a likelihood method that uses the branching patterns of an ultrametric gene tree to establish the transition point between intra- and inter-species branching rates. Single threshold testing defines a unique transition from species to population level in the ultrametric tree, with speciation events reflected before the transition, and coalescence within species reflected in the nodes after the transition. The multiple threshold model reaches the transition boundary and searches alternative models that split and unite species groups in an iterative manner, and compares alternative models with the null model using a log-likelihood ratio test (Fujisawa & Barraclough, 2013). Both single and multiple threshold models were used to estimate the species boundaries in *C. cariosa* group. GMYC was run using ultrametric trees for singe-loci generated in BEAST v 1.8.4 (Suchard et al., 2018) assuming the GTR+G model and an uncorrelated/relaxed clock with 1 partition per locus and with default values for other priors. The analyses ran with 50 000 000 MCMC chains logging every 1000 with a burn-in of 12 000, and was ran with BEAGLE

(Browning et al., 2018). PTP is similar to GMYC, but delimits species using number of substitutions instead of branching rates and does not use ultrametric trees (J. Zhang et al., 2013). GMYC was implemented in The Exelixis Lab web server (https://species.h-its.org/gmyc/). Two approaches of PTP, based on maximum likelihood (PTP) and Bayesian approach (bPTP) were run. PTP analyses were implemented using single-locus maximum likelihood trees, generated with RAxML. The bPTP analyses were run with a MCMC chain length of 500 000 generations, thinning=100, and a burn-in of 25%. PTP/bPTP was implemented in The Exelixis Lab web server (https://species.h-its.org/ptp/). ABGD delimits species by detecting the "barcode gap" using the distribution of genetic pairwise distances (Puillandre et al., 2012). The Jukes-Cantor (JC69) model was used to calculate genetic distances, pmin was set to 0.001, pmax=0.1, steps were set at 10, and number of bins used was 20. ABGD was implemented on ABGD web (https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html).

2.3.5 GBS Dataset

2.3.5.1 GBS Library Preparation

A pilot study was conducted to select a suitable restriction enzyme. Libraries were prepared using ApekI or the combination of PstI/MspI for a selection of five samples. The pilot study determined ApekI would be the most suitable for our target group, and was used to construct the libraries in the whole pool of 96 samples, including seven replicates and 1 outgroup specimen (*C. decorticata*). Libraries were constructed and sequenced at University of Wisconsin Biotechnology Center (Madison, Wisconsin, USA), using 200 ng of DNA and sequencing on an Illumina NovaSeq 6000 platform (150-bp paired-end run).

2.3.5.2 GBS Sequence Assembly Dataset

The reads were de-multiplexed, filtered, and aligned using the iPyrad pipeline (Eaton & Overcast, 2020). Phred scores lower than 33 were filtered out, maximum allowable low quality bases was set at 1, and the 'strict' filter for Illumina adapters and primers was used. Restriction overhangs for ApeKI were filtered (CWGC). The genome of *Cladonia grayi* (Armaleo et al., 2019) was used as a reference genome to filter the mycobiont reads, using BWA (H. Li & Durbin, 2009), converted into fastq file with BEDTools (Quinlan & Hall, 2010), and subsequently subjected to cluster alignment with MUSCLE (Edgar, 2004) within the iPyrad pipeline (Eaton & Overcast, 2020). Maximum 5% Ns per locus filter was applied, minimum samples per locus was set at 4, maximum indels per locus allowable was 8, and a maximum 20% SNPs per locus were allowed.

2.3.5.3 Phylogenetic analysis and species tree

The linked SNP dataset generated by iPyrad was used to estimate the phylogeny of the *Cladonia cariosa* group using Maximum Likelihood analysis, implemented in RAxML v8.2.12 (Stamatakis, 2014), executed through the Cipres Science Gateway HPC2 Workflow on XSEDE (Miller et al., 2010). We used a General Time Reversible nucleotide substitution model with a gamma-distribution (GTR+Gamma) with 100 bootstrap replicates.

We used SVDQuartets (Chifman & Kubatko, 2014) implemented in PAUP* (Swofford, 2001) to infer the species tree under the coalescent model using the unlinked SNP dataset produced by iPyrad. We evaluated all possible quartets without prior assignment to populations, and

completed 100 non-parametric bootstrap replicates. This dataset was not filtered for missingness, because SVDquartets handles missing values well (Nute et al., 2018).

2.3.5.4 Species Delimitation

The five clades inferred by phylogenetic analyses and supported in the species tree were used as our species hypothesis (Clade A, B, C, E, F, Figure 2.2). The species delimitation method BPP (Yang, 2015) based on Bayesian inference was conducted to assess these species hypotheses, and was executed in the iPyrad.api (Eaton & Overcast, 2020). As BPP's species delimitation algorithm is subject to mixing problems with higher numbers of loci, the maxloci parameter was set at 400 loci. As this number of loci is much smaller than our overall dataset, we chose to sample different distributions of randomized loci in replicate BPP analysis with varying random seed values to ensure consistency of the species delimitation analysis. Different starting tree topologies were also used in replicate analysis to ensure consistent delimitation results, as incorrect guide trees can sometimes lead to strongly supported and over-split lineages in BPP (C. Zhang et al., 2014). Ten replicate analyses were run for a total of 110,000 iterations with a burn-in of 10,000 and a sampling frequency of 2. Gamma priors were chosen following Yang (2015). The gamma prior used for theta was (2, 0.017), and for tau was (2, 0.016). Hybridization between the clades was also examined, details are available in Appendix A.2.

2.3.6 Statistical analyses of phenotypic features

Presence/absence of secondary metabolites (atranorin, constictic acid, connorstictic acid, conpsoromic acid, fumarprotocetraric acid, norrangiformic acid, norstictic acid, protocetraric acid, psoromic acid, and rangiformic acid), as well as presence/absence of morphological

characters (podetial squamules, podetial cortex, secondary podetial branching, longitudinal ribbing, podetia, apothecia, apothecial pruina, axil type (open/slit or closed), pycnidia shape (barrel, globular, oblong, urn), branching type (wide or narrow) were analyzed by tabulating contingency tables for each character by species and performing Pearson Chi-squared tests with simulated p-value (2000 replicates) in R (R Core Team, 2019). For significant ($p < 0.05$) Chi-squared tests, post-hoc tests were completed following MacDonald and Gardner (2000). Adjusted Agresti residuals were calculated following Sharpe (Sharpe, 2015), and then compared to a critical z-value of +/- 2.58 to determine which cells contributed most to the chi-squared statistic. The critical z-value was obtained by applying the Bonferroni correction on a p-value = 0.05 and 10 contrasts per test to obtain a new p-value = 0.005, and then finding the associated z-score for the new critical value (2.58). The Bonferroni adjustment is recommended when using adjusted residuals for post-hoc testing, as it adjusts for the number of contrasts, maintains the experimentwise Type I error rate at nominal levels, and normalizes distributions that use simulated p-values for small cell counts (MacDonald & Gardner, 2000). Chi-square tests with p-values <0.05 were considered significant, and adjusted residuals >|2.58| were considered to contribute significantly to the overall chi-squared test.

Differences in continuous features between clades (squamule width, squamule length, incision length, podetia height, podetia width, apothecium diameter, pycnidium width, pycnidium length, algal layer thickness, cortex thickness, epinecral layer thickness, medulla thickness, squamule thickness, spore length, spore width, hymenium height, ascus height, squamule width increase % from dry to wet, squamule length % increase from dry to wet, elevation, latitude and longitude) were tested using ANOVA. Homogeneity of variance was confirmed using Levene's test and

residual plots, and normality was checked visually using histograms. Data were normalized using a log10 transform as needed.

2.3.7 Multivariate Analysis

Non-metric multidimensional scaling (NMDS) was used to visualize specimens in morphometric trait space. Significant characters determined by chi-square tests and ANOVA were used for ordination, and were overlain as vectors for aid in visualization. Data were centered and scaled prior to ordination. NMDS was plotted in two dimensions with PC rotation using Euclidean distance. Specimens were color-coded by clade, and standard error ellipses with a 95% confidence level were added to aid in visualization of clade ordination.

2.4 Results

2.4.1 Sanger Dataset

For the Sanger dataset, 226 sequences were generated: 116 of ITS rDNA and 110 for *rpb2*. The concatenated dataset containing sequences of both loci consisted of 89 taxa and 1263 characters, of which 414 were parsimony-informative. Maximum likelihood analysis based on the concatenated dataset of two loci (ITS rDNA and *rpb2*) produced a tree with the likelihood value -LnL= 5281.05, while the Bayesian analysis produced a tree with an arithmetic mean likelihood value -LnL= 5342.84. The ML and Bayesian analyses yielded trees with similar topologies. The Bayesian tree is presented in Figure 2.1. The basal clade in the tree contained *C. cariosa* specimens, but had poor Bayesian and ML support (0.85/57). A second, more derived clade, contained mostly *C. symphycarpa* specimens, and was well supported in Bayesian analysis but was not supported with ML analysis (1.00/43). The *C. symphycarpa* clade formed a sister clade

to two others, one containing a mix of *C. symphycarpa* and *C. cariosa* specimens, with good Bayesian and poor ML support (0.99/69), and the second clade containing a mix of *C. symphycarpa*, *C. acuminata,* and *C. cariosa* specimens with good Bayesian and poor ML support (1.00/58).

2.4.2 Sanger Species Delimitation

The PTP results for *rpb2* and the MAFFT alignment of ITS were congruent and inferred 5 species, but were not well supported (<0.95). PTP inferred 78 species from the Gblocks ITS alignment. BPTP inferred 72 species for *rpb2*, 72 species for the Gblocks ITS alignment, and 74 species for the Mafft ITS alignment. The GMYC single threshold analyses inferred 1 species for *rpb2* and both ITS alignments. GMYC multiple threshold inferred 1 species for *rpb2*, 14 species for the Gblocks ITS alignment, and 38 species for the MAFFT ITS alignment. The ABGD analysis of *rpb2* estimated 28 species in *rpb2* (prior maximal distance P=0.001), 58 species for ITS Gblocks (P=0.002783), and 47 species for ITS Mafft (P=0.004642). Species delimitation results are summarized in Figure 2.1.

2.4.3 GBS Data and Assembly

In total, 252.2 million reads generated for the specimens in this study, with an average of 3 192 854 reads per specimen (σ=447 213 reads/specimen). After quality filtering, 248.1 million remained (μ=3 140 541 reads/specimen, σ=436 950 reads/specimen), of which 47.6 million reads (μ=602,688 reads/specimen, σ=286 928 reads/specimen) were mapped to the reference. Following clustering within and between samples, 839,802 reads were kept. The final dataset

had in total 18,756 loci and 715,273 SNPs, while the unlinked SNP dataset used to estimate the species tree contained 16,645 unlinked SNPs.

2.4.4 GBS Species Tree Estimation

Maximum likelihood analysis generated a tree with -LnL = 6,771,611.51 (Fig. 2.2). The species tree inferenced by SVDquartets produced similar topology to ML tree (Fig. 2.3). Five well-supported clades were obtained, which we describe as Clades A, B, C, E, and F below. An unrecognized species was identified in a previous study and was titled Clade D (Pino-Bodas et al., 2012), as such we avoided the use of "Clade D" in our naming scheme to avoid confusion.

The clade basal to the phylogenetic tree is referred to as Clade A. Clade A had high ML support (100%) and contained 22 specimens, most of which were morphologically identified as *C. cariosa* (82%), with some specimens that were morphologically identified as *C. symphycarpa* (18%). Clade B is phylogenetically positioned as a sister clade to the clade containing Clades C, E, and F (Fig. 2.2, 2.3). Clade B had high bootstrap support (100%), and contained 19 specimens morphologically identified as *C. symphycarpa* (n=9), *C. dahliana* (n=3), *C. cariosa* (n=5), *C. scotteri* (n=1), and *C. subcariosa* (n=1). Clade C is phylogenetically positioned as a sister clade to Clade E (Fig. 2.2, 2.3). Clade C was well supported with ML analysis (100%), and contained 12 specimens that were mostly morphologically identified as *C. acuminata* (n=11), with one *C. symphycarpa* specimen that was likely due to experimental error. Clade E had 100% bootstrap support, and contained 10 specimens that were morphologically identified as *C. symphycarpa* (n=6), *C. cariosa* (n=2), *C. scotteri* (n=1), and *C. dahliana* (n=1). Clade F is phylogenetically positioned as sister to the clade containing Clade C and E, and had high support (ML=100%).

Clade F contained a mix of specimens that were morphologically identified as *C. cariosa* (n=7) and *C. symphycarpa* (n=5).

2.4.5 Bayesian Phylogenetics and Phylogeography (BPP) Species Delimitation

BPP analysis consistently supported 5 species across replicates with different starting trees, randomized loci selection, and different seed values. The posterior probability that the clades represent distinct species was consistently 1 (Table 2.2).

2.4.6 Morphology and Chemistry

Significant differences were observed between clades in the presence/absence of constictic, connorstictic, norstictic, fumarprotocetraric, psoromic, and rangiformic acids; as well as presence of secondary branching in podetia, longitudinal podetia ribbing, and narrow branching of podetia. The presence/absence of an epinecral layer was not significantly different between groups. Contingency tables with the results of chi-square tests are presented in Table 2.3, with post-hoc testing results available in Appendix A.4. We detected significant differences between clades in squamule width, squamule length, length of squamule incisions, podetia height, apothecia diameter, cortex thickness, squamule thickness, hymenium height, percentage increase in squamule length from dry to wet, and geographical latitude and longitude (ANOVA analyses, Tables 2.4-2.6).

Clade A included specimens with 6 different chemotypes (Fig. 2.2, Table 2.7). The two most frequently occurring chemotypes in our study were the chemotype with atranorin and rangiformic acid, with or without norrangiformic acid as an accessory compound (44%); and the

chemotype with atranorin and fumarprotocetraric acid with or without protocetraric acid (35%). Clade A had significantly shorter squamule width, length, and squamule incisions than Clade B, Clade E and Clade F. Squamules in Clade A were significantly thinner than in Clade B and Clade F, though had significantly thicker hymenium layers in cross-section than Clade B and Clade E. Clade A had larger apothecia diameters than Clade C. When specimens are re-wetted from a desiccated state, Clade A specimens experienced significantly less expansion in squamule length than all other groups (Table 2.4-2.7; Plates 2.1-2.5).

Clade B had 6 chemotypes, the two most frequently occurring were the chemotype containing atranorin and rangiformic acid with or without norrangiformic acid (6 specimens, 32%), and the atranorin only chemotype (5 specimens, 26%). Clade B had shorter and narrower squamules than Clade E, but the two species did not have significantly different squamule incision lengths. Clade B specimens had longer, wider, and thicker squamules with larger incisions than Clade A. In apothecia cross-sections, Clade B specimens had thinner hymenium layers than Clade F and Clade A specimens. Clade B had wider apothecia, shorter podetia, and thicker squamules than Clade C (Table 2.4-2.7; Plates 2.1-2.4, 2.6).

Our Clade C specimens had 2 chemotypes, atranorin and norstictic acid with or without constictic/connorstictic acid (10 specimens, 91%), and atranorin, norstictic acid with or without constictic/connorstictic acid, and rangiformic acid with or without norrangiformic acid (1 specimen, 9%). Clade C had significantly shorter squamule width, length, and squamule incisions than Clades E and F. Podetia were larger in Clade C than in Clade B and Clade E. Apothecia diameters were significantly smaller in Clade C than in Clade A, Clade B, and Clade

E. In this group of specimens, Clade C was the only group where podetia without apothecia were observed. Clade C had significantly thinner squamules than Clade B. Narrow podetial branching was exclusive to Clade C in the specimens included in the study (Table 2.4-2.7; Plates 2.1-2.4, 2.7).

Clade E had 5 chemotypes, the most commonly occurring were atranorin with norstictic acid with or without constictic/connorstictic (3 specimens, 30%), and atranorin with psoromic acid with or without conpsoromic acid (3 specimens, 30%). Clade E squamules were significantly longer in width and length than Clades A, B, and C. Clade E had longer squamule incisions than Clade A and Clade C. Clade E specimens had a significantly thicker cortical layer than Clade A and Clade C, and thicker squamules than Clade C. Podetia were shorter than in Clade C, but had wider apothecia. Clade E had thinner hymenium layers in cross-section than Clade A and Clade F (Table 2.4-2.7; Plates 2.1-2.4, 2.8).

Clade F had 4 chemotypes, the most frequently occurring was the chemotype with atranorin and norstictic acid with or without constictic/connorstictic (9 specimens, 75%). Clade F specimens had significantly longer, wider squamules with longer incisions and thicker cortex than Clades A and C. Clade F had significantly thicker hymenium layers than Clade B and Clade E (Table 2.4-2.7; Plates 2.1-2.4, 2.9).

2.4.7 Multivariate Analysis of Morphological Characters

Phenotypic traits with significant differences among clades in the ANOVA and chi-square analysis were chosen for the NMDS ordination. These traits were: squamule length, squamule

width, length of squamule incisions, rangiformic acid presence, norstictic acid presence, constictic acid presence, connorstictic acid presence, fumarprotocetraric acid presence, psoromic acid presence, width of podetial branching, podetia height, presence of longitudinal ribbing on podetia, secondary podetial branching, apothecia diameter, squamule thickness, hymenium height, cortex thickness, and latitude and longitude.

Clade C was well segregated from all other clades, with narrow podetial branching and presence of connorstictic acid contributing most to the position of Clade C specimens in the ordination. Clade A was close to but segregated from Clades B, E, and F, with presence of fumarprotocetraric and rangiformic acids, tall podetia height, northerly latitude, presence of secondary podetial branching, and longitudinal ribbing on podetia contributing most to Clade A specimen orientation in morphologic space. Clades B, E, and F clusters overlapped in the ordination, with long squamule width, wide squamule length, and long squamule incision length contributing most to their ordination location. The stress of the NMDS ordination was 0.1880951, with two convergent solutions found after 20 tries (Figure 2.4).

2.5 Discussion

2.5.1 Revised taxonomy of the C. cariosa group

Previous phylogenetic studies indicated that there may be undescribed species within the *Cladonia cariosa* group (Pino-Bodas et al., 2012). Building on this work, using genome-wide data and an extended suite of morphological characters, we found strong support for five lineages. According to our morphological and chemical studies, three of these lineages correspond with three previously recognized species, *C. cariosa*, *C. symphycarpa*, and *C.*

acuminata, and two are putative undescribed taxa. Here we describe those clades in detail, as well as the taxonomic implications for the clade Helopodium. In addition, we outline traits that may be useful in discriminating these clades, and place these results within the broader context of species delimitation in phenotypically-plastic lichen forming fungi.

Based on phylogenetic, chemical, and morphological evidence, we considered Clade A to correspond to *Cladonia cariosa* s.s. (Ahti, 2000; Osyczka and Skubała, 2011; Pino-Bodas et al., 2012; Stenroos et al., 2019). The phenotype of specimens in this clade mostly corresponds to previous characterizations of *C. cariosa* s.s., with a few exceptions. *C. cariosa* was previously described as having smaller squamules than *C. symphycarpa*, which we also found in our study. A thick epinecral layer has been previously reported in *C. cariosa* (Pino-Bodas et al., 2012), however in the current study we did not find significant differences in the presence (Table 2.3) or thickness of the epinecral layer between species (Table 2.4), and the average epinecral layer thickness for *C. cariosa* was actually smaller than that found in other clades, except Clade C (Table 2.6). In addition, we found a specimen containing atranorin, norstictic and fumarprotocetraric acid, expanding the known chemotypes of this species (Osyczka & Skubała, 2011). *C. cariosa* has been previously reported to have thinner podetia than *C. symphycarpa* (Ahti, 2000), but we did not find significant differences in podetia thickness or height in our study, although *C. cariosa* podetia were, on average, taller than *C. symphycarpa* podetia. *C. cariosa* has been previously found in Asia, Europe, North America, and South America (Ahti & Hammer, 2002), including Antarctica (Osyczka & Skubała, 2011), mainly in temperate and boreal ecoregions (Ahti & Hammer, 2002; Osyczka & Skubała, 2011). This wide distribution was reflected in our study specimens, which were mainly from boreal and temperate zones, with

some from the polar tundra and arid steppe ecoregions. *C. cariosa* has been previously described in habitats with thin, poor soil (Ahti & Hammer, 2002), and on calcareous substrates (Pino-Bodas et al., 2012). Our specimens were found growing on diverse substrata, including soil, rock, wood, calcareous and acidic substrates, roadside gravel, forest floor, and in grasslands.

In ITS rDNA and *rpb2* analyses for Clade B, these specimens joined in a clade with specimens that Pino-Bodas et al. (2012) considered *C. symphycarpa s.s.* following study of the neotype (Fig. 2.1). Therefore, we consider Clade B to correspond to *Cladonia symphycarpa* s.s. According to literature, *C. symphycarpa* has squamules that are thicker and larger than *C. cariosa* (Ahti, 2000; Ahti & Stenroos 2013) and is more likely to contain psoromic acid than *C. cariosa* (Osyczka & Skubała, 2011), which we also found in our dataset (Fig 2.2, Table 2.3-2.7). It was previously reported that *C. symphycarpa* did not have a squamule epinecral layer (Pino-Bodas et al., 2012), however, we found an epinecral layer present in some specimens (Table 2.3), indicating that a squamule epinecral layer may be a trait moreso indicative of environmental conditions as opposed to a diagnostic character. *C. symphycarpa* is described in literature as having stout podetia as compared to *C. cariosa* (Ahti & Hammer, 2002). In our specimens, we did not find significant differences in podetia thickness or height between *C. cariosa* and *C. symphycarpa*, but on average, the podetia of *C. symphycarpa* specimens tended to be shorter. Interestingly, we encountered six *C. symphycarpa* specimens containing rangiformic and/or norrangiformic acids, which is generally not considered to be a secondary metabolite of this species (Hansen and Ahti, 2011; Huovinen et al., 1989; Osyczka and Skubała, 2011). Although the rangiformic-containing chemotype was frequent in our GBS dataset, given the chemotype frequencies in the Sanger dataset, it is likely that norstictic acid and psoromic acid are more

frequent in the species than our GBS dataset suggests. The distribution of *C. symphycarpa* has been previously described as similar to that of *C. cariosa,* having been found in Asia, Europe, North America, and South America, from polar to temperate regions (Ahti & Hammer, 2002), although not Antarctica (Osyczka & Skubała, 2011), which corroborates with our *C. symphycarpa* specimen distribution (Fig. 2.2). Many of our specimens were growing on calcareous substrata, which is what has been previously found in literature for *C. symphycarpa* (Ahti, 2000; Ahti & Hammer, 2002; Pino-Bodas et al., 2012).

Clade C specimens, which we considered to be *Cladonia acuminata s.s*., were readily distinguished from *C. cariosa* and *C. symphycarpa* by tall, unbranched or sparsely-branched sorediate podetia with very small apothecia (if present) which generally aligned with descriptions in literature (Ahti, 2000; Ahti & Stenroos, 2013; Burgaz et al., 2020). Chemotypes found in our study for *C. acuminata* specimens included atranorin with connorstictic/constictic/norstictic acids, and atranorin with connorstictic/constictic/norstictic and rangiformic/norrangiformic acids (Fig. 2.2), although a larger diversity of chemotypes are known to exist for *C. acuminata* (Ahti, 2000). Rangiformic acid was not previously reported in this species, although Ahti & Stenroos (2013) reported an unidentified fatty acid that could be this substance. The previously reported distribution for *C. acuminata* includes Asia, Europe, North America and South America, mainly in the boreal, in habitats with mineral to humus-rich soil (Ahti & Hammer, 2002), which aligned with our specimen selection, which were all from the boreal (Fig. 2.2), and on exposed humus or soil, although some specimens were found growing on slate rock.

Clade E specimens were easily distinguished from *C. cariosa* and *C. acuminata* by larger squamule size and significantly shorter podetia, but were not as readily distinguished from *C. symphycarpa*. The only characteristic that distinguished Clade E from *C. symphycarpa* was larger squamules, but the range of squamule length and width overlapped between the species, so this character is not considered a reliable diagnostic character for the clade. In addition, Clade E and *C. symphycarpa s.s*. had shared chemotypes, and could not be differentiated through chemical testing. In our multivariate ordination, we found that *C. symphycarpa* and Clade E overlapped in morphometric characteristic space (Fig. 2.4), which may mean that Clade E is largely morphologically and chemically indistinguishable from *C. symphycarpa s.s.*, especially when squamules are on the smaller end of their range, despite being well supported as a separate species in phylogenetic analyses. The distribution for Clade E specimens included Asia, Europe, and North America, although the extension of Clade E's range into South America should be considered for future study. Clade E's distribution across Köppen-Geiger climate zones ranged from fully-humid boreal regions with cool and warm summers or boreal regions with dry and cool summers, but also cold arid steppe and polar tundra ecoregions, but not in warm temperate zones. Growth substrates included soil and rock (Fig. 2.2).

Likewise, Clade F could easily be distinguished from *C. cariosa* and *C. acuminata*, but only be distinguished from *C. symphycarpa* and Clade E by taller hymenium height in most (but not all) specimens. Clade F specimens were more likely to have constictic and/or norstictic acids then *C. symphycarpa* and Clade E, but presence of these secondary compounds was not exclusive to Clade F. Chemically, Clade F shared had the same chemotypes as *C. symphycarpa* and Clade E, except for one specimen that contained atranorin and norstictic and fumarprotocetraric acids. In

multivariate ordination, Clade F overlapped in morphometric trait space with Clade E and *C. symphycarpa* (Fig 2.4). Although Clade F was largely indistinguishable morphologically and chemically from Clade E and *C. symphycarpa*, its distribution was unique in that it was not as widespread as the other species, and found almost exclusively in the Mediterranean region. Clade F was found in warm temperate Köppen-Geiger climate zones that had dry summers that were hot or warm, or fully humid summers that were hot or warm, fully-humid boreal zones with warm summers, or boreal zones with dry and warm summers. (Fig. 2.2), growing on rock or soil, on both acidic and calcareous substrata, and in both open areas and pasture. Although none of the Clade D specimens from the 2012 study (Pino-Bodas et al., 2012) were sequenced using GBS in the current study, ITS and *rpb2* from some specimens were used in the Sanger delimitation, specifically 7CARI, 4SYMP, 10SYMP, 6CARI, 13SYMP, 2CARI, and 1CARI. In the Sanger tree, these specimens formed a group with species largely from the Mediterranean region and included CL1393, CL1371, and CL1386. These 3 specimens were also sequenced using GBS, and grouped with Clade F in the GBS phylogenetic tree. As such, we believe that Clade F identified in this study is likely analogous to Pino-Bodas et al.'s Clade D.

Because features differentiating Clade E and Clade F from other members of the group consist of only statistically supported differences in morphometrics, apparent differences in their range, or microscopic features (as opposed to reliable characters that are useful in the field or in taxonomic keys) they may be considered "near-cryptic" species (Lücking et al., 2021). Popular opinion of the cryptic-species concept ranges from useful, yet inconsistently applied (Struck et al., 2018) to "conceptual and terminological chaos" (Heethoff, 2018). It has been argued that many "cryptic" species are not cryptic at all, and that discerning features likely exist at a fine-scale and are

simply overlooked by well-intentioned researchers (Korshunova et al., 2019). Discovery of cryptic species has become more prevalent with the ongoing advancement in molecular sequencing technology, with cryptic species discovered in many groups of lichen forming fungi, such as Parmeliaceae (Leavitt et al., 2016; Molina et al., 2011), Graphidaceae (Kraichak et al., 2015), Icmadophilaceae (Jørgensen, 2019), and Peltigeraceae (Pardo-De la Hoz et al., 2018). The presence of cryptic species contributes to our understanding of biodiversity and is indicative of evolutionary processes, such as recent speciation or morphological stasis in long-diverged species (Struck et al., 2018), which may be worthwhile exploring in this group in future work. In addition, the presence of cryptic species on the landscape can greatly affect estimates of species richness and endism status (Bickford et al., 2007), and thus have important implications in natural resource protection and management, perhaps an awareness that conservation planners should keep in mind when *Cladonia* is present on the landscape.

2.5.2 Other species

Along with *C. cariosa*, *C. acuminata*, and *C. symphycarpa*, we had targeted *C. scotteri* for inclusion in the study. The specimens studied were polyphyletic. Two *C. scotteri* specimens grouped with other clades (Clades B and E), and two specimens, ML04 and ML06, did not group with any clades. ML06 had >50% missing data, so it is difficult to draw confident conclusions about its phylogenetic placement, however, ML04 grouped separately from other clades and seemed to have ample data for confident placement, indicating the potential phylogenetic position of *C. scotteri* within the group, but more specimens are needed for confident conclusions. Future phylogenetic work within this group should strive to include *C. scotteri* samples, as well as *C. cariosa* specimens with homosekikaic acid, to draw better conclusions

about their positioning within the group. Our findings corroborated with others (Osyczka & Skubała, 2011; Pino-Bodas et al., 2012) that *C. dahliana* is not a separate species, but is a chemotype of several species within the group. Psoromic acid production appears to be a homoplastic trait, as psoromic acid-containing specimens were polyphyletic, and found in Clades E and F as well as in *C. symphycarpa*, and were only absent in *C. cariosa* and *C. acuminata.*

2.5.3 Comparing Sanger and. GBS datasets

Next generation sequencing technologies have proven infinitely useful for estimation of phylogenies, resolution of species trees, and species delimitation (Grewe et al., 2017; Harrison & Kidner, 2011; Massatti et al., 2016). Whereas species delimitations for our SNP dataset had high confidence, species delimitations for our two-locus dataset were not congruent between methods (Fig 2.1). In terms of tree topology, *C. cariosa*, *C. symphycarpa*, and Clade F were placed in a manner consistent with our GBS dataset and a previous study (Pino-Bodas et al., 2012), but *C. acuminata* was not consistently segregated from Clade E specimens. The evolutionary history of individual genes are not always congruent with the species tree, which may be due to various processes including horizontal gene transfer, gene duplication/loss, hybridization, or incomplete lineage sorting (Chifman & Kubatko, 2014). Although incomplete lineage sorting plagues all phylogenetic interpretation regardless of sequencing method, gathering the histories of a few gene regions, as in Sanger methods, may not be fully reflective of the species history. The species tree can be thought of as a cloud-like, fuzzy statistical distribution of gene-trees (Maddison, 1997), so it follows that gathering more data from throughout the genome, as with a GBS approach, can help get a high resolution impression of the true species tree. In addition, it has been identified that the gene region typically used for fungi barcoding - ITS rDNA - is not

ideal for use in *Cladonia* because it lacks a barcode gap in this genus, ie. it lacks a difference between inter- and intraspecific genetic variation (Kelly et al., 2011). As such, ITS rDNA must be paired with another gene region for constructing phylogenies and distinguishing species in *Cladonia*, although other gene regions used for this group (*rpb2*, *ef1a*) are also limited by lack of a barcoding gap (Pino-Bodas et al., 2013). While GBS sequencing can avoid the issue of gene region selection, it also can overestimate species present when using the multispecies coalescent model for species delimitation by detecting population structure instead of species boundaries, which is why genetic evidence should be considered with ecologic or phenotypic evidence when circumscribing species (Leaché et al., 2019; Sukumaran & Knowles, 2017). Nevertheless, next-generation sequencing has demonstrably improved species boundary resolution in other lichen forming fungi, such as the *Usnea aurantiacoatra* - *Usnea antarctica* species pair, where data based on two loci suggested a single species (Wirtz et al., 2008, 2012) and Rad-seq combined with microsatellite sequencing was able to deduce two lineages (Grewe et al., 2017, 2018). This certainly seems to be the case in our study, where improved resolution with a GBS approach revealed Clade E and Clade F as separate, well-supported species.

2.5.4 Future Work

Type specimen study and diagnostic character selection will need to be completed prior to taxonomic determination. Specifically, comparison between these specimens and Vainio's type specimens will need to be evaluated prior to formal species description and naming (Vainio, 1887). Informal description of the putative species, for now Clade E and Clade F, is continued below.

2.6 Conclusions

This study has provided greater insight to the *C. cariosa* group using an integrated taxonomic approach based on GBS data and an extended suite of morphologic and chemical characters. Higher resolution of molecular data has revealed that the group likely contains five species: the three species traditionally recognized, *C. acuminata*, *C. cariosa*, and *C. symphycarpa*; and two undescribed species, one of them previously identified (Pino-Bodas et al., 2012) and a new lineage. Chemical and morphologic features for species identification were identified, but high phenotypic variability within species continues to hinder consistent identification without genome-level molecular data. While genome-level molecular data from GBS provides the best-supported phylogeny, it may not be accessable to all lichen practitioners. Sanger sequencing using ITS and *rpb2* appears to be able to successfully segregate most species in phylogenetic trees (*C. cariosa*, *C. symphycarpa*, and Clade D/Clade F), but has trouble separating *C. acuminata* from Clade E. If reproductive structures are present, *C. acuminata* is readily distinguished from Clade E using morphology.

2.7 Taxonomy

Measurement descriptions are presented as follows: (smallest found) avg-SE to avg+se (largest found).

Cladonia **Clade E** Lewis, Pino-Bodas & Haughland, sp. nov. (Plate 2.1-2.4, 2.8).
Type: CANADA. BRITISH COLUMBIA: ca 48 km N of Stewart, BC, west of Knipple Lake on the Bowser River in the Boundary Range, ca. 56.3709924 º N, 130.052006 º W, 571 m elevation, terricolous on mudstone craig, 2015, *Curtis Björk* 31755 (UBC).

Description. Primary thallus squamulose, persistent; squamules (2.5-) 4.3 - 5.8 (-9.4) mm long and (1.8-) 3.4 - 4.4 (-7.2) mm wide; crenulately lobate along margins, greenish-gray-blue to yellow-green, squamule incisions (0.5-) 1.1 - 1.7 (-3.7) mm length. Squamule anatomy: Algal layer (18.18-) 29.63 - 36.19 (-48.27) µm thick; cortex (24.53-) 40.92 - 47.26 (-50.98) µm thick; epinecral layer rare, ca. 7.0 µm thick; medulla (64.94-) 98.12 - 112.54 (-126.36) µm thick; squamules (124.49-) 165.97 - 186.09 (-217.00) µm thick. Podetia (4.8-) 8.5 - 11.7 (-19) mm in height, (1.1-) 2.2 - 3.8 (-8.2) mm width, arising from squamule center and edges, light green to green color, branched, fissured longitudinally, surface corticated; cortex patchy and disappearing in places, esorediate; branches anastomosing, ascyphose. Apothecia present from early stages, light brown to dark reddish brown, (0.4-) 1.2 - 2.0 (-3.4) mm diameter, often pruinose. Hymenium (29.33-) 34.76 - 38.04 (-42.00) µm long, asci (-17.00) 20.99 - 23.53 (-26.33) µm long. Ascospores not observed. Pycnidia often present on squamule edges, dark brown to black, (0.26-) 0.38 - 0.46 (-0.60) mm diameter, globular or urn-shaped, on primary or secondary (uncommon) squamules. Conidia not observed.

Chemistry: Chemotype 1. C-, K+ yellow, Pd -, UV-. Atranorin. Chemotype 2. C-, K+ yellow, Pd + yellow, UV-. Atranorin and norstictic acid (with or without constictic acid). Chemotype 3. C-, K+ yellow, Pd + yellow, UV-. Atranorin and psoromic acid (with or without conpsoromic). Chemotype 4. C-, K+ yellow, Pd -, UV-. Atranorin and rangiformic acid. Chemotype 5. C-, K+ yellow, Pd -, UV-. Atranorin and an unknown substance (Rf=1-2/2/1, purple).

Habitat and distribution: on soil or rock, in cordillera on mountain steppe and rock outcrops, or grasslands with 25-75% grass and sedge cover. Altitudinal range: 290 - 2050 masl. World distribution: Northern hemisphere, North America, Europe, Asia.

Cladonia **Clade F** Pino-Bodas, Lewis & Haughland, sp. nov. (Plate 2.1-2.4, 2.9).

Type: MONTENEGRO. ROŽAJE: ca 2 km SW of Kalače, Rožaje, off of Berane- Rožaje old road P20 in a wooded area, ca. 42.858889 º N, 20.078611 º E, 1280 m elevation, *Picea abies*, *Fagus sylvatica*, and *Juniperus* community, 2015, *A.R. Burgaz* (MACB 111972).

Description. Primary thallus squamulose, persistent; squamules (1.40-) 3.61 - 4.61 (-6.80) mm long and (1.1-) 2.85 - 3.45 (-4.64) mm wide, deeply crenulate to erosely lobate along margins, long finger-like lobes, light green to green or yellow-green upper side, white lower side, squamule incisions (0.28-) 1.19 - 1.65 (-3.01) mm length. Squamule anatomy: squamules (117.95-) 175.95 - 204.81 (-250.93) µm thick; epinecral layer present or not, (4.5-) 6.38 - 9.48 (-14.12) µm thick; cortex (34.14-) 41.83 - 47.21 (-60.48) µm thick; algal layer (13.83-) 22.93 - 29.01 (-42.55) µm thick; medulla (49.60-) 100.82 - 135.96 (-218.83) µm thick; Podetia (12.0-) 14.33 - 16.79 (-20.9) mm in height, (0.98-) 1.93 - 2.75 (-4.08) mm width, light green to green upper with light brown to brown base, polytomously branched near middle of podetia, fissured longitudinally, surface corticated in patches; esorediate; branches anastomosing, ascyphose. Apothecia light brown to red-brown, (0.3-) 1.32 - 1.84 (-2.2) mm diameter, often lightly pruinose or not. Hymenium (38.33-) 44.43 - 51.11 (-60.66) µm long, asci (-19.66) 24.08 - 27.46 (-31.33) µm long. Ascospores ellipsoid, simple, (10.33-) 10.92 - 11.74 (-12.33) µm long, ca. 3.66 µm wide. Pycnidia often present, on squamule margins or sometimes center of squamule, dark brown to black, (0.10-) 0.20 - 0.28 (-0.40) mm diameter, variable in shape, globular, urn, and oblong observed, on primary squamules (common) or secondary squamules (uncommon). Conidia not observed.

Chemistry: Chemotype 1. C-, K+ yellow, Pd -, UV-. Atranorin. Chemotype 2. C-, K+ yellow, Pd + yellow, UV-. Atranorin and norstictic acid (with or without constictic). Chemotype 3. C-, K+

yellow, Pd + yellow, UV-. Atranorin and psoromic acid (with or without conpsoromic acid). Chemotype 4. C-, K+ yellow, Pd + red, UV-. Atranorin, fumarprotocetraric, and norstictic acids (with or without constictic acid).

Habitat and distribution: In stands with *Fagus sylvatica*, *Quercus*, *Juniperus*; acidic, limestone, schist, calcareous, or rocky substrates; open and forested areas, pastures. Altitudinal range: 260-1686 masl. World distribution: Largely found in the Mediterranean with some outliers in Central Europe.

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Table 2.1. List of taxa, locality, collection, chemistry and GenBank accession numbers of specimens used in this study. Secondary metabolite data was obtained from TLC analysis.

Study Code	Taxon name	Country	Year	Collector	Herbarium and Collection Number	Collection Coordinates	Secondary metabolites	ITS Genbank Assession Number	*rpb2* Genbank Assession Number	GBS Genbank Assession Number
10cari	*Cladonia cariosa*	Norway, Nordland	-	T. Tonsberg	BG L784035		Atranorin, fumarprotocetraric acid	JN621914	JN621946	
10symp	*Cladonia symphycarpa*	Austria, Steiermark	-	J. Hafellner	UPS L135579		Atranorin, constictic acid, norstictic acid	JN621927	JN621960	
11cari	*Cladonia cariosa*	Finland, Uusimaa	-	V. Haikonen	H	60.733333, 26.200000	Atranorin, rangiformic acid	JN621915	JN621905	
11symp	*Cladonia symphycarpa*	Canada, Manitoba	-	T. Ahti, M. Piercey-Normore & T. Booth	H		Atranorin, psoromic acid	JN621928	JN621961	
12cari	*Cladonia cariosa*	Finland, Tavastia Proper	-	V. Haikonen	H	61.066667, 25.816667	Atranorin, fumarprotocetraric acid, rangiformic acid	JN621916	JN621948	
12symp	*Cladonia symphycarpa*	Russia, Tuva Republic	-	T. N. Otnykova	H		Atranorin, fumarprotocetraric acid	JN621929	JN621962	
13cari	*Cladonia cariosa*	Russia, Karelia Republic	-	M.A. OapeeBa	H		Atranorin, fumarprotocetraric acid, rangiformic acid	JN621917	JN621962	
13symp	*Cladonia symphycarpa*	Ukraine, Donts`k Oblast	-	O. Nadeina	H		Atranorin, norstictic acid	JN621930	JN621963	
14cari	*Cladonia cariosa*	Russia, Tuva Republic	-	T. N. Otnykova	H	52.433333, 96.616667	Atranorin	Y	Y	
14symp	*Cladonia symphycarpa*	Bosnia and Herzegonina, Sarajevo	-	A.R. Burgaz	MACB 101124		Atranorin, norstictic acid	JN621931	JN621964	
15cari	*Cladonia cariosa*	Canada, Manitoba	-	T. Ahti, M. Piercey-Normore & T. Booth	H	53.970000, -101.198139	Atranorin, fumarprotocetraric acid, rangiformic acid	JN621934	JN621950	
1acumi	*Cladonia acuminata*	USA, Alaska	-	T. Ahti	H 63278		Atranorin, constictic acid, norstictic acid	JN621932	JN621965	
1cari	*Cladonia cariosa*	Spain, Gerona	-	A.R. Burgaz	MACB 94205	42.394444, 2.156667	Atranorin, norstictic acid	FR695863	HQ340071	
1LATEO	*C. latiloba*	Brazil, Santa Catarina	-	W.B. Sanders	H		Atranorin, norstictic acid	JN621937	JN621967	
1SUBCARI	*Cladonia subcariosa*	USA, North Carolina	-	C. Lendemer, J. A. Macklin & G. Moore	H		Fumarprotocetraric acid	JN621936	JN621968	
1sym	*Cladonia symphycarpa*	Spain, Burgos	-	A.R. Burgaz	MACB 93496	43.096389, -3.357222	Atranorin, conpsoromic acid, psoromic acid	JN621918	JN621951	
2acumi	*Cladonia acuminata*	Canada, Manitoba	-	T. Ahti	H 66130		Atranorin	JN621933	JN621966	
2cari	*Cladonia cariosa*	Portugal, Tras-os-Montes	-	A.R. Burgaz	MACB 93984	41.728889, -6.848889	Atranorin, fumarprotocetraric acid	JN621906	JN621938	
2SUBCARI	*Cladonia subcariosa*	USA, New Jersey	-	J. C. Lendemer, S. Hammer, J. Franklin, S. Herrera & S. Syed	H		Atranorin, norstictic acid	JN621935	JN621969	
2symp	*Cladonia symphycarpa*	Spain, Guadalajara	-	A.R. Burgaz	MACB 93559	41.049167, -2.708889	Atranorin, constictic acid, norstictic acid	JN621919	JN621952	
3cari	*Cladonia cariosa*	Spain, Madrid	-	A.R. Burgaz	MACB 94207	41.772500, 2.437500	Atranorin	JN621907	JN621939	
3symp	*Cladonia symphycarpa*	Chile, Region XII, Magallanes y Antartida	-	A.R. Burgaz	MACB 92017		Atranorin, conpsoromic acid, psoromic acid	JN621920	JN621953	

Study Code	Taxon name	Country	Year	Collector	Herbarium and Collection Number	Collection Coordinates	Secondary metabolites	ITS Genbank Assession Number	*rpb2* Genbank Assession Number	GBS Genbank Assession Number
4cari	*Cladonia cariosa*	Spain, Teruel	-	A.R. Burgaz	MACB 45292	40.382500, -0.640000	Atranorin	JN621908	JN621940	
4symp	*Cladonia symphycarpa*	Spain, Madrid	-	A.R. Burgaz	MACB 92737	40.811667, -3.605556	Atranorin, conpsoromic acid, psoromic acid	JN621921	JN621954	
5cari	*Cladonia cariosa*	Spain, Lerida	-	A.R. Burgaz	MACB 94208	42.550556, 1.733889	Atranorin, fumarprotocetraric acid	JN621909	JN621941	
5symp	*Cladonia symphycarpa*	Spain, Palencia	-	A. R. Burgaz & Rodriguez de Lopez	MACB 92739	42.960278, -4.488056	Atranorin, norstictic acid	JN621922	JN621955	
6cari	*Cladonia cariosa*	Spain, Avila	-	A.R. Burgaz	MACB 93018	40.714167, -4.535278	Atranorin, psoromic acid	JN621910	JN621942	
6symp	*Cladonia symphycarpa*	Sweeden, Oland	-	-	S L50055	56.533333, 16.450000	Atranorin, norstictic acid	JN621923	JN621956	
7cari	*Cladonia cariosa*	Spain, Granada	-	A.R. Burgaz	MACB 92995	37.319722, -2.773056	Atranorin, psoromic acid	JN621911	JN621943	
7symp	*Cladonia symphycarpa*	USA, Michigan	-	C.M. Wetmore	S F53075	46.099444, -84.833611	Atranorin, psoromic acid	JN621924	JN621957	
8cari	*Cladonia cariosa*	USA, Michigan	-	C.M. Wetmore	S F53032	46.408333, -89.700556	Atranorin, fumarprotocetraric acid	JN621912	JN621944	
8symp	*Cladonia symphycarpa*	Germany, Oldenburg	-	H. Sipman & S. Ratzel	B 60 0122320	52.466667, 14.450000	Atranorin, constictic acid, norstictic acid	JN621925	JN621958	
9cari	*Cladonia cariosa*	Norway, Nord-Trondelag	-	T. Tonsberg	BG L79658	64.650000, 13.583333	Atranorin, fumarprotocetraric acid	JN621913	JN621945	
9symp	*Cladonia symphycarpa*	Germany, Oldenburg	-	S. Huneck	B 60 0125267		Atranorin, constictic acid, norstictic acid	JN621926	JN621959	
CL1322	*Cladonia cariosa*	UK, Scotland	-	B.J. & A.M. Coppins 24093	E 00620508	55.816667, -2.733333	Atranorin, norstictic acid, rangiformic acid, norrangiformic acid		Y	
CL1340	*Cladonia symphycarpa*	Montenegro, Podgorica	-	A.R. Burgaz	MACB 111680	42.526111, 19.348333	Atranorin, conpsoromic acid, psoromic acid	Y	Y	
CL1341	*Cladonia symphycarpa*	Montenegro, Žabljak	-	A.R. Burgaz	MACB 111663	43.141389, 19.120556	Conpsoromic acid, norstictic acid, psoromic acid acid, ± atranorin	Y		Y
CL1342	*Cladonia symphycarpa*	Georgia, Mtskheta-Mtianeti	-	A.R. Burgaz	MACB 109401	42.561111, 45.086944	Atranorin, conpsoromic acid, psoromic acid	Y		Y
CL1344	*Cladonia symphycarpa*	Albania, Korçë	-	A.R. Burgaz	MACB 111461	40.521667, 20.822778	Atranorin, norstictic acid, ± atranorin	Y		Y
CL1345	*Cladonia cariosa*	Albania, Korçë	-	A.R. Burgaz	MACB 111460	40.521667, 20.822778	Atranorin, rangiformic acid	Y		Y
CL1346	*Cladonia cariosa*	Albania, Korçë	-	A.R. Burgaz	MACB 111455	40.523889, 20.801667	Atranorin, rangiformic acid	Y		Y
CL1347	*Cladonia symphycarpa*	Croacia, Istria	-	A. R. Burgaz	MACB 108249	45.126944, 14.121944	Atranorin, norstictic acid	Y		
CL1353	*Cladonia cariosa*	Montenegro, Žabljak	2018	A. R. Burgaz	MACB 111950	43.188611, 19.060000	Atranorin, rangiformic acid		Y	
CL1354	*Cladonia cariosa*	Greece, West Macedonia	2017	A.R. Burgaz	MACB	40.715553, 21.1852784	Atranorin, constictic acid, norstictic acid		Y	Y
CL1355	*Cladonia cariosa*	Greece, West Macedonia	2017	A. R. Burgaz	MACB	40.715556, 21.185278	Atranorin, constictic acid, norstictic acid		Y	Y
CL1356	*Cladonia cariosa*	Montenegro, Rožaje	2018	A. R. Burgaz	MACB 111972	42.858889, 20.078611	Atranorin, constictic acid, norstictic acid		Y	Y

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CL1357	*Cladonia cariosa*	Greece, West Macedonia	2017	A. R. Burgaz	MACB 112505	40.033611, 21.164444	Atranorin, constictic acid, norstictic acid, fumarprotocetraric acid		Y	Y
CL1358	*Cladonia cariosa*	Montenegro, Rožaje	2018	A. R. Burgaz	MACB 111955	42.862222, 20.0580556	Atranorin, rangiformic acid	Y	Y	Y
CL1359	*Cladonia cariosa*	Montenegro, Plav	2018	A. R. Burgaz	MACB 111940	42.658056, 19.9261111	Atranorin, rangiformic acid	Y	Y	Y
CL1360	*Cladonia cariosa*	Montenegro, Žabljak	2018	A. R. Burgaz	MACB 111656	43.141389, 19.120556	Atranorin, rangiformic acid	Y	Y	
CL1361	*Cladonia symphycarpa*	Montenegro, Žabljak	2018	A. R. Burgaz	MACB 112027	43.170281, 19.0783281	Atranorin, rangiformic acid, norrangiformic acid		Y	Y
CL1362	*Cladonia cariosa*	Italy, Sicily	2018	A.R. Burgaz	MACB	37.925554, 14.6683322	Atranorin, rangiformic acid	Y		Y
CL1363	*Cladonia symphycarpa*	Greece, Central Greece	2017	A. R. Burgaz	MACB	38.942500, 21.806111	Atranorin, constictic acid, norstictic acid	Y	Y	
CL1364	*Cladonia symphycarpa*	Greece, Epirus	2017	A.R. Burgaz	MACB	39.788889, 21.226667	Atranorin	Y		Y
CL1366	*Cladonia symphycarpa*	Croatia, Istria	2015	A.R. Burgaz	MACB 108308	45.238889, 13.755278	Atranorin, constictic acid, norstictic acid			Y
CL1367	*Cladonia symphycarpa*	Montenegro, Cetinje	2018	A. R. Burgaz	MACB 111693	42.374167, 18.869167	Atranorin, constictic acid, norstictic acid	Y	Y	
CL1368	*Cladonia symphycarpa*	Montenegro, Cetinje	2018	A. R. Burgaz	MACB 111691	42.379444, 18.864167	Atranorin, constictic acid, norstictic acid	Y	Y	
CL1369	*Cladonia cariosa*	Albania, Shkodër County	-	A. R. Burgaz	MACB 111298	42.362778, 19.632222	Atranorin, rangiformic acid	Y	Y	
CL1370	*Cladonia symphycarpa*	Greece, Epirus	2017	A. R. Burgaz	MACB 112427	39.788889, 21.226667	Atranorin, constictic acid, norstictic acid	Y	Y	
CL1371	*Cladonia symphycarpa*	Greece, Thessaly	2017	A. R. Burgaz	MACB	39.4075, 23.0388889	Atranorin, constictic acid, norstictic acid	Y	Y	
CL1372	*Cladonia symphycarpa*	Montenegro, Žabljak	2018	A.R. Burgaz	MACB	42.304444, 18.9169444	Atranorin, constictic acid, norstictic acid	Y	Y	Y
CL1373	*Cladonia symphycarpa*	Greece, West Macedonia	2017	A. R. Burgaz	MACB		Atranorin, rangiformic acid	Y		
CL1374	*Cladonia symphycarpa*	Montenegro, Žabljak	2018	A. R. Burgaz	MACB 111668	43.170278, 19.078333	Atranorin, constictic acid, norstictic acid	Y	Y	
CL1375	*Cladonia symphycarpa*	Greece, Central Macedonia	2017	A. R. Burgaz	MACB 112455	40.769722, 21.907500	Atranorin, constictic acid, norstictic acid	Y		
CL1376	*Cladonia cariosa*	Albania, Kukës District	-	A. R. Burgaz	MACB 111433	42.071667, 20.342222	Atranorin, rangiformic acid	Y	Y	
CL1377	*Cladonia cariosa*	Greece, Laconia	2018	A.R. Burgaz	MACB	36.611111, 22.949444	Atranorin, fumarprotocetraric acid, protocetraric acid, rangiformic acid	Y		Y
CL1378	*Cladonia cariosa*	Greece, Central Macedonia	2015	A.R. Burgaz	MACB	40.769722, 21.9075	Atranorin, constictic acid, norstictic acid	Y	Y	Y
CL1379	*Cladonia acuminata*	Georgia, Mtskheta-Mtianeti	2015	A.R. Burgaz	MACB 109378	42.581165, 45.103503	Atranorin, constictic acid, norstictic acid	Y	Y	Y
CL1385	*Cladonia acuminata*	Georgia, Mtskheta-Mtianeti	2015	A.R. Burgaz	MACB 109379	42.522652, 45.055797	Atranorin, constictic acid, norstictic acid, rangiformic acid	Y	Y	Y
CL1386	*Cladonia cariosa*	Spain, Girona	2014	R. Pino Boadas	H 9217318	41.776283, 2.76855278	Atranorin, conpsoromic acid, psoromic acid	Y	Y	Y
CL1387	*Cladonia cariosa*	Russia, Volgograd	-	D. V. Sukhovs	H 9 217 316	-	Atranorin, norstictic acid	Y		

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CL1389	*Cladoia cariosa*	Turkey	2014	T. Ahti 75761, M. Kocakaya, R. Pino-Bodas	H 9217 309	-	Atranorin, fumarprotocetraric acid, protocetraric acid, rangiformic acid	Y	Y	
CL1390	*Cladonia symphycarpa*	Turkey	2014	T. Ahti 74837, M. Kocakaya & R. Pino-Bodas	H 9 217 330	-	Atranorin, constictic acid, norstictic acid	Y	Y	
CL1391	*Cladonia cariosa*	Turkey, Kayseri	2014	R. Pino Bodas, T. Ahti, M. Kocakaya, Z. Kocakaya, and I. Seven	H 9217318	38.627222, 35.5272222	Atranorin, constictic acid, norstictic acid		Y	Y
CL1392	*Cladonia cf. symphycarpa*	Turkey	2014	T. Ahti 74843, M. Kocakaya, R. Pino-Bodas	H 9217 313	-	Atranorin, constictic acid, norstictic acid, fumarprotocetraric acid		Y	
CL1393	*Cladonia cariosa*	Turkey, Kayseri	2014	T. Ahti, M Kocakaya, R Pino-Bodas	H 9217313	38.627222, 35.5772222	Atranorin, constictic acid, norstictic acid	Y	Y	Y
CL1394	*Cladonia symphycarpa*	Spain, Huesca, Benasque	2014	R. Pino-Bodas	H 9 217 326	-	Atranorin, constictic acid, norstictic acid, fumarprotocetraric acid	Y		
CL1395	*Cladonia cariosa*	Canada, British Columbia	2013	T. Ahti, D. Haughland	H 9217311	51.883333, -120	Atranorin, constictic acid, norstictic acid	Y	Y	Y
CL1396	*Cladonia cariosa*	Canada, New Brunswick	2014	T. Ahti, S.R. Clayden	H 9217314	45.1986, -66.2301	Atranorin, fumarprotocetraric acid, protocetraric acid, rangiformic acid	Y	Y	Y
CL1397	*Cladonia cariosa*	USA, Alaska	-	T. Ahti 69713	H 9204056	-	Atranorin, fumarprotocetraric acid, protocetraric acid, rangiformic acid	Y	Y	
CL1398	*Cladonia cariosa*	Finland, Päijänne Tavastia	2016	V. Haikonen	H 9214313	60.93817, 25.641034	Atranorin, rangiformic acid	Y	Y	Y
CL1399	*Cladonia acuminata*	USA, AK	2011	T. Ahti	H 9217308	64.859167, -147.84139	Atranorin, constictic acid, norstictic acid			Y
CL1400	*Cladonia acuminata*	Georgia, Mtskheta-Mtianeti	2015	T.Ahti, AR Burgaz, I Kupradze, A. Jorjadze	H 9217322	42.588889, 45.0961111	Atranorin, constictic acid, norstictic acid		Y	Y
CL1401	*Cladonia acuminata*	Georgia, Mtskheta-Mtianeti	2015	T.Ahti, AR Burgaz, I Kupradze, A. Jorjadze	H 9217321	42.283333, 44.8666667	Atranorin, constictic acid, norstictic acid	Y	Y	Y
CL1402	*Cladonia cariosa*	Japan, Nagano	2015	Y. Ohmura	H 9217310	36.056667, 138.338889	Atranorin, fumarprotocetraric acid	Y	Y	Y
CL1403	*Cladonia symphycarpa*	Russia, Altai Krai	2017	EA Davydov, T. Ahti	H 9217331	51.152361, 81.6375	Atranorin, conpsoromic acid, psoromic acid	Y	Y	Y
CL1404	*Cladonia symphycarpa*	Czech Republic	2014	T. Ahti, J Steinova, J Liska, Z Palice	H 9217324	50.065833, 14.3233333	Atranorin, norstictic acid	Y		Y
CL1426	*Cladonia cariosa*	Canada, Newfoundland	2014	T. Ahti	H 9217315	49.483333, -58.116667	Atranorin, rangiformic acid			Y
ML01	*Cladonia scotteri*	Canada, Nunavut	2012	C. Bjork	UBC, 28291	65.532998, -106.67401	Atranorin, unknown	Y	Y	Y
ML02	*Cladonia scotteri*	Canada, Nunavut	2012	C. Bjork	UBC, 28464	66.636106, -107.68029	Atranorin, rangiformic acid, norrangiformic acid	Y	Y	Y
ML04	*Cladonia scotteri*	Canada, Nunavut	2012	C. Bjork	UBC, 28635	65.937993, -107.50801	Atranorin, rangiformic acid	Y	Y	Y
ML06	*Cladonia scotteri*	Canada, Alberta	2009	C. Bjork	UBC, 20090624.11	53.833977, -119.24392	Fumarprotocetraric acid (possible fungal contaminant)			Y
ML08	*Cladonia symphycarpa*	Canada, British Columbia	2015	T. Goward	UBC, 15-038	51.866663, -119.98334	Atranorin, psoromic acid	Y	Y	Y
ML09	*Cladonia cariosa*	Canada, British Columbia	2018	D. Haughland	PMAE, 2018-86	49.878718, -119.2745	Atranorin, rangiformic acid	Y	Y	Y
ML11	*Cladonia cariosa*	Canada, British Columbia	2018	D. Haughland	PMAE, 2018-29	50.814524, -119.73958	Atranorin	Y	Y	Y

Study Code	Taxon name	Country	Year	Collector	Herbarium and Collection Number	Collection Coordinates	Secondary metabolites	ITS Genbank Assession Number	*rpb2* Genbank Assession Number	GBS Genbank Assession Number
ML12	*Cladonia cariosa*	Canada, Alberta	2013	A. Hillman	PMAE, ABMI.L505314	56.634958, -115.01727	Atranorin (trace), rangiformic acid, norrangiformic acid(?)		Y	
ML13	*Cladonia symphycarpa*	Canada, Alberta	2013	L. Marcoux	PMAE, ABMI.L.5319	51.066055, -109.99809	Atranorin	Y	Y	
ML14/15	*Cladonia symphycarpa*	Canada, Yukon Territory	2018	D. Haughland	PMAE, 2018-405	64.578084, -138.25787	Atranorin	Y	Y	Y
ML16	*Cladonia symphycarpa*	Canada, Northwest Territories	2017	D. Haughland	PMAE, 2017-001	60.500982, -116.27963	Atranorin		Y	
ML17	*Cladonia symphycarpa*	Canada, Alberta	2018	D. Haughland	PMAE, 2018-54	52.581199, -112.52415	Atranorin, unknown peach terpene		Y	Y
ML18B_ SUBCARI	*Cladonia subcariosa*	Canada, British Columbia	2007	T. Goward	UBC, 07-233	54.648576, -122.55342	Atranorin, unknown R: 2/4/6	Y		
ML21	*Cladonia symphycarpa*	Canada, Alberta	2010	T. Grainger	PMAE, ABMI.l.248053	54.452568, -115.63843	Atranorin, rangiformic acid		Y	Y
ML22	*Cladonia symphycarpa*	Canada, British Columbia	2016	D. Haughland	PMAE, 2016-224	59.374951, -129.10566	Atranorin, constictic acid, norstictic acid	Y	Y	Y
ML23	*Cladonia symphycarpa*	Canada, British Columbia	2011	C. Bjork	UBC, 25192	53.316665, -120.13334	Atranorin, rangiformic acid			Y
ML24	*Cladonia symphycarpa*	Canada, British Columbia	2012	D. Haughland	PMAE, 2012-388	50.507359, -120.50501	Atranorin	Y	Y	Y
ML25	*Cladonia symphycarpa*	Canada, Alberta	2017	D. Haughland	PMAE, 2017-990	52.259995, -117.10001	Atranorin, norstictic acid		Y	Y
ML26	*Cladonia symphycarpa*	Canada, British Columbia	2007	T. Goward	UBC, 07-196	54.160081, -121.66413	Atranorin, rangiformic acid, fumarprotocetraric acid		Y	Y
ML27	*Cladonia symphycarpa*	Canada, Yukon Territory	2013	D. Haughland	PMAE, DH-20130916	60.198773, -130.06544	Atranorin		Y	
ML29	*Cladonia symphycarpa*	Canada, Northwest Territories	2017	D. Haughland	PMAE, 2017-541	60.775036, -116.56446	Atranorin, rangiformic acid	Y	Y	Y
ML30	*Cladonia cariosa*	Canada, Yukon Territory	2018	D. Haughland	PMAE 2018-298	64.578084, -138.25787	Atranorin, rangiformic acid, fumarprotocetraric acid		Y	Y
ML31	*Cladonia cariosa*	Canada, Yukon Territory	2018	D. Haughland	PMAE, 2018-160	64.052867, -139.42549	Atranorin, rangiformic acid, fumarprotocetraric acid			Y
ML32	*Cladonia symphycarpa*	Canada, Northwest Territories	2017	D. Haughland	PMAE, 2017-543	60.775036, -116.56446	Atranorin	Y	Y	Y
ML32	*Cladonia cariosa*	Canada, Northwest Territories	2017	D. Haughland	PMAE-DH-NWT-7012-NWT	60.775036, -116.56446	Atranorin	Y		
ML33	*Cladonia cariosa*	Canada, Yukon Territory	2018	D. Haughland	PMAE, 2018-169	64.008413, -138.62782	Atranorin, rangiformic acid, fumarprotocetraric acid	Y	Y	Y
ML34	*Cladonia cariosa*	Canada, Alberta	2015	P. De Carvalho	PMAE, ABMI.L.680599	51.225535, -112.27	Atranorin	Y	Y	Y
ML35	*Cladonia cariosa*	Canada, Alberta	2012	R. Braun	PMAE, ABMI.L.93194	50.830571, -110.99121	Atranorin	Y	Y	Y
ML36	*Cladonia cariosa*	Canada, Alberta	2017	D. Haughland	PMAE, 2017-38	52.294733, -113.8013	Atranorin, rangiformic acid, fumarprotocetraric acid, protocetraric acid (trace)	Y		Y
ML37	*Cladonia cariosa*	Canada, Yukon Territory	2018	D. Haughland	PMAE, 2018-186	64.354858, -138.40921	Atranorin, rangiformic acid, fumarprotocetraric acid	Y	Y	
ML38	*Cladonia cariosa*	Canada, British Columbia	2016	C. Bjork	UBC, 42012	53.801274, -121.31097	Atranorin, rangiformic acid	Y		Y
ML39	*Cladonia cariosa*	Canada, British Columbia	2015	T. Goward	UBC, 15-097	51.849993, -120.05	Atranorin, rangiformic acid	Y	Y	Y

Study Code	Taxon name	Country	Year	Collector	Herbarium and Collection Number	Collection Coordinates	Secondary metabolites	ITS Genbank Assession Number	*rpb2* Genbank Assession Number	GBS Genbank Assession Number
ML40	*Cladonia symphycarpa*	Canada, British Columbia	2009	C. Bjork	UBC, 19269	51.256667, -117.48167	Atranorin, RAM unknown 48 (1-2/2/1)	Y		Y
ML41	*Cladonia cariosa*	Canada, Alberta	2016	J. Ritz	PMAE, ABMI.L.794186	56.762224, -117.03965	Atranorin, rangiformic acid, fumarprotocetraric acid	Y	Y	Y
ML42	*Cladonia cariosa*	Canada, Alberta	2009	M. Birkigt	PMAE, ABMI.L.151288	59.67749, -112.60443	Atranorin		Y	
ML43	*Cladonia subcariosa*	Canada, Northwest Territories	2012	C. Bjork	UBC 26999	64.083332, -111.23334	Atranorin	Y		Y
ML44	*Cladonia dahliana*	Canada, Alberta	2018	M. Lewis	PMAE, ML08	52.580947, -112.52482	Atranorin, psoromic acid (trace)		Y	Y
ML46	*Cladonia symphycarpa*	Canada, British Columbia	2007	T. Goward	UBC, 07-314	51.76667, -119.9667	Atranorin, RAM unknown 48 (1-2/2/1)			Y
ML47	*Cladonia dahliana*	Canada, Yukon Territory	2016	D. Haughland	PMAE, 2016-223	60.819276, -137.48303	Atranorin, psoromic acid	Y	Y	Y
ML49	*Cladonia dahliana*	Canada, Alberta	2012	R. Braun	PMAE, ABMI.L.121829	50.672462, -111.05797	Atranorin, rangiformic acid	Y	Y	Y
ML50	*Cladonia dahliana*	Canada, Alberta	2011	L. Brown	PMAE, ABMI.L.303483	52.166245, -111.17393	Atranorin, psoromic acid	Y	Y	Y
ML51	*Cladonia symphycarpa*	Canada, Alberta	2015	S. O'Donovan	PMAE, ABMI.L.680998	51.934284, -112.05797	Atranorin, psoromic acid	Y	Y	
ML52	*Cladonia cariosa*	Canada, British Columbia	2015	C. Bjork	UBC, 31755	56.370992, -130.05201	Atranorin, norstictic acid	Y	Y	Y
ML53	*Cladonia symphycarpa*	Canada, Alberta	2017	S. Townson	PMAE, ABMI.L.1345223	52.087721, -112.00993	Atranorin, rangiformic acid	Y	Y	Y
ML54B	*Cladonia acuminata*	Canada, Alberta	2013	L. Pelletier-Ahmed	PMAE, ABMI Lichen # 1656	52.011165, -111.47167	Atranorin, norstictic acid, connorstictic acid (trace)			Y
ML55	*Cladonia acuminata*	Canada, Alberta	2013	A. Hillman	PMAE, ABMI.L.64718	56.634958, -115.01727	Atranorin, norstictic acid, connorstictic acid			Y
ML56	*Cladonia acuminata*	Canada, Alberta	2012	M. Lindholm	PMAE, ABMI.L.87441	51.522911, -111.88457	Atranorin, norstictic acid	Y	Y	Y
ML57	*Cladonia acuminata*	Canada, Alberta	2016	A. Cyr	PMAE, ABMI.L.1232061	54.045707, -111.6912	Atranorin, norstictic acid, connorstictic acid			Y
ML58	*Cladonia acuminata*	Canada, Alberta	2015	D. Haughland	PMAE, 2015-458	64.05374, -139.43977	Atranorin, norstictic acid, connorstictic acid	Y		Y
ML59	*Cladonia acuminata*	Canada, Alberta	2014	D. Minocher	PMAE, ABMI.L.483157	59.810755, -119.45875	Atranorin, norstictic acid, connorstictic acid, constictic acid (?)(trace)	Y		Y
ML61	*Cladonia decorticata*	Canada, Alberta	2013	C. Pachkowski	PMAE, ABMI.L.15010	51.93428, -112.05797	Atranorin, norstictic acid (trace), unknown fatty acids?, connorstictic acid (trace)	Y		
ML63	*Cladonia decorticata*	Canada, Alberta	2016	J. Lowther	PMAE, ABMI.L.761658	59.319573, -111.28409	Perlatolic acid, fumarprotocetraric acid ? (trace), unknown (4-5/3/4-5)			Y
ML69	*Cladonia cariosa*	Canada, Northwest Territories	2017	D. Haughland	PMAE, 2017-542		Atranorin, fumarprotocetraric acid	Y	Y	
ML70	*Cladonia cariosa*	Canada, British Columbia	2018	C. Bjork	ER18P11	49.783881, -114.83306	Atranorin, fumarprotocetraric acid	Y	Y	Y
ML75	*Cladonia cariosa*	Canada, Alberta	2019	D. Haughland	PMAE, 2019-5	50.885883, -111.8951	Atranorin, fumarprotocetraric acid, norstictic acid		Y	Y

Table 2.2. Bayesian Phylogenetics and Phylogeography (BPP) species validation results. Two replicates of 400 randomized loci with varying starting seed values were completed for each of five different starting trees. Ten replicate analyses were run for a total of 110,000 iterations with a burn-in of 10,000 and a sampling frequency of 2. The gamma prior used for theta was (2, 0.017), and the gamma prior used for tau was (2, 0.016).

((((ACU, E),F),SYM),CAR)					
		Delimitation	Prior	Posterior	NSpecies
Replicate 1	0	0	0.2	0	1
	1	1000	0.2	0	2
	2	1100	0.2	0	3
	3	1110	0.2	0	4
	4	1111	0.2	1	5
Replicate 2	0	0	0.2	0	1
	1	1000	0.2	0	2
	2	1100	0.2	0	3
	3	1110	0.2	0	4
	4	1111	0.2	1	5
(((SYM, CAR),(ACU,F)),E)					
		Delimitation	Prior	Posterior	NSpecies
Replicate 1	0	0	0.16667	0	1
	1	1000	0.16667	0	2
	2	1100	0.16667	0	3
	3	1110	0.16667	0	4
	4	1110	0.16667	0	4
	5	1111	0.16667	1	5
Replicate 2	0	0	0.16667	0	1
	1	1000	0.16667	0	2
	2	1100	0.16667	0	3
	3	1110	0.16667	0	4
	4	1110	0.16667	0	4
	5	1111	0.16667	1	5
(((F, E),(SYM,CAR)),ACU);					
		Delimitation	Prior	Posterior	NSpecies
Replicate 1	0	0	0.166667	0	1
	1	1000	0.166667	0	2
	2	1100	0.166667	0	3
	3	1101	0.166667	0	4
	4	1110	0.166667	0	4
	5	1111	0.166667	1	5
Replicate 2	0	0	0.166667	0	1
	1	1000	0.166667	0	2
	2	1100	0.166667	0	3
	3	1101	0.166667	0	4
	4	1110	0.166667	0	4
	5	1111	0.166667	1	5
(((SYM, E),(ACU,CAR)),F)					
		Delimitation	Prior	Posterior	NSpecies
Replicate 1	0	0	0.166667	0	1
	1	1000	0.166667	0	2
	2	1100	0.166667	0	3
	3	1101	0.166667	0	4
	4	1110	0.166667	0	4

	5	1111	0.166667	1	5
Replicate 2	0	0	0.166667	0	1
	1	1000	0.166667	0	2
	2	1100	0.166667	0	3
	3	1101	0.166667	0	4
	4	1110	0.166667	0	4
	5	1111	0.166667	1	5
((((CAR, SYM),F),E),ACU)					
		Delimitation	Prior	Posterior	NSpecies
Replicate 1	0	0	0.2	0	1
	1	1000	0.2	0	2
	2	1100	0.2	0	3
	3	1110	0.2	0	4
	4	1111	0.2	1	5
Replicate 2	0	0	0.2	0	1
	1	1000	0.2	0	2
	2	1100	0.2	0	3
	3	1110	0.2	0	4
	4	1111	0.2	1	5

Table 2.3. Contingency tables of chemical and qualitative morphological features. Number of specimens with the feature absent are listed under "0" columns, and number of specimens with the feature present are under the "1" column. Bolded values indicate that the presence of the feature is significantly higher than expected in the clade, italicized values indicate that the feature presence is significantly lower than expected, as determined by chi-square testing and a significance value of p=<0.05. Post hoc-testing determined which cells contributed most to the chi-squared statistic by calculating Agresti residuals following Sharpe (Sharpe, 2015), and then comparing adjusted residuals to a critical z-value of +/- 2.58. The critical z-value was found by applying a Bonferroni correction to the p-value of 0.05, and adjusting for 10 contrasts.

	Atranorin			Constictic			Connorstictic			Conpsoromic			Fumarprotocetraric		
	0	1	%	0	1	%	0	1	%	0	1	%	0	1	%
Clade A (*C. cariosa*)	0	24	100	**24**	*0*	0	24	0	0	24	0	0	*12*	**12**	47.82
Clade B (*C. symphycarpa*)	0	19	100	16	2	10.53	19	0	0	17	2	10.53	19	0	0
Clade C (*C. acuminata*)	0	12	100	6	6	50	*7*	**5**	41.67	12	0	0	12	0	0
Clade E	0	10	100	9	1	10	10	0	0	8	2	20	10	0	0
Clade F	0	12	100	*4*	**8**	66.67	12	0	0	11	1	8.33	11	1	8.333
$\chi 2$	NaN			27.947			28.964			6.0803			27.71		
P	n/a			**0.0004998**			**0.0004998**			0.1814			**0.0004998**		

	Norrangiformic			Norstictic			Protocetraric			Psoromic			Rangiformic		
	0	1	%	0	1	%	0	1	%	0	1	%	0	1	%
Clade A (*C. cariosa*)	23	1	4.35	**23**	*1*	0	21	3	13.04	24	0	0	*5*	**19**	82.61
Clade B (*C. symphycarpa*)	18	1	5.26	16	3	15.79	19	0	0	14	5	26.32	13	6	31.58
Clade C (*C. acuminata*)	12	0	0	*1*	**11**	91.67	12	0	0	12	0	0	11	1	8.33
Clade E	10	0	0	7	3	30	10	0	0	7	3	30	9	1	10
Clade F	12	0	0	*2*	**10**	83.33	12	0	0	11	1	8.33	**12**	*0*	0
$\chi 2$	1.674			41.703			6.8936			12.083			33.607		
P	1			**0.0004998**			0.2019			**0.01599**			**0.0004998**		

	Podetial Squamules			Podetial Cortex			Secondary Branching			Longitudinal Ribbing			Apothecia			Soredia			Epinecral Layer		
	0	1	%	0	1	%	0	1	%	0	1	%	0	1	%	0	1	%	0	1	%
Clade A (*C. cariosa*)	4	11	73.33	0	15	100	2	13	86.67	0	15	100	0	15	100	20	0	0	6	10	63
Clade B (*C. symphycarpa*)	5	6	54.55	0	11	100	6	5	45.45	0	11	100	0	11	100	16	0	0	9	4	31
Clade C (*C. acuminata*)	2	10	83.33	1	11	91.67	8	4	33.33	**4**	*8*	66.67	3	9	75	*4*	**7**	64	0	1	100
Clade E	3	3	50	0	6	100	1	5	83.33	0	6	100	0	6	100	9	0	0	5	1	17
Clade F	2	2	50	0	4	100	2	2	50	1	3	75	0	4	100	10	0	0	3	3	50
$\chi 2$	3.7773			3.0638			10.531			11.386			9.6			39.153			6.2656		
P	0.4783			0.6912			**0.02599**			**0.02299**			0.06547			**0.0004998**			0.1569		

	Apothecia Pruina			Pycnidia			Axils		Pycnidia Shape				Branch Width	
	0	1	%	0	1	%	Open/Slit	Closed	Barrel	Globular	Oblong	Urn	Wide	Narrow
Clade A (*C. cariosa*)	2	13	86.67	13	10	43.48	13	2	2	3	0	5	15	0
Clade B (*C. symphycarpa*)	1	10	90.91	7	12	63.16	9	0	1	2	0	9	11	0
Clade C (*C. acuminata*)	0	9	100	8	4	33.33	4	5	1	2	0	1	*0*	**10**
Clade E	0	6	100	3	7	70	5	1	0	3	0	4	6	0
Clade F	1	3	75	8	4	33.33	3	1	0	2	1	1	4	0
$\chi 2$	3.1118			5.9252			9.5314		15.233				46	
P	0.5637			0.2159			0.06697		0.2394				**0.0004998**	

Table 2.4. Analysis of variance test results for continuous features of members of the *C. cariosa* group. Bolded values indicate significantly different values of the feature between clades. ($p < 0.05$).

Feature	Df	F-value	Pr(>F)
Squmule Width	4	7.1595	**0.0000654**
Squamule Length	4	8.5464	**1.06E-05**
Squamule Incision Length	4	3.9645	**0.006338**
Podetia Height	4	2.7567	**0.03948**
Podetia Thickness	4	2.2426	0.07975
Apothecium Diameter	4	3.0218	**0.02839**
Pycnidia Width	4	1.5587	0.2091
Pycnidia Length	4	1.5403	0.2142
Algal Layer Thickness	4	1.7405	0.1613
Cortex Thickness	4	4.2822	**0.005872**
Epinecral Layer Thickness	4	1.1414	0.3773
Medulla Thickness	4	1.9191	0.1271
Squamule Thickness	4	3.1444	**0.02503**
Hymenium Height	4	3.3513	**0.0258**
Ascus Height	4	2.0886	0.1138
Squamule Width Percent Increase from Dry to Wet	4	0.2109	0.9316
Squamule Length Percent Increase from Dry to Wet	4	4.0519	**0.005098**
Elevation (masl)	4	1.8338	0.1318
Geographical Latitude	4	5.9317	**0.000355**
Geographical Longitude	4	5.0826	**0.001168**

Table 2.5. Analysis of variance pairwise comparisons of continuous features for members of the *C. cariosa* group. Bolded values indicate statistically significant ($p<0.05$) differences between clades.

Clade	Squamule Width	Squamule Length	Squamule Incision Length	Podetia Height	Apothecium Diameter	Cortex Thickness	Squamule Thickness	Hymenium Height	Squamule Length % Increase Dry to Wet	Latitude	Longitude
A-B	**0.0188**	**0.0017**	**0.0481**	0.067	0.5356	0.0737	**0.0196**	**0.0292**	**0.0141**	0.1058	0.2114
A-C	0.2768	0.134	0.8068	0.3995	**0.0016**	0.3332	0.1695	0.3905	**0.0281**	0.3707	0.7564
A-E	**<0.0001**	**<0.0001**	**0.005**	0.06	0.5893	**0.0068**	0.1218	**0.0058**	**0.0322**	0.1696	0.3792
A-F	**0.0004**	**0.0002**	**0.0024**	0.6606	0.5096	**0.0047**	**0.0532**	0.685	**0.0004**	**0.0023**	**0.0017**
B-C	0.3449	0.2109	0.1633	**0.0147**	**0.0133**	0.0689	**0.0139**	0.6237	0.9566	0.6134	0.45
B-E	**0.0108**	**0.0204**	0.2473	0.712	0.9753	0.1744	0.7598	0.2985	0.8987	0.9641	0.8869
B-F	0.1246	0.2681	0.1907	0.0994	0.8285	0.1369	0.9269	**0.0564**	0.1463	**<0.0001**	**0.0001**
C-E	**0.002**	**0.0018**	**0.0262**	**0.0163**	**0.0353**	**0.0139**	**0.0329**	0.2524	0.9448	0.6339	0.602
C-F	**0.0267**	**0.0351**	**0.0171**	0.8934	0.0923	**0.0114**	**0.0187**	0.3095	0.2057	**0.0007**	**0.0026**
E-F	0.2976	0.2335	0.938	0.0764	0.8638	0.9084	0.7341	**0.0143**	0.2547	**0.0003**	**0.0008**

Table 2.6. Means and standard error of measured morphological features.

CLADE	sqwidth.avg (mm)	sqwidth.se (mm)	sqlen.avg (mm)	sqlen.se (mm)	incision.avg (mm)	incision.se (mm)	podht.avg (mm)	podht.se (mm)	podwidth.avg (mm)	podwidth.se (mm)
A car	1.865	0.231	2.246	0.256	0.729	0.115	15.728	1.704	2.245	0.172
B symp	2.490	0.223	3.362	0.321	1.010	0.117	10.370	0.717	2.613	0.406
C acumi	2.256	0.380	2.816	0.382	0.698	0.090	16.078	1.081	1.413	0.191
E	3.924	0.567	5.136	0.763	1.408	0.317	10.103	1.573	3.020	0.860
F	3.153	0.298	4.113	0.492	1.424	0.228	15.563	1.231	2.340	0.409

CLADE	apodiam.avg (mm)	apodiam.se (mm)	pycwid.avg (mm)	pycwid.se (mm)	pyclen.avg (mm)	pyclen.se (mm)	algthick.avg (µm)	algthick.se (µm)	cortexthick.avg (µm)	cortexthick.se (µm)
A car	1.903	0.153	0.287	0.027	0.309	0.038	25.320	1.121	29.070	1.714
B symp	1.687	0.206	0.307	0.042	0.335	0.040	31.263	2.763	36.912	3.316
C acumi	0.661	0.199	0.240	0.029	0.215	0.029	20.612	2.363	23.398	3.003
E	1.673	0.429	0.423	0.037	0.420	0.046	32.916	3.285	44.088	3.167
F	1.575	0.259	0.235	0.042	0.235	0.043	25.971	3.042	44.519	2.690

CLADE	epithick.avg (µm)	epithick.se (µm)	medthick.avg (µm)	medthick.se (µm)	squamthick.avg (µm)	squamthick.se (µm)	sporelen.avg (µm)	sporelen.se (µm)	sporewid.avg (µm)	sporewid.se (µm)
A car	5.469	0.435	87.786	8.040	143.795	9.648	12.498	0.252	3.330	0.055
B symp	7.872	1.350	122.455	13.615	189.962	13.674	12.500	0.162	2.995	0.109
C acumi	2.180	NA	61.970	13.695	110.036	19.677	13.000	NA	2.660	NA
E	6.860	NA	105.328	7.209	176.025	10.056	NA	NA	NA	NA
F	7.927	1.552	118.385	17.573	190.382	14.426	11.330	0.408	3.660	0.000

CLADE	hyemenht.avg (µm)	hyemenht.se (µm)	ascusht.avg (µm)	ascusht.se (µm)	Squamwidthdiff%.avg	Squamwidthdiff%.se	Squamlendiff%.avg	Squamlendiff%.se
A car	45.364	0.824	27.996	0.946	27.200	2.962	20.462	2.059
B symp	39.331	1.020	22.628	1.237	28.798	4.093	30.851	2.305
C acumi	42.000	3.266	29.330	2.723	30.727	6.473	31.561	3.953
E	36.398	1.643	22.264	1.275	29.236	5.530	36.286	8.548
F	47.773	3.336	25.773	1.690	24.382	3.573	34.261	8.823

Table 2.7. Summary of secondary chemistry and key morphological features of members of the *C. cariosa* group from study specimens in the GBS dataset. X indicates the presence of a metabolite in the chemotype.

Species	*Chemotype*	*Atranorin*	*Rangiformic/ Norrangiformic*	*Norstictic/Connorstictic /Constictic*	*Fumarprotocetraric/ Protocetraric*	*Psoromic/Compsoromic*	*Unknown compound*	*% of specimens*	*Morphological Characteristics*
Clade A = C. cariosa	**1**	x						4%	-Significant differences in podetia height and thickness not detected in specimens chosen, but on average, podetia were taller than those of *C. symphycarpa.*
	2	x	x					44%	-Squamules shorter in length and width than *C. symphycarpa*. Clade E, and Clade F, with shorter squamule incisions.
	3	x					x	4%	- Squamules thinner than *C. symphycarpa* and Clade F
	4	x	x		x			35%	- Thicker hymenium layer than *C. symphycarpa* and Clade E
	5	x			x			9%	- Larger apothecia than *C. acuminata.*
	6	x		x	x			4%	- When re-wetted from a desiccated state, experience less expansion in squamule length than others. - Found in Asia, Europe, North America, South America, Antarctica - Taller podetia than Clade E, usually taller than *C. symphycarpa*. - Range: Asia, Europe, North America, South America, Antarctica
Clade B =C. symphycarpa	**1**	x						26%	- Podetia significantly shorter than those of *C. acuminata*. On average, podetia shorter than *C. cariosa*, but not significantly so.
	2	x	x					32%	- Shorter and narrower squamules than Clade E
	3	x		x				11%	- Longer, wider, thicker squamules with longer incisions than *C. cariosa.*
	4*	x				x		21%	- Thinner hymenium layer than *C. cariosa* and Clade F
	5	x					x	5%	- Wider apothecia, shorter podetia, and thicker squamules than *C. acuminata*
	6	x		x		x		5%	- Range: Asia, Europe, North America, South America
Clade C =C. acuminata	**1**	x		x				91%	- Podetia sorediate, mostly unbranched (rarely dichotomously branched near tips) - Podetia may or may not bear apothecia. Podetia significantly taller than those of *C. symphycarpa* and Clade E. - Smaller apothecia than *C. cariosa, C. symphycarpa*, and Clade E. - Shorter squamule width, length, and squamule incisions than Clade E and Clade F - Thinner squamules than *C. symphycarpa* - Range: Asia, Europe, North America, South America
	2	x	x	x				9%	
Clade E	**1**	x						10%	- Significantly shorter podetia than *C. acuminata*, with wider apothecia. On average, podetia shorter than *C. cariosa*, but not significantly so. - Squamules longer and wider than *C. cariosa, C. symphycarpa*, and *C. acuminata*, with longer incisions than *C. cariosa* and *C. acuminata*. Thicker squamules than *C. acuminata* - Thicker primary squamule cortex than *C. cariosa* and *C. acuminata* - Thinner hymenium layers than *C. cariosa* and Clade F - Range: Asia, Europe, North America
	2	x	x					10%	
	3	x				x		30%	
	4	x					x	20%	
	5	x		x				30%	
Clade F	**1**	x						8%	-On average, podetia similar in height to *C. cariosa*, and taller than *C. symphycarpa* and Clade E, though height differences not statistically significant. -Longer, wider primary squamules with longer incisions and thicker cortex than *C. cariosa* and

	2	x		x				75%	*C. acuminata.* - Significantly thicker hymenium layers than *C. symphycarpa* and Clade E - Found largely in the Mediterranean region
	3	x		x	x			8%	
	4	x				x		8%	

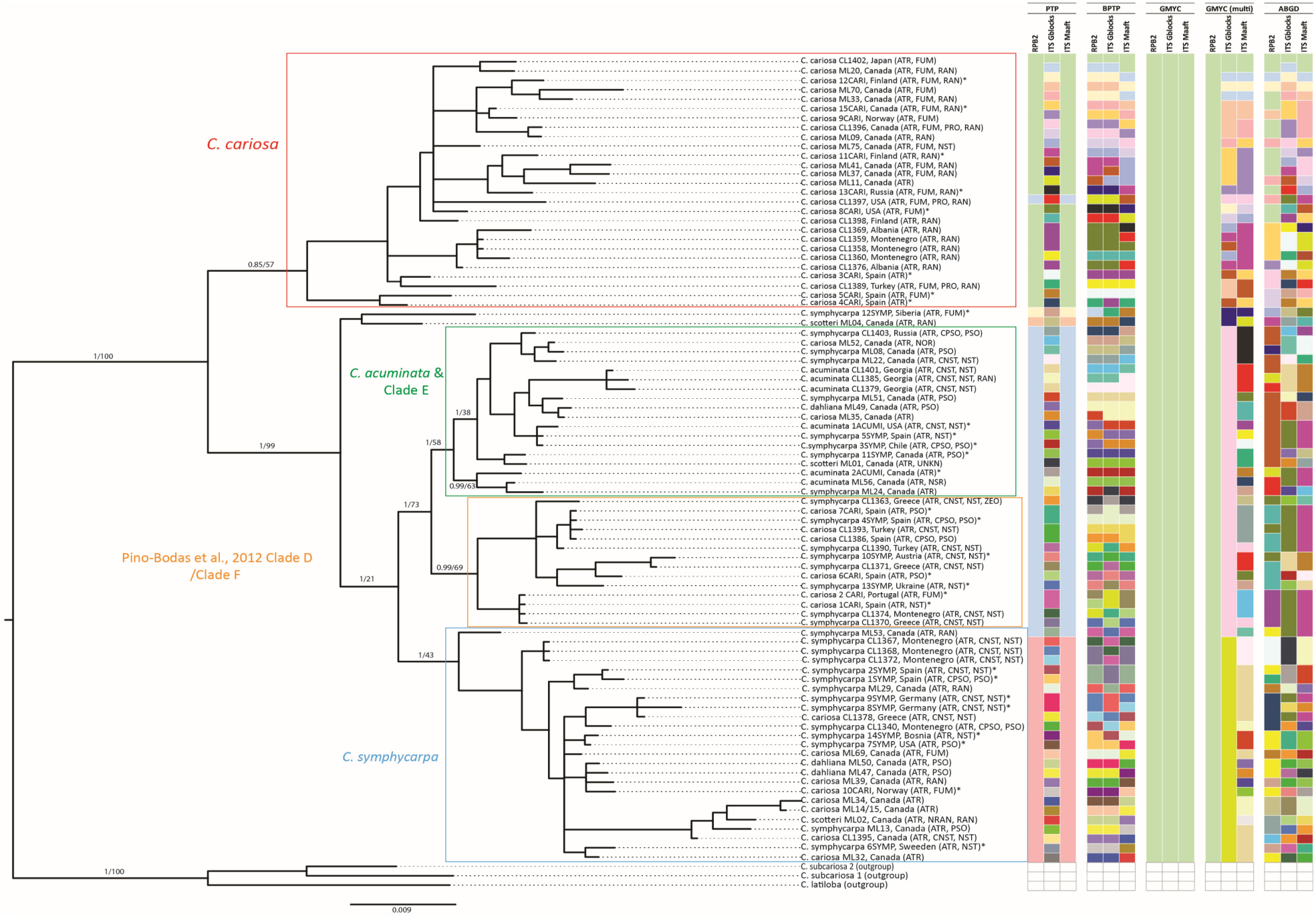

Figure 2.1. Bayesian tree of concatenated complete alignments of RBP2 and ITS gene regions in the *C. cariosa* group. Branch numbers indicate posterior probability/maximum likelihood values (LnL= -5342.84). ATR = atranorin, CNST = constictic acid, CNRST = connorstictic, CPSO = conpsoromic acid, FUM = fumarprotocetraric acid, NORANG = norrangiformic acid, NST = norstictic acid, PSO = psoromic acid, RANG = rangiformic acid, UNKN = unknown compound, ZEO = Zeorin. Coloured boxes indicate identity of clades according to previously identified specimens from Pino-Bodas et al. (2012), specimens are indicated by *.

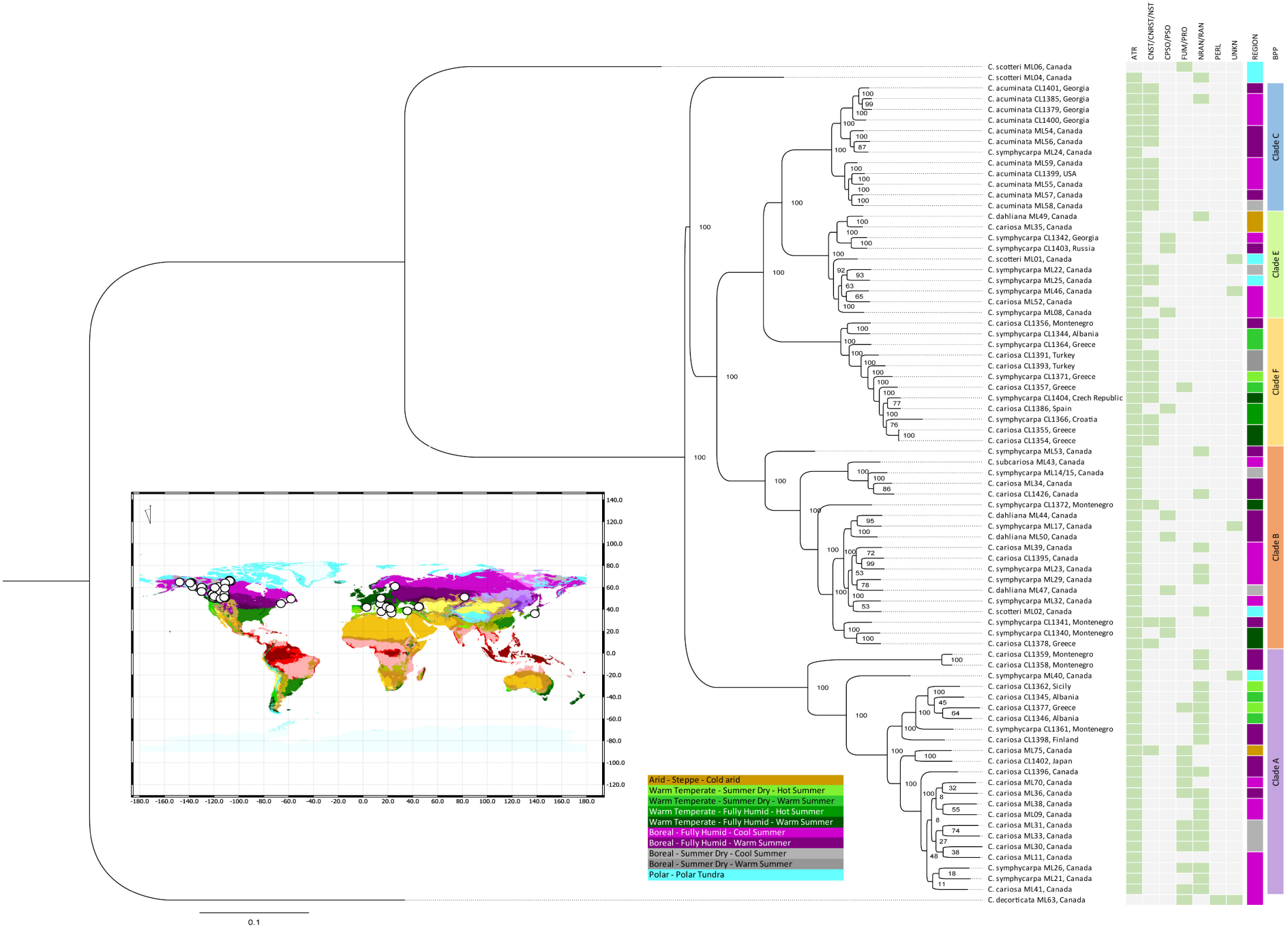

Figure 2.2. Phylogeny of the *Cladonia cariosa* group based on ML analysis of a single nucleotide polymorphism (SNP) dataset produced by a Genotyping by Sequencing (GBS) method (LnL= -6,771,611.51). Bootstrap values are noted at tree nodes. ATR = atranorin, CNST = constictic acid, CNRST = connorstictic, CPSO = conpsoromic acid, FUM = fumarprotocetraric acid, NORANG = norrangiformic acid, NST = norstictic acid, PSO = psoromic acid, RANG = rangiformic acid, UNKN = unknown compound.

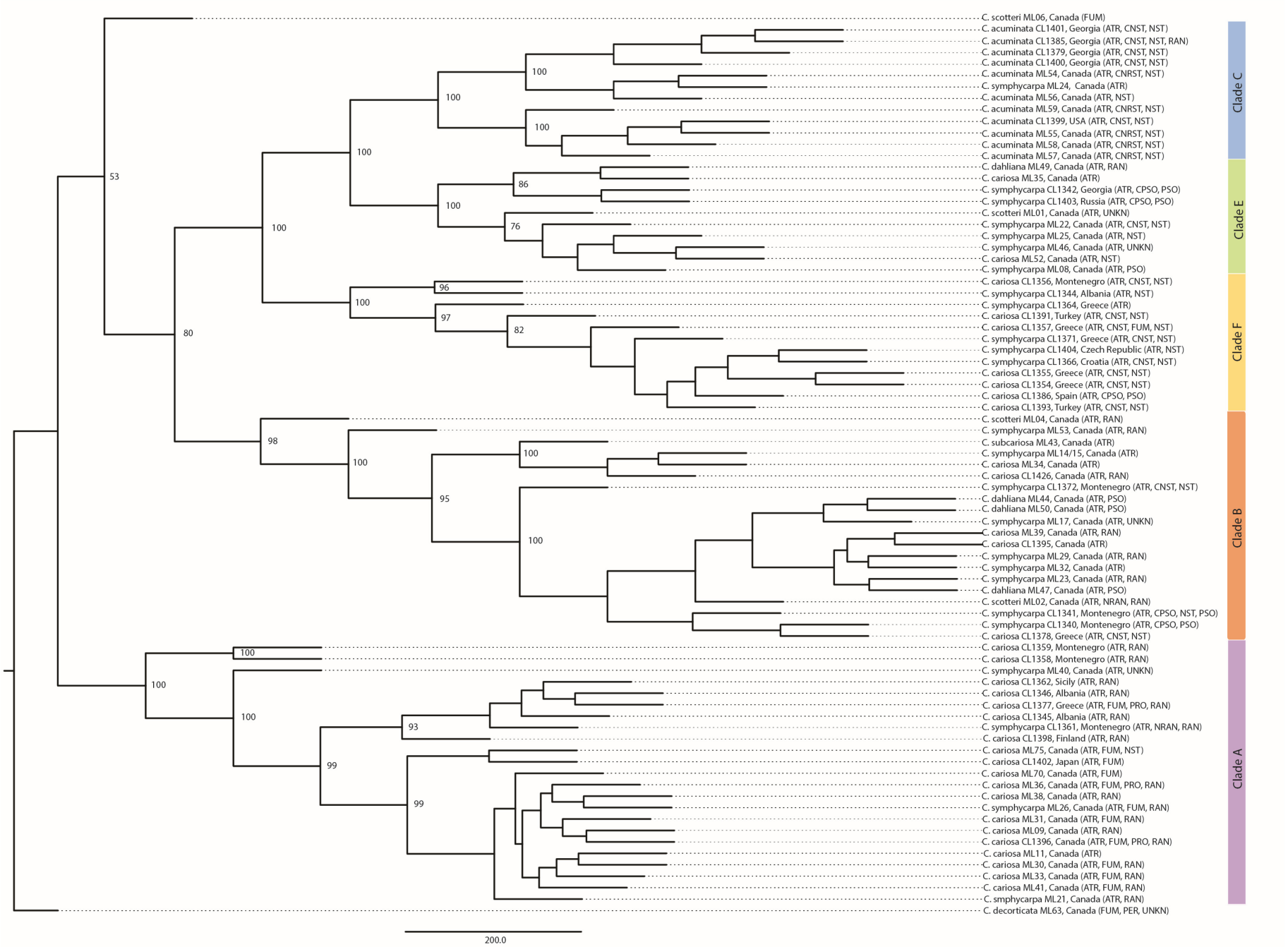

Figure 2.3. Species tree of the *Cladonia cariosa* group estimated by SVDQuartets. Tree is based on an unlinked single nucleotide polymorphism (USNP) dataset produced by a Genotyping by Sequencing (GBS) method. Numbers at tree nodes denote bootstrap support values, tree depicts the majority-rule consensus tree over 100 bootstrap samples.

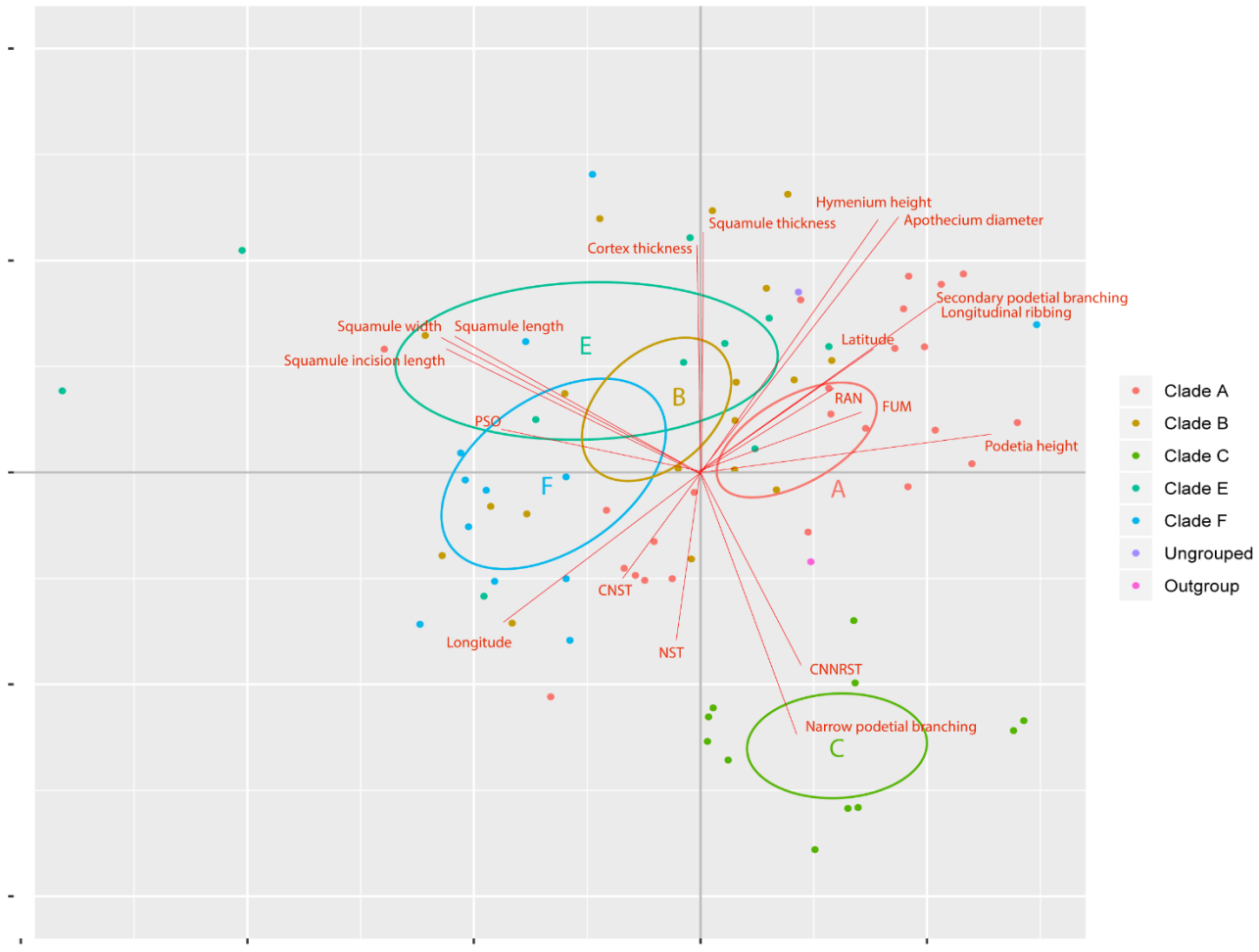

Figure 2.4. Non-metric multidementional scaling (NMDS) ordination of specimens in morphological characteristic space. Significant characters determined by chi-square tests and ANOVA were used for ordination, and were overlain as vectors for visualization. NMDS plotted in two dimensions with PC rotation using Euclidean distance. Data were centered and scaled. Stress = 0.1880951, with two convergent solutions found after 20 tries. Ellipses are standard error ellipses with 95% confidence.

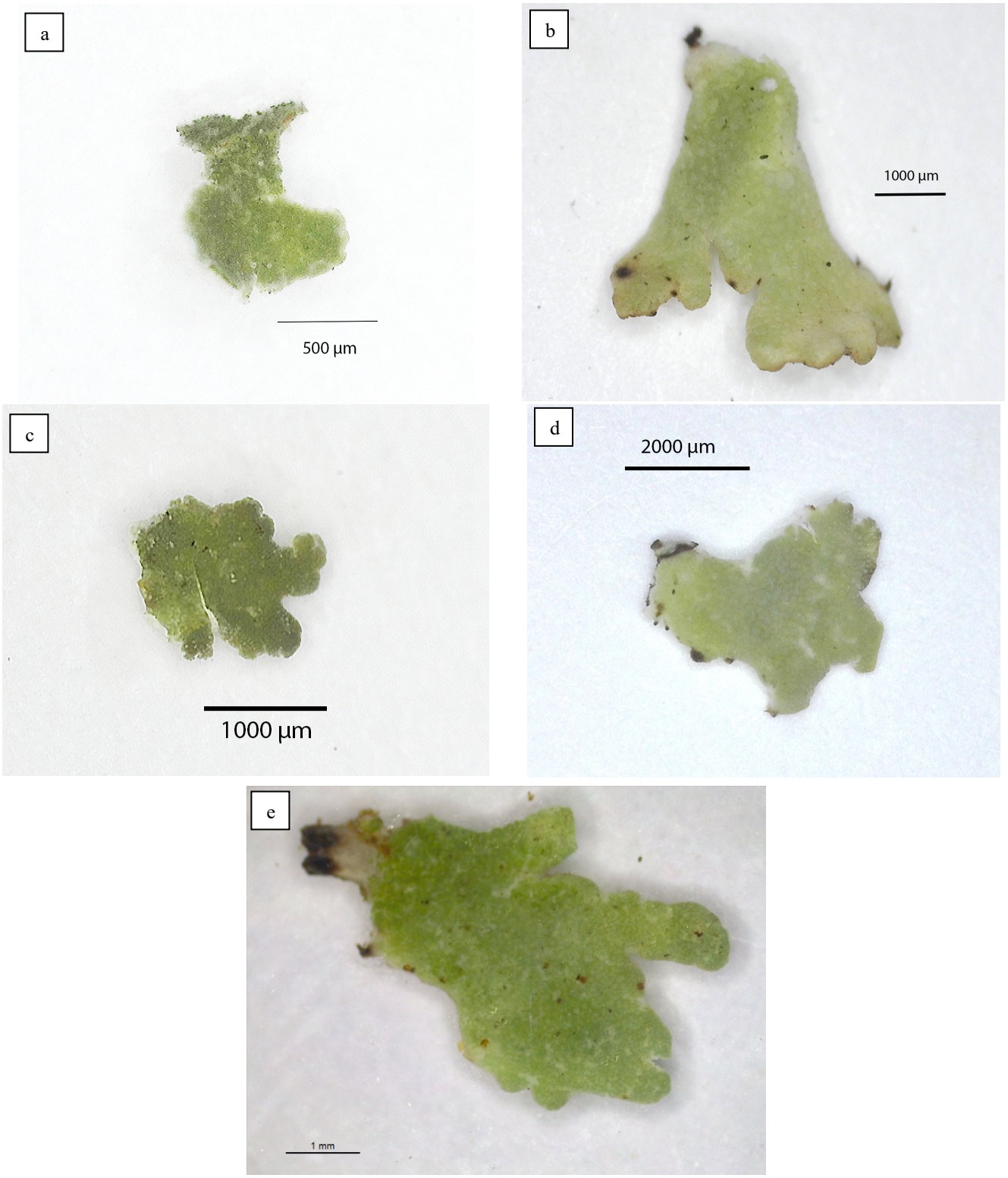

Plate 2.1. Side-by-side photographs of representative squamules from delineated genetic species *C. cariosa* (a), *C. symphycarpa* (b), *C. acuminata* (c), Clade E (d), and Clade F (e). Specimens displayed are (a) ML36, (b) ML39, (c) ML58, (d) ML52, and (e) CL1356.

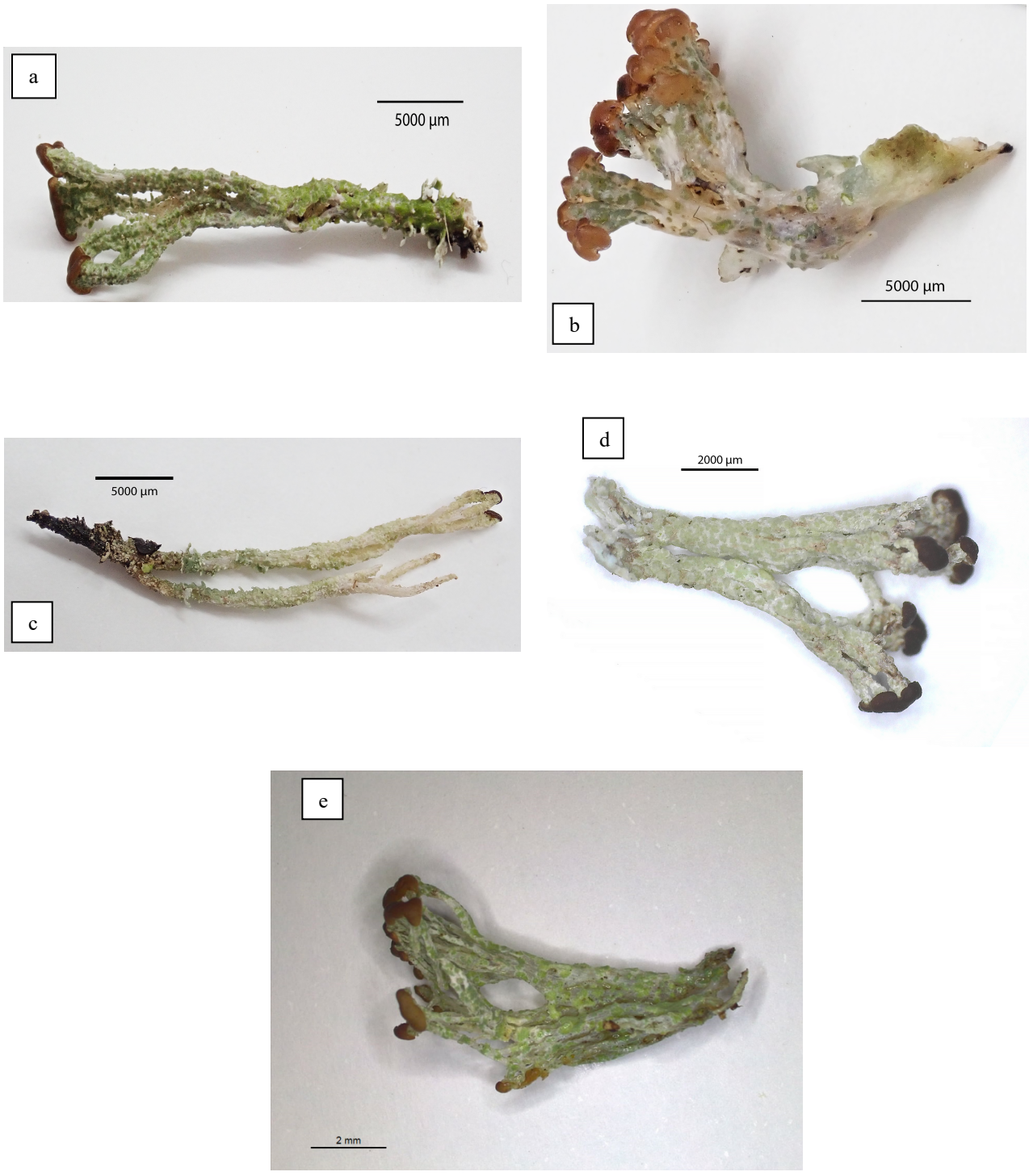

Plate 2.2. Side-by-side photographs of representative podetia from delineated genetic species *C. cariosa* (a), *C. symphycarpa* (b), *C. acuminata* (c), Clade E (d), and Clade F (e). Specimens displayed are (a) ML36, (b) ML39, (c) ML58, (d) ML52, and (e) CL1356.

Plate 2.3. Side-by-side photographs of representative apothecia from delineated genetic species *C. cariosa* (a), *C. symphycarpa* (b), *C. acuminata* (c), Clade E (d), and Clade F (e). Specimens displayed are (a) ML36, (b) ML39, (c) ML58, (d) ML52, and (e) CL1356.

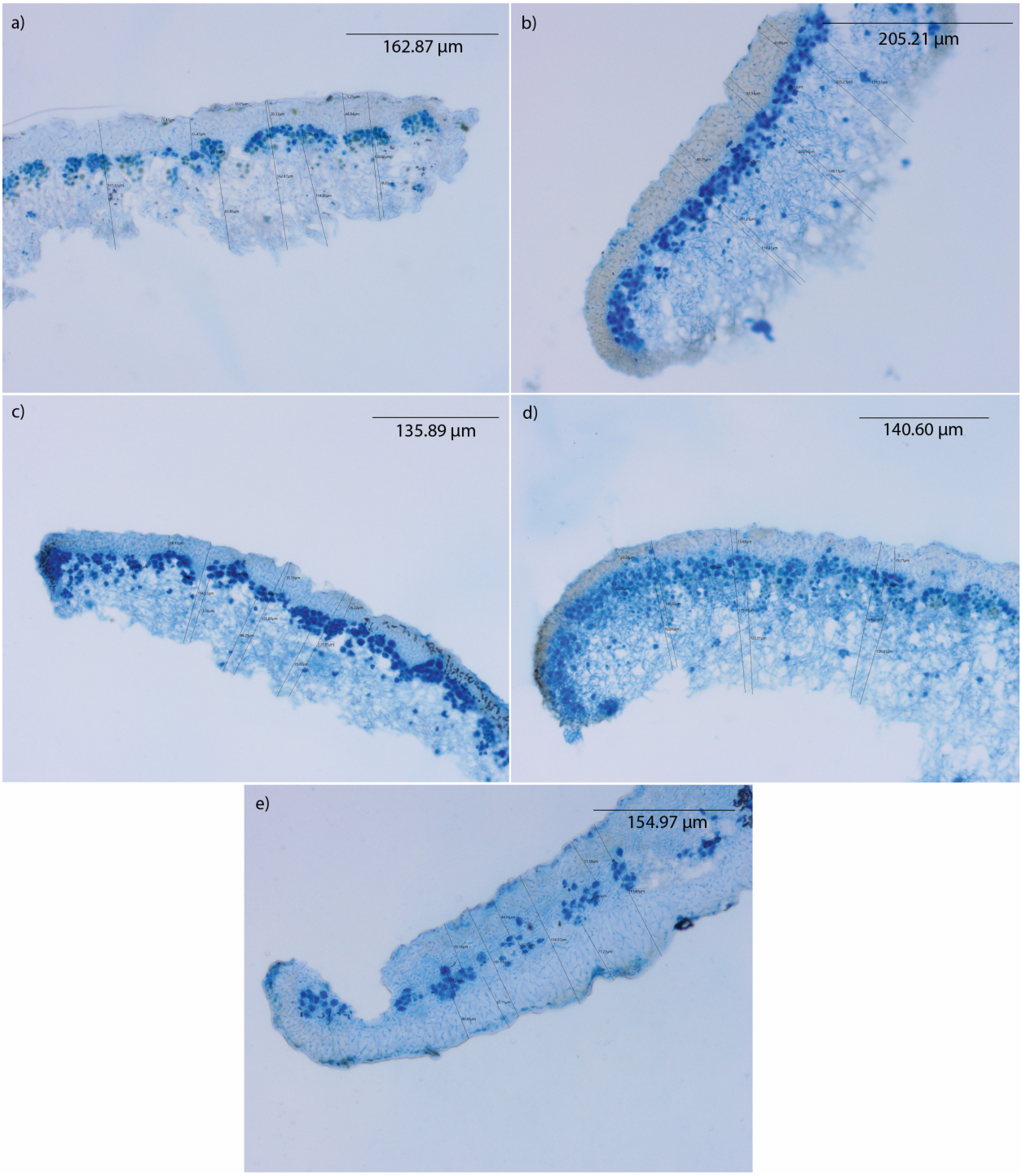

Plate 2.4. Side-by-side photographs of representative squamule cross-sections from delineated genetic species *C. cariosa* (a), *C. symphycarpa* (b), *C. acuminata* (c), Clade E (d), and Clade F (e) under 20x magnification. Specimens displayed are (a) ML33, (b) ML39, (c) ML58, (d) ML52, and (e) CL1356.

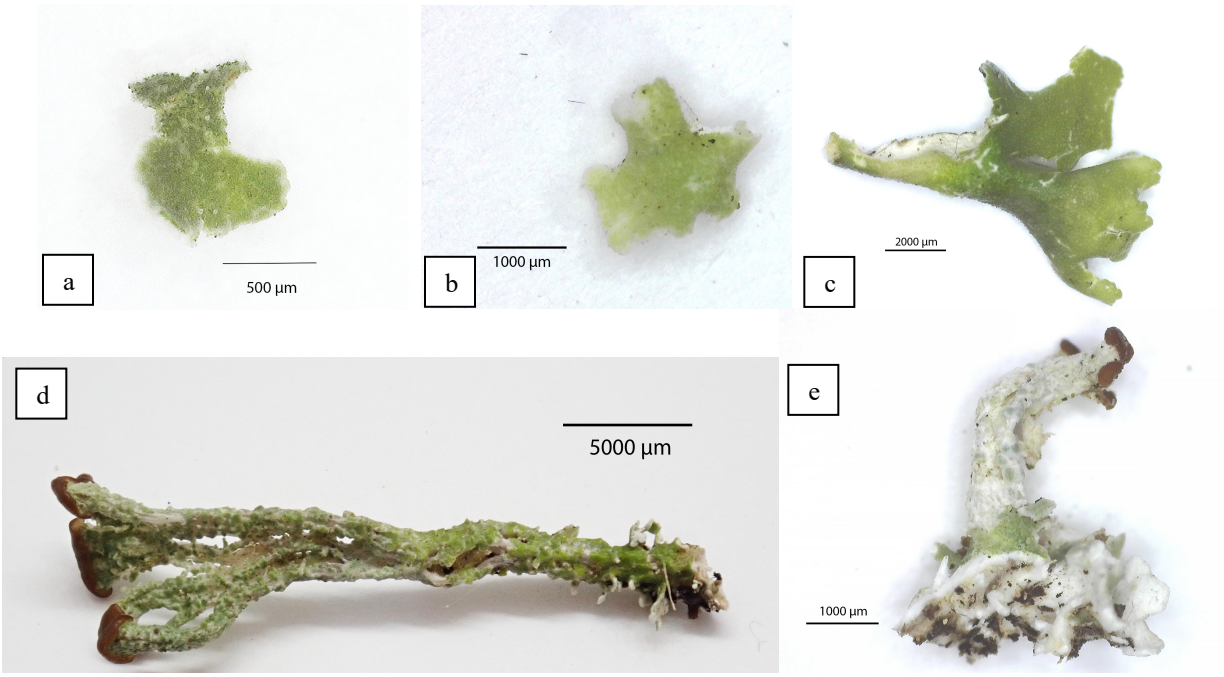

Plate 2.5. Morphological variation in the (a-c) squamules and (d-e) podetia of *C. cariosa.* Specimens displayed (a) ML36, (b) ML31, (c) ML40, (d) ML36, and (e) ML31.

Plate 2.6. Morphological variation in the (a-c) squamules and (d-f) podetia of *C. symphycarpa.* Specimens displayed are (a) ML39, (b) ML23, (c) ML17, (d) ML39, (e) ML23, (f) ML17.

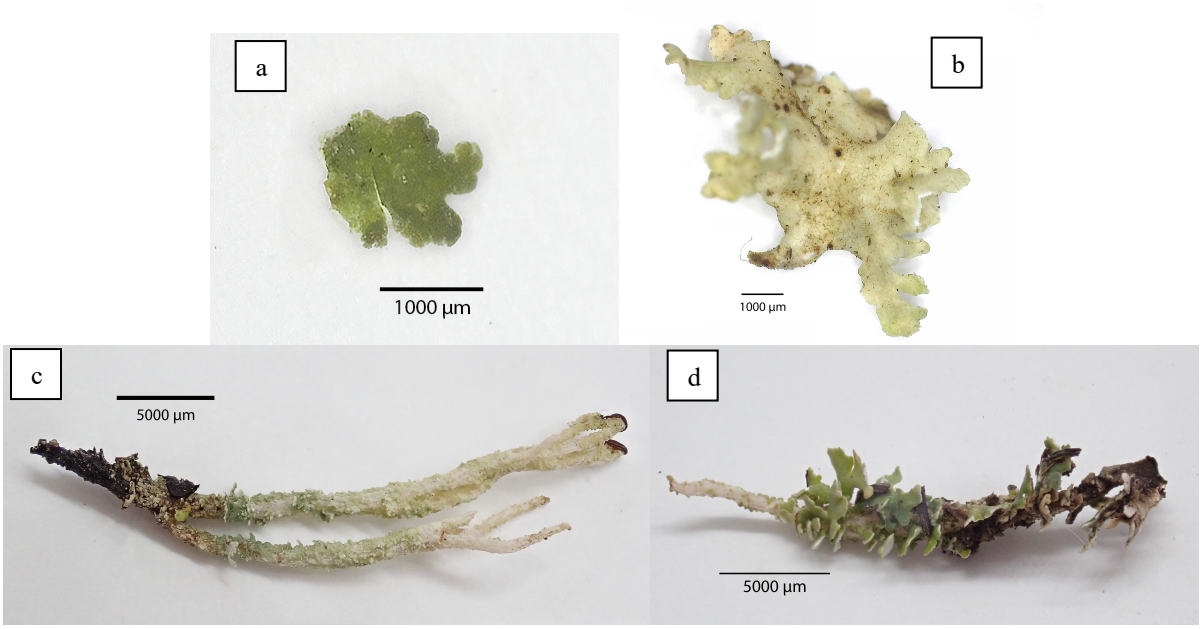

Plate 2.7. Morphological variation in the (a-b) squamules and (c-d) podetia of *C. acuminata*. Specimens displayed are (a) ML58, (b) ML54, (c) ML58, (d) ML54.

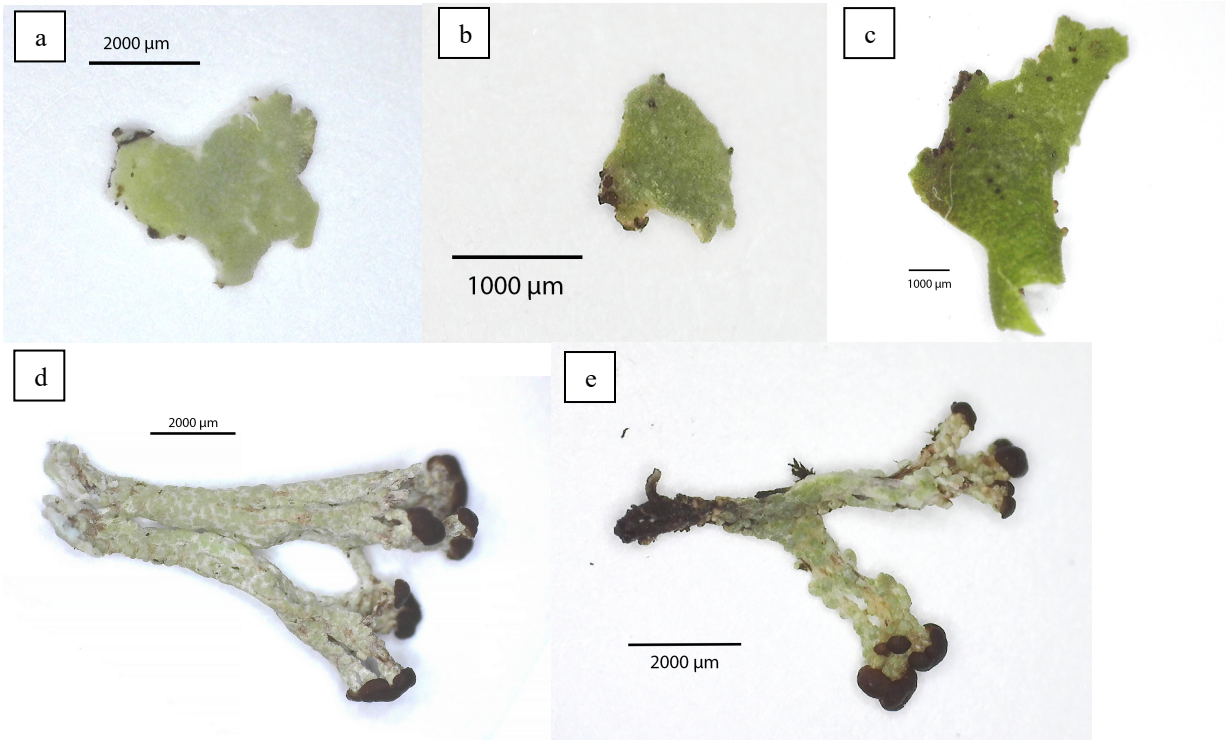

Plate 2.8. Morphological variation in the (a-c) squamules and (d-e) podetia of Clade E. Specimens displayed are (a) ML52, (b) ML01, (c) ML49, (d) ML52, (e) ML01.

Plate 2.9. Morphologic variation in the (a-b) squamules and (c-d) of Clade F. Specimens displayed are (a) CL1357, (b) CL1366, (c) CL1357, (d) CL1357.

Chapter 3

Biological Soil Crust Response to Simulated Drought and Defoliation in the Northern Great Plains

3.1 Abstract

Biological soil crusts (BSCs) are key components of dryland ecosystems that influence crucial ecosystem processes, like nutrient cycling, soil stabilization, and infiltration. Land use intensification combined with changes in climate may alter the cover, structure, and composition of BSC, threatening ecosystem function in drylands. While a growing body of literature exists for BSC of arid regions like the Great Basin and Colorado Plateau, BSC of the Great Plains has been comparatively understudied. Using a manipulative experiment conducted over 4 years at seven North American temperate grasslands sites, we evaluated how BSC species cover, richness, and community composition responded to a 45% reduction in precipitation, and defoliation simulating four common grazing management systems. We observed that effects differed by site, with BSC lichen and moss increasing in cover with defoliation at some sites. *Selaginella densa*, which often acts as a growing medium for biocrust lichen, was found to be sensitive to combined effects of drought and defoliation, decreasing the most under drought and spring+fall defoliation. Moss and lichen cover were found to have negative associations with litter, and lichen had positive associations with light at sites with high ANPP, and negative associations with light at sites with low ANPP. BSC beta-diversity was significantly higher in undefoliated treatments and lower in spring+fall defoliated treatments. Our results indicate that climate change and land use do impact BSC. Given the many demonstrated benefits of BSC,

land management strategies in the Great Plains should seek to include BSC when assessing range health.

3.2 Introduction

Biological soil crusts (BSCs) are communities of organisms that occur on or within a few centimeters of the soil surface, consisting of variable assemblages of lichen, moss, microarthropods, cyanobacteria, algae, fungi, and bacteria (Belnap et al., 2016). BSCs play important roles in soil stabilization and formation (Belnap & Büdel, 2016), regulation of the hydrologic cycle (Chamizo et al., 2016), regulation of seed establishment and germination (X.-R. Li et al., 2005), and in carbon and nitrogen cycling (Porada et al., 2014; Poulter et al., 2014). Members of BSC communities are often poikilohydric and tolerant to conditions to which vascular plants are sensitive, and can occupy up to 75% of cover in arid and semi-arid landscapes (Ferrenberg et al., 2017). BSCs are critical contributors to ecosystem function in drylands; however, projected changes in climate and land use intensification may alter these communities (Ferrenberg et al., 2015), threatening the sustainability of current dryland ecosystem function.

BSCs in the Canadian Prairies, at the northernmost extent of the North American Great Plains, are not as well characterized as in other regions of the world. The Canadian Prairie ecozone represents 16% of the Great Plains of North America, but little of the original grassland habitat remains as about 75% of this ecosystem has been converted to other land use (Gauthier & Wiken, 2003). The region supports extensive cattle ranching and cultivation, with arable land generally allocated to cultivation and the remainder used for cattle grazing. Rangelands in the Great Plains commonly host BSC communities, although they are less visually prominent than in more arid regions (Warren et al., 2021). Work to date has determined that biocrusts in this region are dominated by chlorolichen: lichen-forming fungi that have green algae as a photosynthetic partner (Freebury, 2014; Haughland et al., 2018; Looman, 1964a, 1964b), differing from the cyanobacterial and cyanobacterial-green algal crusts studied in Australia, Colorado Plateau, and

Sonoran Desert (Colesie, Felde, et al., 2016). Surveys completed in Grasslands National Park in Saskatchewan found a rich diversity of lichen (194 lichen species), of which common terricolous macrolichen species included *Cladonia pocillum, Physcia adscendens, Xanthoparmelia wyomingica,* and *Circinaria hispida* growing on a *Selaginella densa*-dominated turf (Freebury, 2014). The limited research that exists suggest that BSC communities in this ecosystem are sensitive to anthropogenic disturbances and slow to recover, with BSCs in disturbed areas consisting of *Nostoc* species, *Cladonia pyxidata, Selaginella densa,* and *Phaeophyscia constipata* and undisturbed late-seral BSC consisting of a high cover of fruticose and foliose lichens such as *Cetraria aculeata, Cladonia cariosa,* and *Xanthoparmelia wyomingica* (Pyle, 2018).

While BSC communities can thrive in drylands like the semi-arid regions of the Canadian Prairies, prolonged drought conditions may reduce BSC functionality. Many species that constitute BSCs are poikilohydric and are dormant when desiccated. Physiological function can be achieved only through absorption of liquid water (in the form of rain, fog, dew) for some organisms such as cyanobacteria, cyanolichens, and bryophytes, while water vapour (humidity) is enough to activate others (Gauslaa, 2014; Proctor et al., 2007). The chlorolichens dominant in Alberta Grassland biocrusts can photosynthesize with humidity alone, suggesting that these types of biocrusts may be more resilient to decreases in annual precipitation (Belnap, Phillips, & Miller, 2004). Well-developed BSC-forming bryophytes often have roughened surfaces that trap water, allowing them to maintain humid conditions for longer and buffer against extreme environmental conditions (Eldridge et al., 2010; Chamizo et al., 2016), although generally are absent from deserts that do not have regular seasonal rainfall or night-time dew deposition (Proctor et al., 2007). Despite the desiccation tolerance imparted by poikilohydry, observations

made on BSC-forming lichens and mosses have shown limits to this physiological adaptation, particularly with prolonged drought conditions that caused significantly slower reactivation of photosynthetic activity during re-hydration cycles (Munzi et al., 2019). Prolonged desiccation of lichen after a period of 9 weeks was found to lead to a reduction of 45% and 40% in chlorophyll α in *Lobaria pulmonaria* and *Peltigera polydactyla* lichen (Kranner et al., 2003). Similarly, in mosses, *Anomodon vitculosus* was found to have slower and less complete desiccation recovery with increased number of days in a desiccated state, with major metabolic disruption and problematic recovery occurring after six to seven weeks (Proctor et al., 2007).

As cattle grazing and BSC presence are often concurrent on Prairie landscapes, land managers may be able to mitigate some of the climatic effects of prolonged drought on BSC dryland communities through altered grazing regimes. Physical disturbance by hooves is well-known to be detrimental to the structure and functionality of BSCs (Mallen-Cooper et al., 2018; Muscha & Hild, 2006; Warren & Eldridge, 2001). The extent of trampling damage has been shown to relate to seasonality, with grazing more detrimental to BSC in dry seasons when they are brittle and slower to recover (Marble & Harper, 1989). Studies of BSC response to the removal of vascular plant biomass isolated from the effects of trampling, however, are fewer in number. In general, a negative correlation has been found between vascular plant cover and BSC, with BSC cover increasing when vascular plant cover declines (Warren & Eldridge, 2001), and decreasing in cover when removal of above-ground vascular biomass via grazing is discontinued (Looman, 1964a, 1964b). In some systems moss cover has been more susceptible to grazing disturbance than lichens (Muscha & Hild, 2006), but has had greater tolerance to grazing than lichens in other systems (Ponzetti et al., 2007; Read et al., 2011). However, most research on BSC composition and how BSCs respond to changes in climate or grazing are based on semi-arid and

arid ecosystems in the southern United States, Europe, or Australia (Büdel, 2001; Warren et al., 2021; Warren & Eldridge, 2001; Weber et al., 2016) and little is known about the resilience of BSCs in the northern grassland systems of the Canadian Prairies.

Projected climate conditions for the next 50 years in the Canadian Prairies are anticipated to include increased drying (Tam et al., 2019) and increased climatic variability, with concomitant increases in extreme wet and dry years, and an increase in the frequency of extreme weather events (Kharin et al., 2007; Kharin & Zwiers, 2000; Mladjic et al., 2010). Grazing effects should be considered concurrently with drought effects on BSC in the Great Plains, as areas where BSC is present are often used for grazing cattle. Given the essential role of BSC in ecosystem function, understanding the impacts of land-use decisions like grazing practice in conjunction with projected increase in climatic drought on BSC will be important considerations for managing these landscapes effectively and sustainably. We examined the response of BSC cover and diversity (namely lichens, bryophytes, and *Selaginella densa*) to drought and different defoliation regimes at seven sites across a climate gradient to address the following questions: (1) how are BSC communities affected by drought and defoliation, and (2) are these effects consistent in BSC communities across an environmental gradient? We hypothesized that a decrease in precipitation would cause a decrease in biocrust cover and would shift community composition due to the negative physiological impacts of prolonged desiccation (Munzi et al., 2019), and that defoliation would have positive effects on biocrust cover and species richness and would cause community shifts due to canopy opening and litter reduction (Corbin & Thiet, 2020).

3.3 Methods and Materials

3.3.1 Site Descriptions

Impacts of drought and grazing on biocrust community composition were assessed at seven native grassland sites in Alberta, Canada. Relative cover of vascular vegetation and BSC varied between sites (Table 3.1). Mean annual temperature (MAT) ranges from 1.9 to 4.9 °C. Mean annual precipitation (MAP) ranges from 312 to 533 mm. Soil types at the sites consist of varying degrees of Chernozemic (Boroll) soils (AGRASID, 2019). Communities that were BSC-dominant consisted of chlorolichens, commonly *Cladonia pocillum, Cladonia rei, Cladonia robbinsii, Cladonia symphycarpa*, *Physconia muscigena, Xanthoparmelia wyomingica,* as well as *Polytrichum* moss and *Selaginella densa*. Communities that were dominated by vascular plants had non-BSC cryptogamic communitites consisting mainly of mosses in *Amblystegiaceae*, *Bryaceae*, and *Pottiaceae* families, with trace amounts or low cover of *Cladonia multiformis, C. pocillum,* and *C. rei* growing on surface litter. Sites were rested from grazing for a minimum of one year prior to applying drought and grazing treatments. Site details and location are available in Table 3.1 and Figure 3.1. Photo plates of the sites are available in Appendix B.8.

3.3.2 Experimental Drought and Grazing Plots

Plots were established in 2016 in upland pasture that had been under grazing exclusion for the preceding year. At each site, 2.5 m x 2.5 m plots were randomly established and each treatment was replicated 4 times, except at Kinsella and Mattheis which had 5 replicates, for a total of 300 plots. A vascular plant study (Batbaatar et al., 2021) was completed concurrently at the plots, and environmental data were shared between studies. Drought and vascular plant defoliation treatments were applied in a fully factorial design, with two levels of precipitation (drought simulated by a 45% reduction in precipitation vs. ambient precipitation) and four levels of

defoliation (3 treatments mimicking common grazing practises and an undefoliated treatment, Table 3.2). Biocrust constituents were not subject to defoliation. Drought was simulated with rainout shelters modelled after Gherardi & Sala (2013), which were removed each October for winter and re-installed the following May.

Environmental variables, including light at ground level, litter quantity, above-ground net primary productivity, soil water content, and soil temperature were assessed at plot level. Soil water content and soil temperature were measured using Decagon 5TM sensors attached to EM50 ECH20 loggers, set at a 2 hour logging interval. Sensors were placed at a 5 cm depth near the center of each plot, but outside of the biocrust cover subplots. Readings were averaged to produce a growing-season mean soil water content and soil temperature. Above-ground net primary productivity (ANPP) and litter quantity were measured at each plot between July 10-30th each year to coincide with peak vascular plant biomass. ANPP and litter were sampled from within a 0.1 x 1 m strip quadrat within the larger 2.5 x 2.5 m plot, but outside of biocrust assessment inner plots. Different locations were sampled each year for ANPP and litter biomass to avoid bias associated with cumulative year-to-year impacts. To determine biomass, samples were dried at 65 °C for at least 48 hours before weighing and all biomass values were converted to g/m^2. For more details on environmental variable measurements and vascular vegetation assessment, see Batbaatar et al. (2021). Soil samples were collected from within the 2.5 m x 2.5 m plots and analysed for texture, pH, total nitrogen, and total carbon.

3.3.3 Species Composition

BSC species composition was assessed prior to the application of experimental treatments in 2016, and during the final year of the experiment in 2019. Assessments were completed by different observers in 2016 and 2019. To estimate inter-observer variability, both observers

assessed 10% of plots in 2019 in order to standardize methodology and to investigate observer effects. Further details on the evaluation of observer effects can be found in Appendix B.2. Lichens, bryophytes, and *Selaginella densa* cover were assessed in 50 cm x 50 cm plots nested within the 2.5 m x 2.5 m treatment plots. *Selaginella densa*, a prostrate lycophyte, was assessed as a part of biocrust constituents because it is drought-tolerant, is functionally similar to biocrust, and often acts as a growth substrate for biocrust algae, cyanobacteria, lichens and bryophytes. The biocrust cover plots were assessed by quadrant (SE, SW, NW, NE), and then quadrant covers were combined to get final cover for each species within the 50 x 50 cm plot. Absolute cover was used to assess change in biocrust constituents and was measured using a ruler to avoid subjectivity associated with percent cover estimates, and to decrease observer variability. Plots were shaded with an umbrella and sprayed with water prior to assessment to increase species detectability, except the Onefour site which was assessed dry in 2016 and 2019. Representative bryophyte specimens were collected from all bryophyte species observed at the site level, and were lab identified by Teri Hill and Brittney Miller at the Alberta Biodiversity Monitoring Institute. When separate bryophyte species were not reliably differentiated at the field-level, species were lumped into species groups for community and species richness analyses. Representative specimens of lichen were also collected for identification confirmation in lab.

3.3.4 Data Analysis

Analyses were conducted in R version 1.3.1073 (R Core Team, 2019) with mixed modelling completed using nlme (Pinheiro et al., 2020) or the base stats package (R Core Team, 2019), and ANOVAs completed using car (Fox & Weisberg, 2019). Cover analyses were modelled by cover stratum (total BSC cover, lichen, moss, and *S. densa*) separately at each site using generalized least squares linear models with identity variance structures for drought and defoliation to test

cover and richness responses to treatments. Biocrust, lichen, moss, and *S. densa* response to environmental variables (light at ground surface measured under litter, litter biomass, annual net primary production, soil moisture content, and soil temperature) were also modelled. We chose to analyze each site separately because our study sites had high ecological dissimilarity, and we suspected that treatments would have different effects at different sites. We found biocrust composition to be highly variable at the microtopographic scale, and plots were found to differ significantly from each other in 2016, before experimental treatments began. To account for natural variation in the plots prior to experimental treatment, we used the 2016 cover/richness of the respective response stratum as a covariate in the model, and 2016 data are presented in figures to illustrate changes during the course of the study. Normality and heteroscedasticity were confirmed visually with plots of model residuals. Transformations were used on response variables only if they improved residual plots. If model residuals did not meet normality and heteroscedasticity requirements following use of identity variance structures and transformations, absolute cover values were converted to proportions, arcsine transformed, and modelled using a Gamma distribution. Specific transformations and models used are defined in Appendix B.5.

Biocrust community responses to treatments and associations with environmental gradients were visualized with NMDS ordinations of Bray-Curtis dissimilarity indices of community abundance matrices using vegan (Oksanen et al., 2019). A dummy species with a cover of 1 was included as a zero-adjustment to improve the erratic behavior of the Bray-Curtis coefficient in sites with empty and depauperate plots, following Clarke et al. (2006). Permutational Analysis of Variance (PerMANOVA, n.perm = 10,000) was completed in vegan (Oksanen et al., 2019) on Bray-Curtis dissimilarity indices to determine whether biocrust community composition significantly differed

between treatments. Post-hoc testing was completed using the RVAideMemoire package with 10,000 permutations (Hervé, 2021).

Betadisper (Oksanen et al., 2019) was used to test homogeneity of group dispersions (beta-diversity) between treatment groups using spatial medians as the group centroid. If negative eigenvalues were produced in the underlying Principal Coordinates Analysis for Betadisper, values were corrected using the Lingoes method (Legendre & Anderson, 1999).

ANOVAs (Fox & Weisberg, 2019) on Bray-Curtis dissimilarity between 2016 and 2019 plots were used to determine if the magnitude of shift in community composition was significantly different between treatment groups.

3.4 Results

3.4.1 Community Characterization

BSC species by site in 2016 and 2019 are presented in Figure 3.2. BSC at Onefour, Mattheis, and Twin River sites tended to be lichen and *Selaginella*-dominant, while Oyen, Kinsella, Stavely and Sangudo sites tended to have higher moss cover at the ground surface. Dominant biocrust vegetation at Onefour included *Xanthoparmelia wyomingica, Xanthoparmelia camtschadalis, Cladonia robinsii, Cladonia symphycarpa,* and *Selaginella densa.* The study plot at Mattheis was similar to Onefour, also containing high cover of *S. densa, X. wyomingica*, and *C. robbinsii* in the biocrust, as well as *Physconia mucigena*, *Cladonia rei*, and members of the *Cladonia pocillum* group. The biocrust at Twin River primarily consisted of *S. densa*, with some *Cladonia* species. Biocrusts at Onefour, Mattheis, and Twin River were evenly-distributed for the most part, and present at all plots. Biocrust at the Oyen study site contained *Cladonia rei, Polytrichum*

juniperinum, Cladonia multiformis, Selaginella densa, and members of *Amblystegiaceae* and the *C. pocillum* group. Stavely had only one plot containing surface cryptogams, which consisted of trace amounts of moss in the Bryaceae family, but did not have a continuous, discernable biocrust. Kinsella's biocrust was moss-dominant, and consisted mainly of moss in the families *Amblystegiaceae*, *Brachytheciaceae*, *Hypnaceae*, *Thuidiaceae* as well as *Ceratodon purpureus* and small amounts of the lichen *C. rei*. Sangudo also had a moss-dominant crust, consisting mainly of moss in the *Amblystegiaceae* and *Brachytheciaceae* families. Biocrusts in Oyen, Kinsella, and Sangudo tended to have lower cover and be patchier than those in the southern study sites, and did have some plots with zero percent cover of cryptogamic crust. Biocrust presence in plots at Oyen and Stavely did not meet thresholds for plot replication, so we removed these sites from subsequent analyses. A full list of species by site found during the study is available in Appendix B.1. Because the sites were ecologically distinct, and in fact had almost no overlap in multivariate space (Figure 3.3), we analysed each of the remaining 5 sites separately. We then examined the statistically significant changes across sites for overarching commonalities.

3.4.2 Do drought and defoliation influence biocrust cover and richness?

At Onefour, the site with the highest % biocrust cover and the second lowest ANPP, biocrust cover increased significantly in ambient plots in 2019 (Table 3.3). In comparison, biocrust cover remained relatively constant in rain shelter plots (Fig. 3.4a). These differences were largely driven by increases in *S. densa* cover (Fig. 3.4b) under ambient conditions. Defoliation did not significantly impact biocrust cover at Onefour.

Conversly, in Sangudo, a site with low biocrust cover and relatively high ANPP, biocrust cover was not impacted by drought but it was impacted by defoliation. This site did not exhibit a real biocrust, instead we initially documented low cover of a diversity of bryophytes. All defoliation treatments as well as the control exhibited increases in moss cover from 2016 to 2019 (Fig. 3.4d). The highest moss cover increase was observed in plots subjected to spring+fall (HH) defoliation, and was lowest in undefoliated plots (NN) or only spring-defoliated (HN), though error was high in NN plots, and HH defoliated plots started with higher cover in 2016.

Treatment effects were most complex at Matthias, the site with the highest lichen richness, a well-developed, relatively extensive biocrust, and the lowest ANPP. Lichen cover at Mattheis (X^2=9.833, p=0.020) increased across most treatments, and the greatest increases were observed in undefoliated (NN) plots, while the lowest was observed in fall-defoliated (NH) treatments (Fig. 3.4c). All defoliation treatments increased lichen cover, except for the NH where lichen cover remained relatively stable. In addition, interactive effects of drought and defoliation on cover were observed at Mattheis on total BSC cover (X^2=20.86, p=1.12E-04, Fig. 3.4e) and *S.densa* (X^2=21.47, p=8.42E=05, Fig. 3.4f). For biocrust cover as a whole at Mattheis, defoliation treatments were not significantly different under AM, but did differ significantly from each other under RS. Under drought, NN plots experienced an increase in cover from 2016, and had significantly higher cover than NH and HH, which conversely decreased in cover from 2016. Interestingly, total BSC cover in RS-NN plots increased from 2016, in contrast to AM-NN, AM-HN, AM-NH treatments, all of which experienced cover decreases from 2016 (although AM-HN plots started with higher cover in 2016, so this decrease brought total BSC cover levels down to be approximately equal with RS-NN plots). When subjected to HH grazing, BSC increased under AM, but decreased under RS. Trends in total BSC cover appeared to be

attributable to *S.densa*, which follows the same response patterns as total BSC cover, but with higher effect magnitude in some treatments, as total BSC cover responses were likely buffered by relative stability in lichen cover responses. Under AM conditions, *S. densa* experienced a large decrease in cover in AM-NN plots and increased in AM-HH plots from 2016 to 2019, with higher cover in AM-HH treatments. Interestingly, the inverse effect was observed in RS plots; where RS-HH decreased cover and RS-NN plots increased in cover, with higher cover in RS-NN plots in 2019. RS-NN plots also had significantly higher cover than RS-NH, which decreased in cover substantially from 2016.

Biocrust cover did not differ significantly across treatments at Twin River or Kinsella, and biocrust richness did not differ significantly between drought and defoliation treatments at any of the sites. Complete results are presented in Table 3.3 and Appendix B.3.

3.4.3 Is biocrust community composition influenced by drought and defoliation?

PerMANOVA found a drought x year interaction at Onefour in BSC community composition, indicating that 2019-AM plots were significantly different from 2019-RS, 2016-RS, and 2016-AM plots ($F=3.43$, $p=0.050$, Fig. 3.5). AM plots in 2019 at Mattheis had greater beta-diversity than RS plots, however, they also had greater beta-diversity to begin with in 2016 ($F=7.45$, $p<0.001$, Fig. 3.6a). Beta-diversity increased in both AM and RS treatments from 2016 to 2019 at Mattheis, but communities also differed compositionally between 2016 and 2019, although this didn't appear to be associated with treatment ($X^2=5.36$, $p=0.002$). HH defoliation at Mattheis had significantly lower beta-diversity than other defoliation treatments in 2019, and NN plots significantly increased in beta-diversity from 2016 to 2019 ($F=2.91$, $p=0.009$, Fig. 3.6b). Although communities themselves were not compositionally different between treatments, the

magnitude of community compositional shift from 2016 to 2019 was higher in HH defoliation treatments than in NN treatments at Mattheis, but only under RS conditions (X^2=8.53, p=0.036, Fig. 3.7a).

At Twin River, treatments between years didn't appear to result in significant differences in beta-diversity or community composition, but of the compositional shifts that occurred, RS-HH experienced the most compositional shift in relation to other treatments. Compositional shift in RS-HH plots was significantly higher than in RS-HN and RS-NH, as well as AM-NH and AM-HH plots (X^2=8.74, p=0.032, Fig. 3.7b).

Plots in 2016 differed compositionally from 2019 plots at Kinsella and Sangudo, although differences didn't appear to be associated with treatments (F=2.39, p=0.040; F=6.89, p=0.002). Plots subjected to RS treatment had significantly larger shifts in community composition than AM plots at Kinsella (X^2=10.46, p=0.001, Fig. 3.7c). At Sangudo, NH plots had larger community shifts than NN and HN (Fig. 3.7d). Results of community analysis are presented in Table 3.4, and Appendices B.4. and B.6.

3.4.4 Are the effects of drought and defoliation on biocrust cover constituents and ecosystem components related to eachother?

Lichen cover had a negative association with light at ground level at Mattheis (p=0.01191, Fig. 3.8a), a positive association with light levels at Kinsella (p=0.0153, Fig. 3.8b), and a negative association with litter at Kinsella (p=0.0001, Fig. 3.8c). Moss cover was negatively associated with litter at Sangudo (p<0.001, Fig. 3.8d), and *S. densa* was found to be negatively associated with soil moisture content at both Twin River (p=0.02, Fig. 3.8e) and Mattheis (p=0.029, Fig. 3.8f). Results are available in Table 3.5.

Statistically significant environmental gradients identified in NMDS and vector analysis included soil temperature, litter quantity, annual net primary production (ANPP), precipitation, soil moisture content, and light (Fig. 3.3). Direct gradient analysis showed associations between increased light levels and *C. symphycarpa, C. robbinsii*, the *C. pocillum* group, *P. constipata, C. acuminata/rei/cornuta*, and *X. wyomingica*. Mosses were associated with high litter cover, high ANPP, grass cover, and increased precipitation. *S. densa, X. camtschadalis, X. wyomingica, C. robbinsii,* and *C. symphycarpa* were negatively associated with litter, ANPP, precipitation, and grass cover.

3.5 Discussion

BSC cover and community responses to grazing and drought varied across sites and across functional groups, which was not surprising given the ecological differences between sites. Plant-biocrust interactions are highly dependent on ecological context and community composition of biocrusts and vascular plant communities (Havrilla et al., 2019), an important detail to keep in mind when considering the results of our manipulative ecological experiment across differing natural subregions. Study locations with higher precipitation, higher vascular plant productivity, and higher litter tended to have a cryptogamic cover composed primarily of mosses, whereas locations with lower precipitation, low vascular plant productivity, and increased light at ground level tended to have lichen-dominated BSC consisting primarily of foliose lichen patches interspersed with, or growing on, *Selaginella densa*, which was consistent with other descriptions of BSC distribution (Belnap et al., 2016; West, 1990). Our dry southern sites had a fairly continuous, dominant biocrust, but moving north into more mesic areas, biocrusts gave way to fragmented surface cryptogamic individuals prevailing under shaded grass

and litter canopies. One site with particularly high litter and ANPP (Stavely) had no cryptogamic cover at all. Biocrust communities have been observed to exist along a gradient: in semi-arid regions, dry conditions limit primary productivity, and create the sparse litter conditions and open canopy that allows BSC to thrive (Corbin & Thiet, 2020), and even in more mesic areas, BSC can exist in the interspaces between bunchgrasses and shrubs (Warren et al., 2021), eventually succumbing to shading out by live biomass and litter deposits of well-established vascular plant communities (Sedia & Ehrenfeld, 2003). Light intensity and moisture seem to be important factors that drive surface moss and lichen abundance, which are largely influenced by the overlying vascular plant community (Sedia & Ehrenfeld, 2003), which in turn is influenced by climate and disturbances such as grazing.

Lichen cover under drought did not significantly differ from lichen cover under ambient conditions in our experiment. Lack of lichen response to drought is not surprising, given their poikilohydrous nature and adaptations to moisture availability. Lichen are often dry and dormant during extreme environmental conditions, like high temperature and low moisture conditions, using dormancy as an 'avoidance' survival strategy (Green et al., 2018). Lichen also have physical adaptations driven by water availability. Physical adaptations of lichen such as rhizines, and similar structures, bind lichen to their growth substrate and also enable them to access stored water within the soil (Colesie, Green, et al., 2016). In addition to accessing soil moisture, lichen are able to utilize moisture from rain, dew, and fog for photosynthesis (Gauslaa, 2014). Some studies have found that prolonged desiccation can have negative impacts on lichen photosystem recovery (Kranner et al., 2003; Munzi et al., 2019). While we did not examine photosynthetic response in this study, it appears that the threshold for negative impact from desiccation was

either not reached, or if lichen were negatively impacted, it did not result in corresponding impacts on cover.

Lichen cover increased under most grazing treatments at Mattheis, except for fall-defoliation where it appeared to stay the same. Responses to light were also examined, as one of the main impacts of defoliation is an increase in light levels at ground surface. Interestingly, lichen had different responses to light at different sites. Lichen at Kinsella, a mesic site with relatively high vascular plant cover, had a positive association with light levels, whereas lichen at Mattheis, a semi-arid site with relatively low vascular plant cover, had a negative association with light levels. Light levels did differ substantially between the sites, sub-litter light levels were mostly less than 1000 µmol $m^{-2}s^{-1}$, whereas sub-litter light at Mattheis tended to be greater than 1500 µmol $m^{-2}s^{-1}$, which indicates that higher light levels associated with defoliation could be advantageous for lichen, but may be detrimental at high levels. Lichens, while adapted to high-light conditions, have been found to mainly be photosynthetically active at low light levels (Green et al., 2018) and benefit from the photoprotection and reduction in desiccation that partial shading provides (Y. Zhang et al., 2016).

Moss increased in response to all defoliation treatments at Sangudo, a mesic site with high ANPP, with fall defoliation and spring+fall treatments increasing moss cover the most. Moss was also found to have a negative relationship with surface litter in this study. BSC constituents are generally thought of as poor competitors against established vascular foliage, and thrive in arid and semi-arid climates where drought limits primary productivity (Y. Zhang et al., 2016), but in temperate biomes, biocrusts require plant-limiting edaphic conditions or periodic disturbances to the vascular plant community to maintain an open canopy (Corbin & Thiet,

2020). Defoliation disturbance reduces litter accumulation and, as mentioned previously, also allows for infiltration of light to the ground surface. In some ecological contexts, like in deserts, biocrust moss has been found to prefer shaded habitats over open canopies (Eldridge et al., 2000), as exposure can lead to drying and heat-accelerated water loss (Xu et al., 2009). Differences seen here might be explained by differences in climate, as the Sangudo site has higher precipitation (492 mm MAP), cooler mean annual temperature (2.2°C), and experienced comparatively low light levels at ground surface (285 μmol $m^{-2}s^{-1}$). Mosses were likely light-limited here and, once exposed by defoliation of vascular plant cover, did not experience the heat and photo-stress conditions present in arid/semi-arid sites.

Though we did not observe significantly different biocrust community composition between treatments, it was found that drought alone, drought accompanied by spring+fall defoliation, and spring+fall or fall defoliation alone caused some shifts in communities over time, depending on the site. Beta-diversity, i.e. species diversity within a treatment, was significantly lower in spring+fall defoliation treatments than other treatments, and highest beta-diversity was observed in undefoliated treatments. This may be demonstrating that competition becomes less important in habitats characterized by high disturbance or abiotic stress (Grime, 2001), increasing the importance of niche-based processes - specifically, a species' ability to cope with extreme abiotic conditions versus its ability to compete. Disturbances like defoliation of vascular plants twice a year, as in our spring+fall defoliation treatment, cause increasingly extreme abiotic stress, and can cause cover reduction of disturbance-intolerant species that are otherwise strong competitors, leaving high-disturbance communities with disturbance-tolerant species that are inferior competitors, increasing community similarity among disturbed sites (Jiang & Patel, 2008). Undefoliated treatments leave live vascular biomass and accumulated litter in place, albeit very

little of each in semi-arid sites, which may have created shady microsites for competitive shade-tolerant BSC constituents while also hosting disturbance-tolerant BSC constituents, explaining higher beta-diversity in undefoliated treatments. The treatments causing the largest abiotic stress, drought combined with spring+fall defoliation, resulted in the largest community shifts from 2016 to 2019, with the most extreme defoliation treatments resulting in more similar communities, ie. those made up of disturbance-tolerant BSC constituents.

Club moss *(Selaginella densa)*, a low-growing vascular cryptogam, is common in our grassland systems, providing up to 80% ground cover in many plant communities in our region (Colberg & Romo, 2003). It has low water-use requirements and often is found growing in areas where precipitation is insufficient for sustaining cover of forbs and grasses (Webster and Steeves 1964). Cover afforded by clubmoss may have positive ecosystem benefits, such as the prevention of soil erosion, regulation of soil surface temperature, and the increase of soil organic matter and water availability (Naeth et al., 1991; Shay et al., 2000). While the conventionally accepted definition of biocrust does not include clubmoss as the majority of the biomass is above the soil surface, the boundaries between club moss mats and soil crusts can be considered fluid (Belnap et al., 2001). As clubmoss often acts as a growing medium for biocrust lichen, performs similar functions as biocrust, and occupies a great deal of ground cover in our grassland systems, we believe there is a strong case to consider club moss as part of biocrust. While known to increase with grazing and be tolerant of drought (Van Dyne & Vogel, 1967), club moss preferred ambient conditions at our driest site, Onefour, but had a negative association with soil moisture at the other two semi-arid sites, indicating perhaps an intolerance to wetter conditions, or decreased competitiveness in habitats with higher soil moisture contents. Club moss appeared to do well with some disturbance, and was able to handle high levels of defoliation under ambient conditions, but

under drought conditions did best with un-defoliated treatments and poorly with high levels of defoliation, suggesting it may not be as resilient to drought and grazing as other BSC constituents, despite its ubiquitous presence in our southernmost sites.

Precipitation during the growing season varied among sites and across study years. Growing season precipitation was lower than the long-term (115-year) average growing season precipitation in most sites and years, except in 2019 when growing season precipitation was higher than the long term average at Kinsella, Sangudo, and Stavely (Batbaatar et al., 2021). As most sites were already experiencing drought, treatments with rainout shelters applied accordingly experienced an extreme reduction in growing precipitation. Despite extreme drought conditions in drought-treatment plots, lichen and moss cover did not differ from ambient plots, demonstrating the resilience of these organisms to dry conditions.

3.6 Conclusion

In this study, we found that the effects of drought and defoliation on BSC were variable by site. BSC was largely adapted to a reduction in precipitation and defoliation, but may experience sensitivity to extremes in light intensity or litter quantity, and for *S. densa*, wet conditions. The change in environmental conditions for BSC resulting from defoliation of vascular plants may cause an increase in abundance of BSC, but of note, our study isolated defoliation effects from those of trampling, which is often detrimental to BSC (Warren & Eldridge, 2001). In addition, beta-diversity of BSC communities at some sites was lower in plots where defoliation disturbance of vascular plants was more intense. The presence of BSC on the landscape may be an indicator of ecosystem health in arid systems, but in more mesic environments the occurrence of BSC organisms can signal a degraded or early-successional status (Warren & Eldridge,

2001), or limiting soil conditions (Warren et al., 2021). While excessive grazing disrupts the balance between ungulates, the vascular plant community, and BSC, total removal of livestock may also constitute a disturbance - especially in ecosystems like the Great Plains that evolved under a disturbance regime - ultimately resulting in a loss of productivity (Milchunas et al. 1990). Given that effects of climate and landscape management strategies on BSC communities are largely dependent on the ecosystems in which BSC is found, successful land management strategies should strive to incorporate ecological knowledge of the particular site, range monitoring programs that include assessment of BSC cover, and use stocking rates and grazing systems that mimic the historic disturbance regime for the ecosystem.

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Table 3.1. Ecologic and climate characteristics of seven study sites in the Grassland ecoregion of Alberta, Canada, adapted from Batbaatar et al. (2021). Mean annual precipitation (MAP) and temperature (MAT) are based on 115-years (1901-2015) of data obtained from ClimateNA v6.3 software. The annual (PPT) and growing season precipitation (GSP) for the ambient treatment during the experiment are based on data obtained from weather stations nearby the study sites. Values in parentheses are standard deviations. Sites are ordered from arid to mesic according to MAP (Batbaatar et al., 2021). Total carbon (TC), total nitrogen (TN), pH are site averages obtained by soil samples in 2016.

Site	Coordinates	MAT (°C)	MAP (mm)	GDD	TC	TN	Soil pH	Soil Texture	% Bare Ground Cover	% BSC Cover	ANPP	Dominant Vascular Vegetation	Dominant biocrust vegetation
Mattheis	50.9, -111.88	3.6 (±1.04)	312 (±64)	1690	1.61	0.13	6.52	Sandy loam	3	43	4.76	*Bouteloa gracilis, Hesterostipa comata, Koeleria macrantha, Artemesia frigida*	*Selaginella densa, Cladonia magycarica, Cladonia pocillum, Xanthoparmela. wyomingica, Physconia muscigena*
Onefour	49.05, -110.44	4.9 (±1)	318 (±73)	1690	2.13	0.18	6.43	Clay loam	10	47	5.25	*Hesterostipa comata, Carex, Koeleria macrantha, Artemesia frigida*	*Selaginella densa, Xanthoparmelia wyomingica*, *Xanthoparmelia camtschadalis*, *Cladonia robbinsii, Cladonia symphycarpa*
Oyen	51.58, -110.5	3.27 (±1.1)	321 (±67)	1490	3.84	0.31	5.84	Loam	1	0.6	17.46	*Pascopyron smithii, Pascopyron dasystachyum, Stipa comata, Pulsatilla patens*	*Cladonia rei*, *Polytrichum juniperinum, Syntrichia ruralis*, Amblystegiaceae moss, Brachytheciaceae moss
Twin River	49.03, -112.35	4.7 (±0.9)	358 (±87)	1578	3.15	0.28	6.61	Clay loam	1	13	9.78	*Pascopyon dasystachyum, Hesterostipa comata, Festuca, Poa pratensis, Achillea millefolium*	*Cladonia*, *Selaginella densa,*
Kinsella	53.03, -111.56	1.9 (±1.1)	401 (±68)	1412	7.23	0.59	5.79	Clay loam	0	5	27.08	*Festuca hallii, Pascopyron dasystachum, Poa pratensis, Stipa comata, Artemesia frigida*	*Ceratodon purpureus, Syntrichia ruralis*, Amblystegiaceae moss, Brachytheciaceae moss, *Cladonia rei,*
Sangudo	53.83, -114.8	2.2 (±0.9)	492 (±74)	1301	5.96	0.45	6.92	Loam	2	0.5	27.93	*Pascopyon smithii, Pascopyron dasystachyum, Poa pratensis, Taraxacum officinale, Trifolium repens*	*Barbula concoluta, Barbula unguiculata, Bryum argenteum, Ceratodon purpureus*
Stavely	50.19, -113.9	3.4 (±0.9)	533 (±110)	1388	9.60	0.80	6.36	Silty clay loam	0	Trace	67.15	*Poa pratensis, Festuca campestris, Danthonia parryi, Gallium boreale, Achillea millefolium, Lathyrus ochroleucus*	None

Table 3.2. Grazing simulation treatments. Treatments were applied in either Spring or Fall or both by trimming vegetation with shears to the height reported.

Treatment Code	Simulated Grazing System	Spring (June) Defoliation Treatment	Fall (September) Defoliation Treatment
HH	Continuous	to 3 cm height	to 3 cm height
HN	Early Spring	to 3 cm height	no treatment
NH	Fall Deferred	no treatment	to 3 cm height
NN	Control	no treatment	no treatment

Table 3.3. Analysis of Variance results for biocrust cover, lichen cover, moss cover, *S. densa* cover, and biocrust species richness across five study sites. Main and interactive effects of drought and grazing on species cover and richness were tested. Biocrust cover includes lichen, moss, and *S. densa* cover. Statistically significant values ($p<0.05$) are denoted in bold.

Analysis of Variance Tables - Univariate Cover & Richness		**Biocrust Cover**			**Lichen Cover**			**Moss Cover**			***S.densa* Cover**			**Biocrust Species Richness**		
Site	**Test**	df	Chisq	P	df	Chisq	P	df	Chisq	P	df	Chisq	P	df	Chisq	P
Onefour	drought	1	6.214	**0.013**	1	0.010	0.920	1	4.985	**0.026**	1	6.743	**0.009**	1	0.003	0.958
	defoliation	3	1.832	0.608	3	4.909	0.179	3	1.218	0.749	3	1.725	0.631	3	2.668	0.446
	drought:defoliation	3	2.281	0.516	3	6.857	0.077	3	3.517	0.319	3	3.424	0.331	3	7.207	0.066
Mattheis	drought	1	0.455	0.500	1	1.962	0.161				1	2.300	0.129	1	0.234	0.629
	defoliation	3	9.516	**0.023**	3	9.833	**0.020**				3	3.187	0.364	3	2.077	0.557
	drought:defoliation	3	20.863	**1.12E-04**	3	1.433	0.698				3	21.467	**8.418E-05**	3	0.870	0.833
Twin River	drought	1	1.617	0.204							1	1.599	0.206	1	0.345	0.557
	defoliation	3	0.947	0.814							3	1.404	0.705	3	6.361	0.095
	drought:defoliation	3	0.617	0.893							3	5.498	0.139	3	1.670	0.644
Kinsella	drought	1	0.047	0.828	1	0.001	0.974	1	0.086	0.770				1	1.382	0.240
	defoliation	3	5.336	0.149	3	3.148	0.369	3	3.502	0.320				3	7.538	0.057
	drought:defoliation	3	2.868	0.412	3	6.051	0.109	3	3.555	0.314				3	7.401	0.060
Sangudo	drought	1	0.271	0.603				1	0.582	0.446				1	0.000	1.000
	defoliation	3	17.196	**0.001**				3	15.162	**0.002**				3	6.408	0.093
	drought:defoliation	3	1.025	0.795				3	2.219	0.528				3	2.218	0.528

Table 3.4. Community analysis results for five study sites. Differences in community composition between treatments were determined using PerMANOVA on Bray-Curtis dissimilarity indices of community matrices with 10,000 permutations. Dummy species with a cover of 1 were utilized to improve erratic behavior of Bray-Curtis coefficients in sites with empty or depauperate plots, following Clarke et al. (2006). Differences in homogeneity of group dispersion (beta-diversity) was evaluated between treatments using Betadisper, using spatial medians as group centroids. Negative eigenvalues were corrected using the Lingoes method (Legendre and Anderson, 1999). Finally, differences in Bray-Curtis distance movement between 2016 and 2019 between groups were evaluated using ANOVA. Communities did not have to be significantly different between treatment groups (perMANOVA) to have significantly different shifts in magnitude from 2016 to 2019.

Community Analyses		**PerMANOVA**			**Betadisper**			**Bray-Curtis Distance Moved 2016 to 2019**		
Site	**Test**	**Df**	**F.Model**	**Pr(>F)**	**Df**	**F**	**Pr(>F)**	**Df**	**Chisq**	**Pr (>Chisq)**
Onefour	DROUGHT	1	1.074	0.311	1	0.832	0.367	1	1.210	0.271
	DEFOLIATION	3	0.789	0.541	3	1.165	0.331	3	1.292	0.731
	YEAR	1	2.486	0.096	1	0.036	0.847			
	DROUGHT:DEFOLIATION	3	0.305	0.931	7	0.948	0.477	3	4.755	0.191
	YEAR:DROUGHT	1	3.430	**0.050**	3	1.001	0.403			
	YEAR:DEFOLIATION	3	0.484	0.795	7	0.311	0.960			
	YEAR:DROUGHT:DEFOLATION	3	0.600	0.697	15	0.578	0.891			
Mattheis	DROUGHT	1	5.167	**0.003**	1	14.900	**0.000**	1	0.238	0.626
	DEFOLIATION	3	2.481	**0.010**	3	1.700	0.175	3	3.469	0.325
	YEAR	1	5.357	**0.002**	1	10.285	**0.002**			
	DROUGHT:DEFOLIATION	3	1.970	**0.039**	7	2.360	**0.032**	3	8.533	**0.036**
	YEAR:DROUGHT	1	0.385	0.841	3	7.453	**0.000**			
	YEAR:DEFOLIATION	3	0.455	0.944	7	2.911	**0.009**			
	YEAR:DROUGHT:DEFOLATION	3	1.279	0.223	15	1.268	0.243			
Twin River	DROUGHT	1	1.023	0.368	1	2.974	0.090	1	0.195	0.659
	DEFOLIATION	3	2.247	**0.010**	3	2.298	0.085	3	6.209	0.102
	YEAR	1	2.050	0.079	1	1.509	0.223			
	DROUGHT:DEFOLIATION	3	1.502	0.115	7	3.205	**0.007**	3	8.741	**0.033**
	YEAR:DROUGHT	1	0.143	0.990	3	1.177	0.332			
	YEAR:DEFOLIATION	3	0.595	0.874	7	1.167	0.337			
	YEAR:DROUGHT:DEFOLATION	3	0.402	0.977	15	1.220	0.278			
Kinsella	DROUGHT	1	1.531	0.155	1	0.835	0.359	1	10.458	**0.001**
	DEFOLIATION	3	2.248	**0.009**	3	2.915	**0.041**	3	2.882	0.410

Community Analyses		PerMANOVA			Betadisper			Bray-Curtis Distance Moved 2016 to 2019		
Site	**Test**	**Df**	**F.Model**	**Pr(>F)**	**Df**	**F**	**Pr(>F)**	**Df**	**Chisq**	**Pr (>Chisq)**
	YEAR	1	2.387	**0.040**	1	3.528	0.065			
	DROUGHT:DEFOLIATION	3	1.456	0.121	7	0.854	0.552	3	6.711	0.082
	YEAR:DROUGHT	1	0.492	0.820	3	1.394	0.257			
	YEAR:DEFOLIATION	3	0.594	0.891	7	1.718	0.121			
	YEAR:DROUGHT:DEFOLATION	3	0.450	0.975	15	0.744	0.729			
Sangudo	DROUGHT	1	0.851	0.431	1	0.004	0.947	1	1.001	0.317
	DEFOLIATION	3	2.890	**0.009**	3	0.675	0.568	3	14.615	**0.002**
	YEAR	1	6.891	**0.002**	1	0.690	0.405			
	DROUGHT:DEFOLIATION	3	3.391	**0.003**	7	1.146	0.347	3	0.309	0.958
	YEAR:DROUGHT	1	1.355	0.239	3	1.066	0.374			
	YEAR:DEFOLIATION	3	1.312	0.229	7	0.600	0.754			
	YEAR:DROUGHT:DEFOLATION	3	1.632	0.126	15	0.416	0.972			

Table 3.5. Results of testing for significant relationships between BSC constituents and environmental factors using ANOVA. Statistically significant relationships ($p<0.05$) are denoted in bold.

Site		Light			Litter			ANPP			*S. densa* cover			SMC			Soil Temperature		
		df	F	p	df	F	p	df	F	p	df	F	p	df	F	p	df	F	p
Onefour	Lichen cover	30	0.037	0.849	30	1.110	0.324	30	0.495	0.487	30	0.176	0.678	22	0.041	0.841	22	0.974	0.334
	S. densa cover	30	0.383	0.540	30	1.007	0.324	30	0.824	0.371				22	0.147	0.705	22	0.153	0.700
Mattheis	Lichen cover	38	6.978	**0.012**	38	1.015	0.320	38	0.333	0.568	38	0.846	0.364	37	2.276	0.140	37	2.354	0.133
	S. densa cover	38	1.640	0.208	38	2.554	0.118	38	0.241	0.627				37	5.165	**0.029**	37	1.098	0.301
Twin River	*S. densa* cover	29	0.176	0.446	29	3.682	0.065	29	0.002	0.961				26	6.122	**0.020**	26	0.584	0.452
Kinsella	Lichen cover	38	11.660	**0.002**	38	-4.388 (t-val)	**0.0001**	38	3.205	0.081				37	1.075	0.307	37	1.564	0.219
	Moss cover	38	0.073	0.789	38	0.329	0.570	38	0.056	0.814				37	0.514	0.478	37	1.146	0.291
Sangudo	Moss cover	29	2.400	0.132	29	15.910	**0.0004**	29	3.173	0.085				28	0.687	0.414	28	0.198	0.660

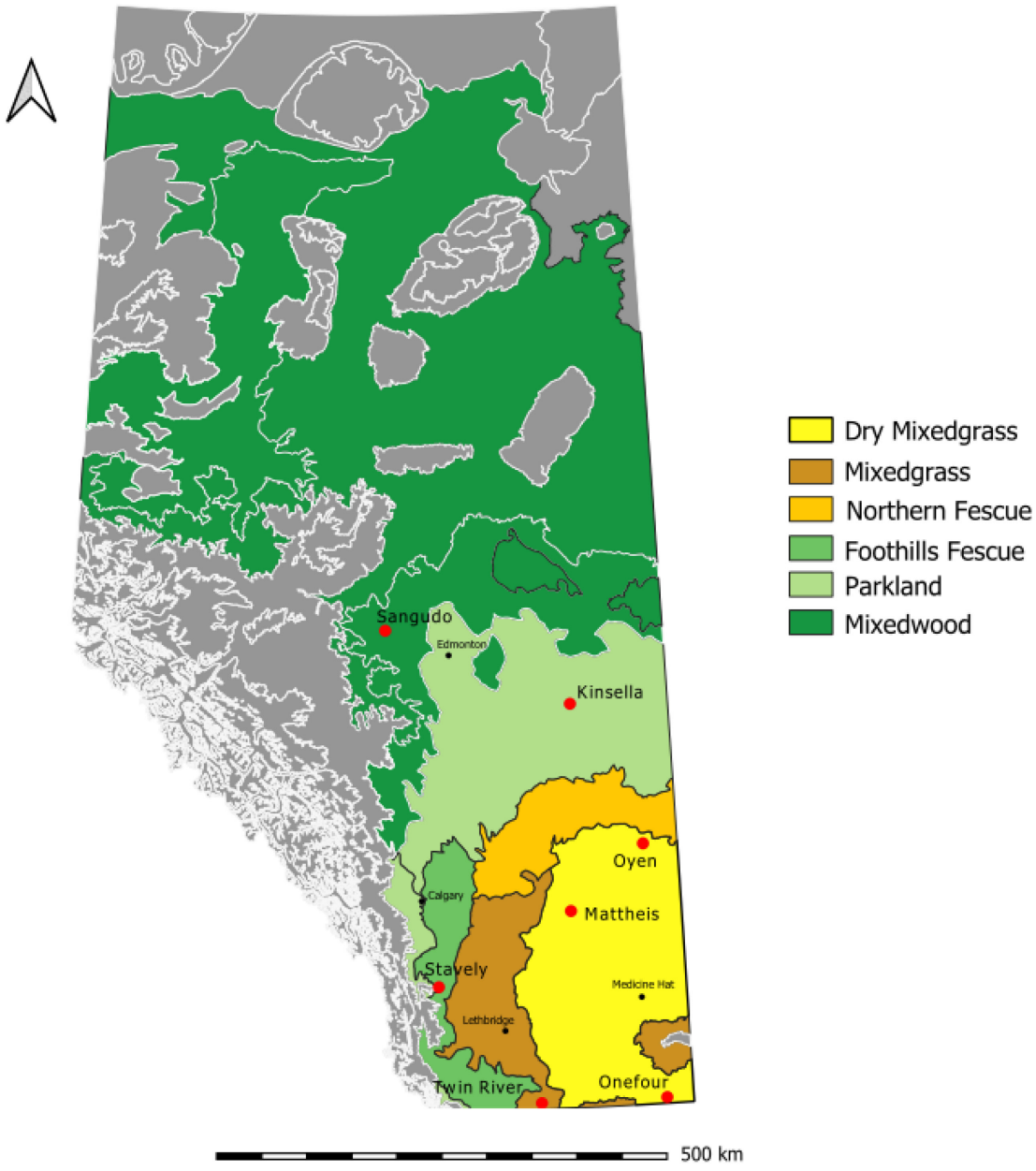

Figure 3.1. Map of study sites and location within Natural Subregions of Alberta, Canada.

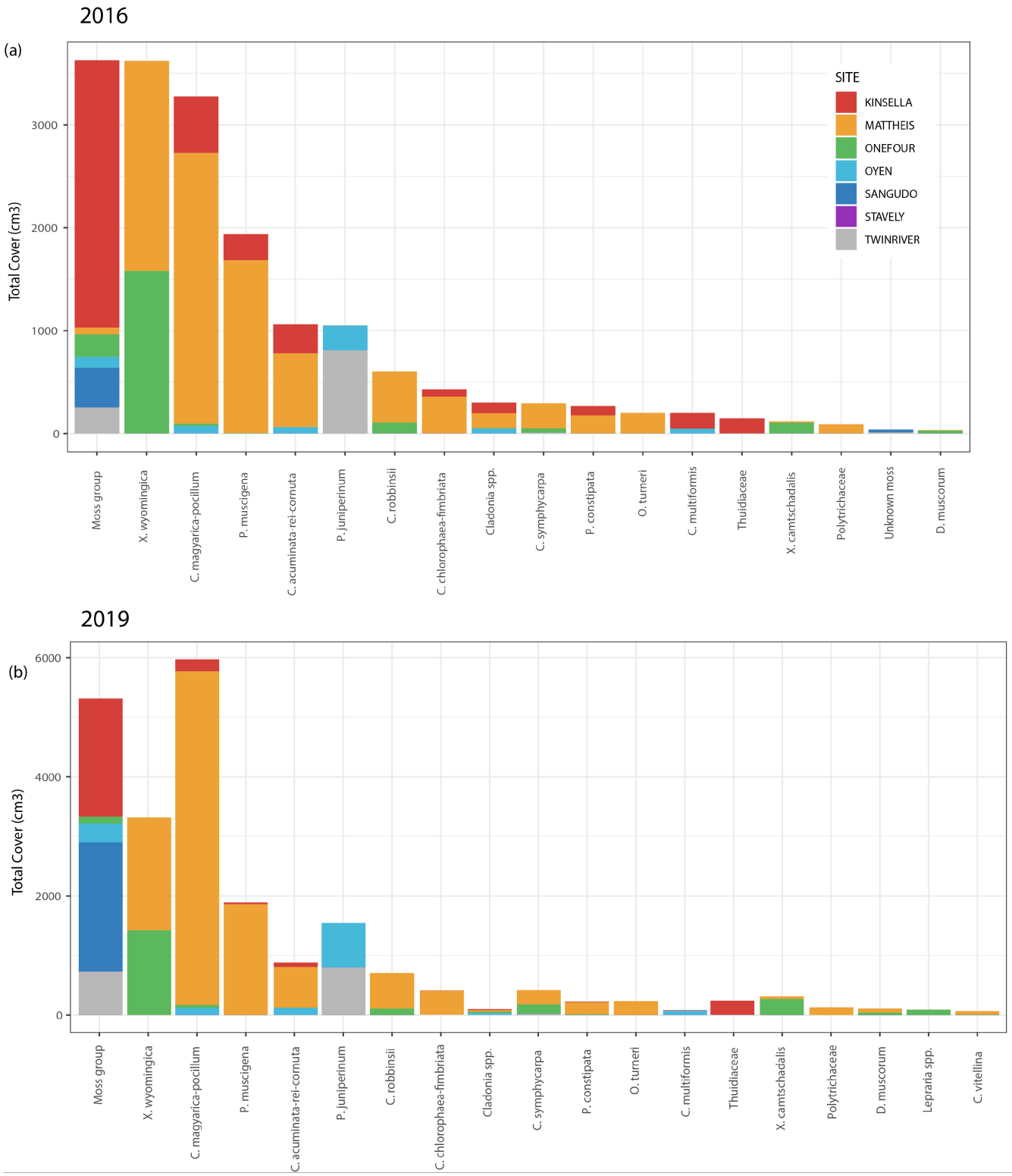

Figure 3.2. Absolute cover (cm^2) of biocrust constituent species by site in (a) 2016 and (b) 2019. Bryophyte species not reliably differentiated at the field-level were lumped into "Moss group", and included mosses in the Amblystegiaceae, Brachytheciaceae, Bryaceae, Hypnaceae, Pottiaceae, *Bryum* sp., *Ceratodon purpureus*, and *Syntrichia ruralis*.

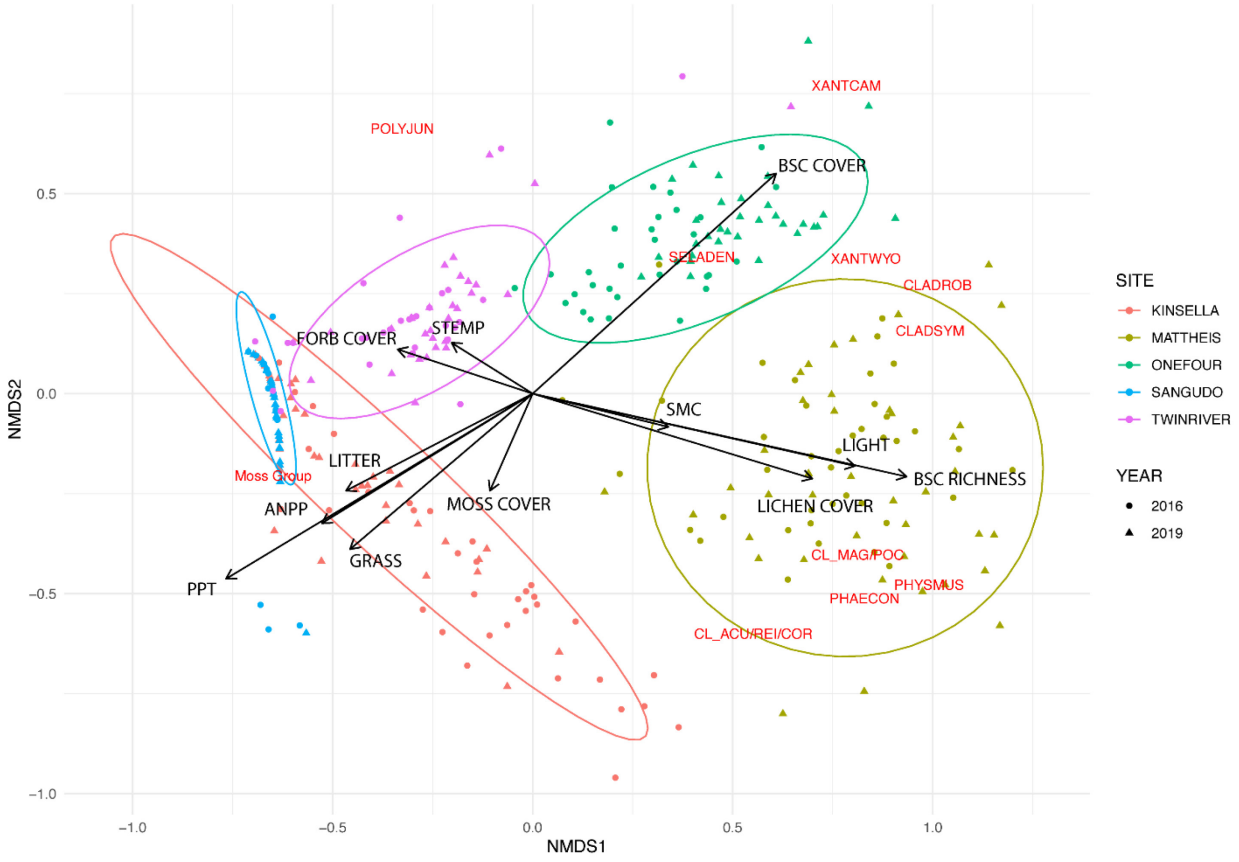

Figure 3.3. Global NMDS of all sites. Analysis was completed at the site level because of the large ecological disparity between sites. Stress= 0.1165446, best solution reached after 1000 tries. Data standardized with Wisconsin double standardization and were square-root transformed. Configuration rotated to maximize variance of points on first dimension, origin centered to the average of the axes, and halfchange scaling. Environmental vectors with $p<0.05$ were plotted. BSC cover=total BSC cover, SMC=soil moisture content, GRASS=cover of grasses, PPT=precipitation, ANPP=annual net primary productivity, STEMP=soil temperature). Bryophyte species not reliably differentiated at the field-level were lumped into "Moss group", and included mosses in the Amblystegiaceae, Brachytheciaceae, Bryaceae, Hypnaceae, and Pottiaceae families; *Bryum* sp., *Ceratodon purpureus*, and *Syntrichia ruralis*.

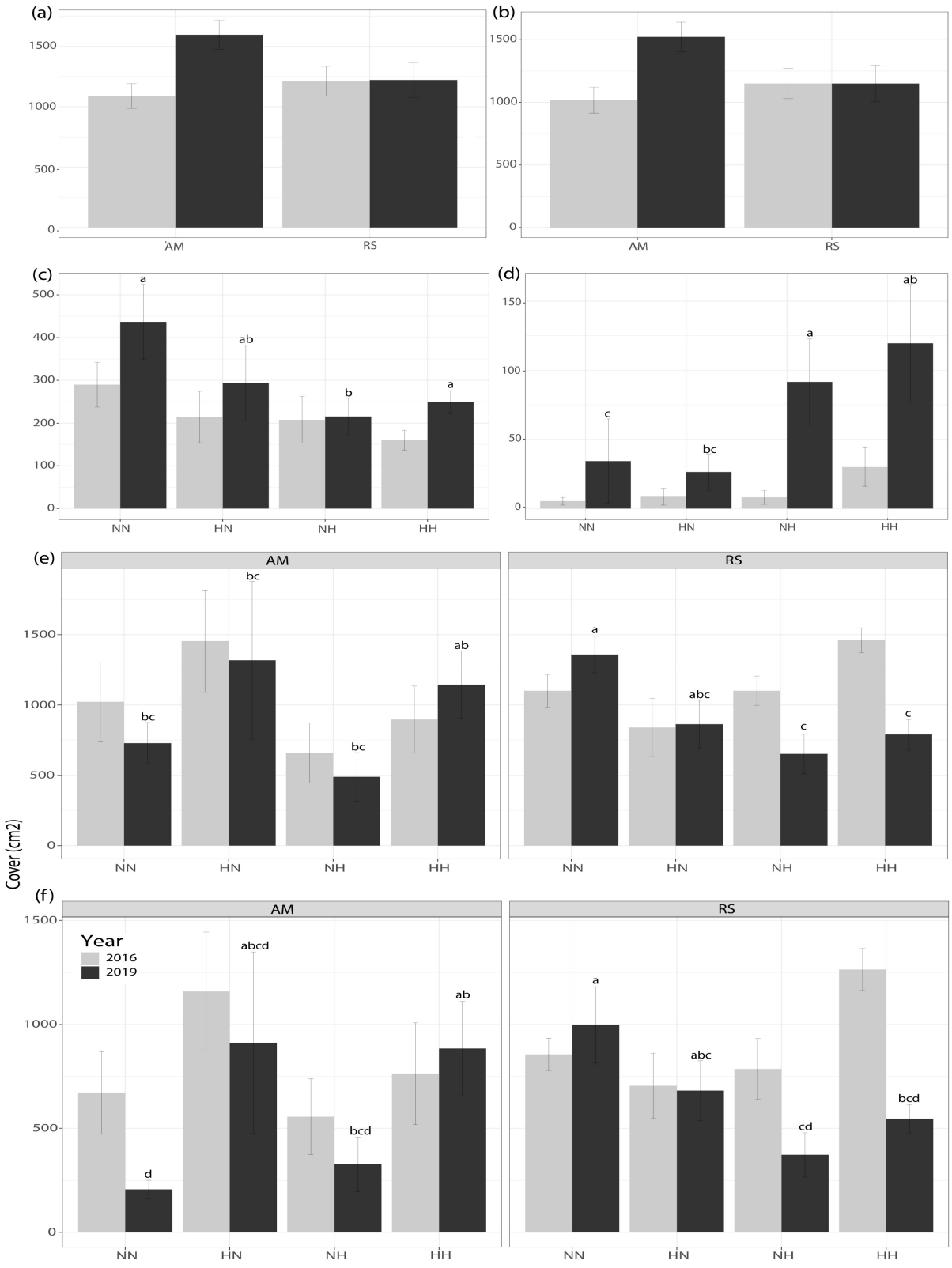

Figure 3.4. Effects of ambient (AM) and drought (RS) treatments on (a) total biocrust cover (cm^2) and (b) *S. densa* cover (cm^2) at Onefour; effects of undefoliated (NN), spring (HN), fall (NH) and spring + fall (HH) defoliation on (c) total lichen cover (cm^2) at Mattheis and (d) total moss cover (cm^2) at Sangudo; and interactive effects of treatment on (e) total biocrust cover (cm^2) and (f) *S. densa* cover (cm^2) at Mattheis.

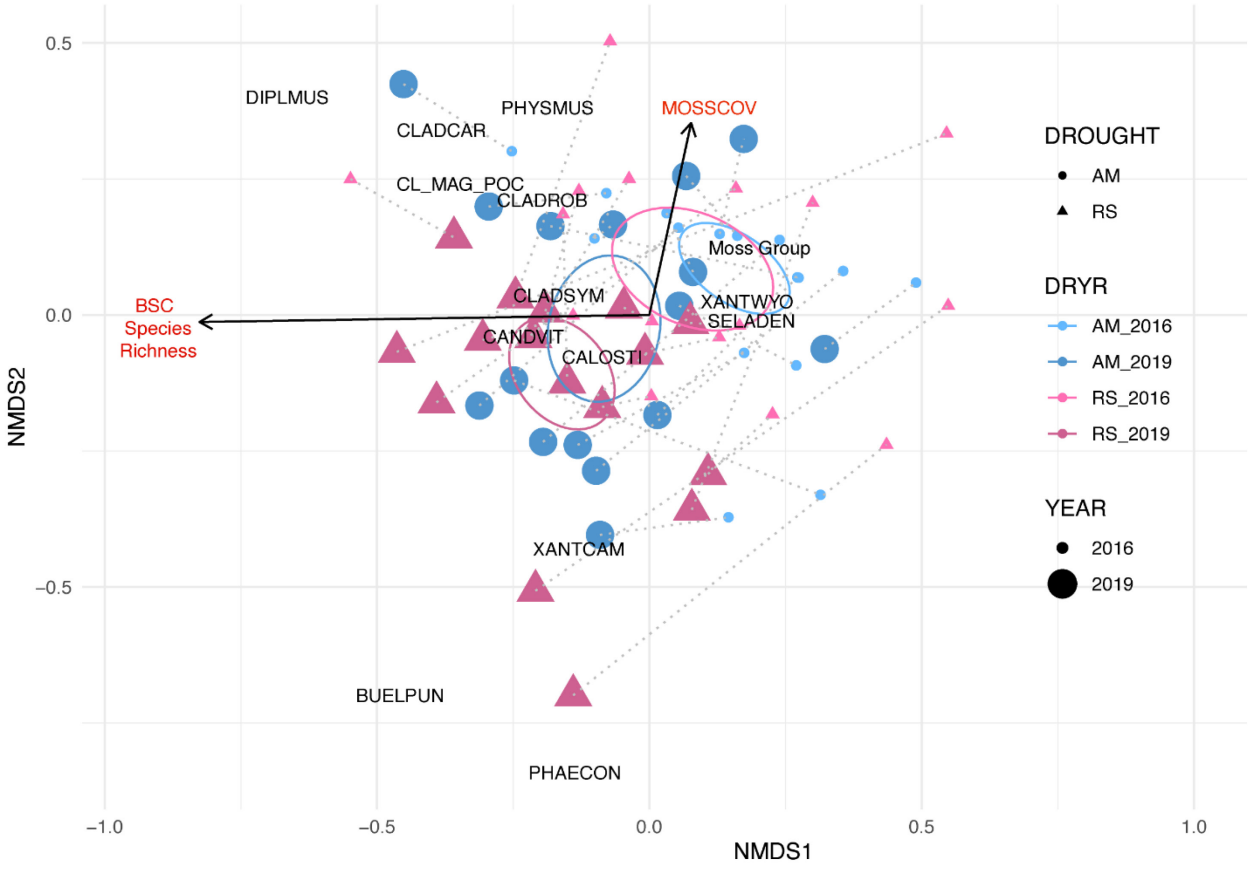

Figure 3.5. NMDS Onefour drought-year interaction. Stress = 0.2312236, 2 dimensions, with two convergent solutions after 1000 tries. Expanded species scores based on square-root Wisconsin transformation. Halfchange scaling used with PC rotation and centring. Ellipses represent the standard error of group centroids to 95% confidence. Significantly different community composition differences were detected between 2019 ambient (AM2019) and 2019 drought (RS2019) treatment plots, and between 2016 drought (RS2016) and 2019 ambient (AM2019) plots in perMANOVA test post-hocs. Shifts appear to be associated with *C. symphycarpa*, *X. wyomingica*, *S. densa*, *C. vitellina*, *C. stillicidiorum*, and the Moss Group. The Moss Group consisted of bryophyte species not reliably differentiated at the field-level, and included mosses in the Amblystegiaceae, Brachytheciaceae, Bryaceae, Hypnaceae, and Pottiaceae families; *Bryum* sp., *Ceratodon purpureus*, and *Syntrichia ruralis*.

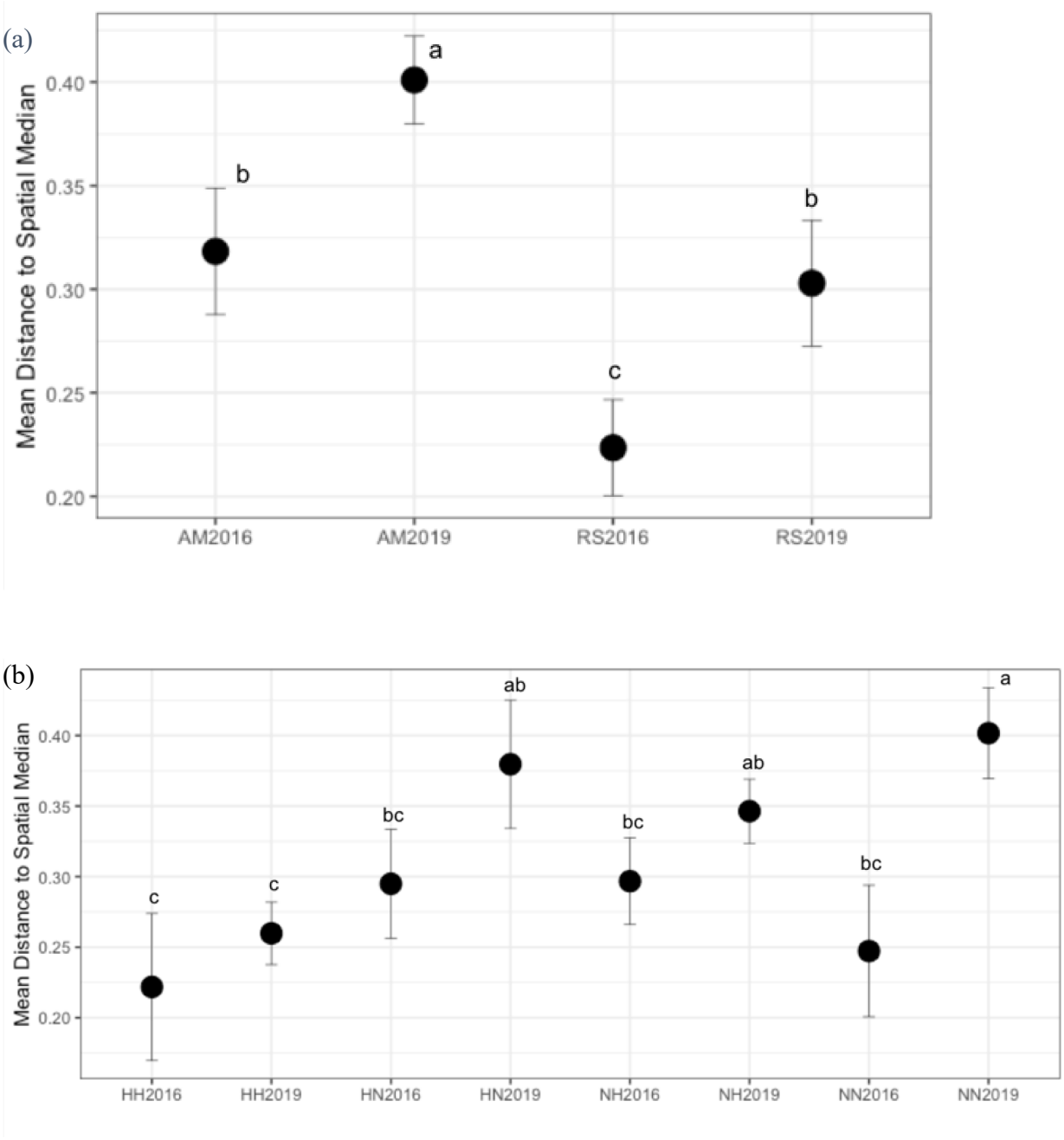

Figure 3.6. Beta-diversity, as measured by mean non-euclidean distances between principal coordinates of plots and group spatial medians at Mattheis by (a) by year in ambient (AM) and drought (RS) treatment, and (b) by year and undefoliated (NN), spring (HN), fall (NH), and spring + fall (HH) defoliation treatment. Principal coordinates were obtained from Bray-Curtis distances of the species matrix.

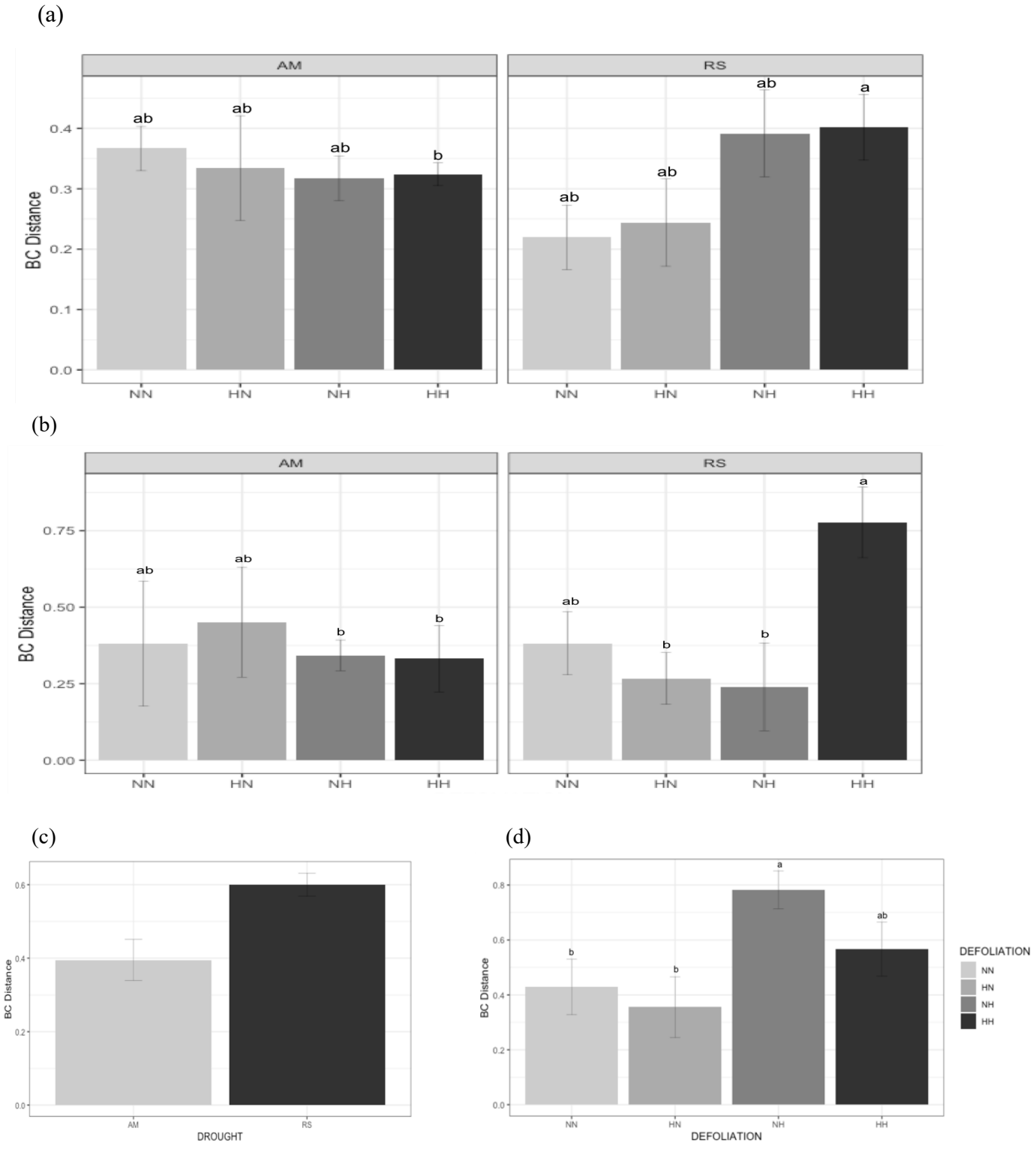

Figure 3.7. Magnitude of biocrust community shifts from 2016 to 2019 measured with Bray-Curtis distance. (a) Interactive effect of ambient (AM) and drought (RS) treatments with undefoliated (NN), spring (HN), fall (NH), and spring + fall (HH) defoliation treatments at Mattheis, (b) interactive effects of drought and defoliation treatments at Twin River, (c) precipitation reduction treatments at Kinsella, and (d) defoliation treatments at Sangudo.

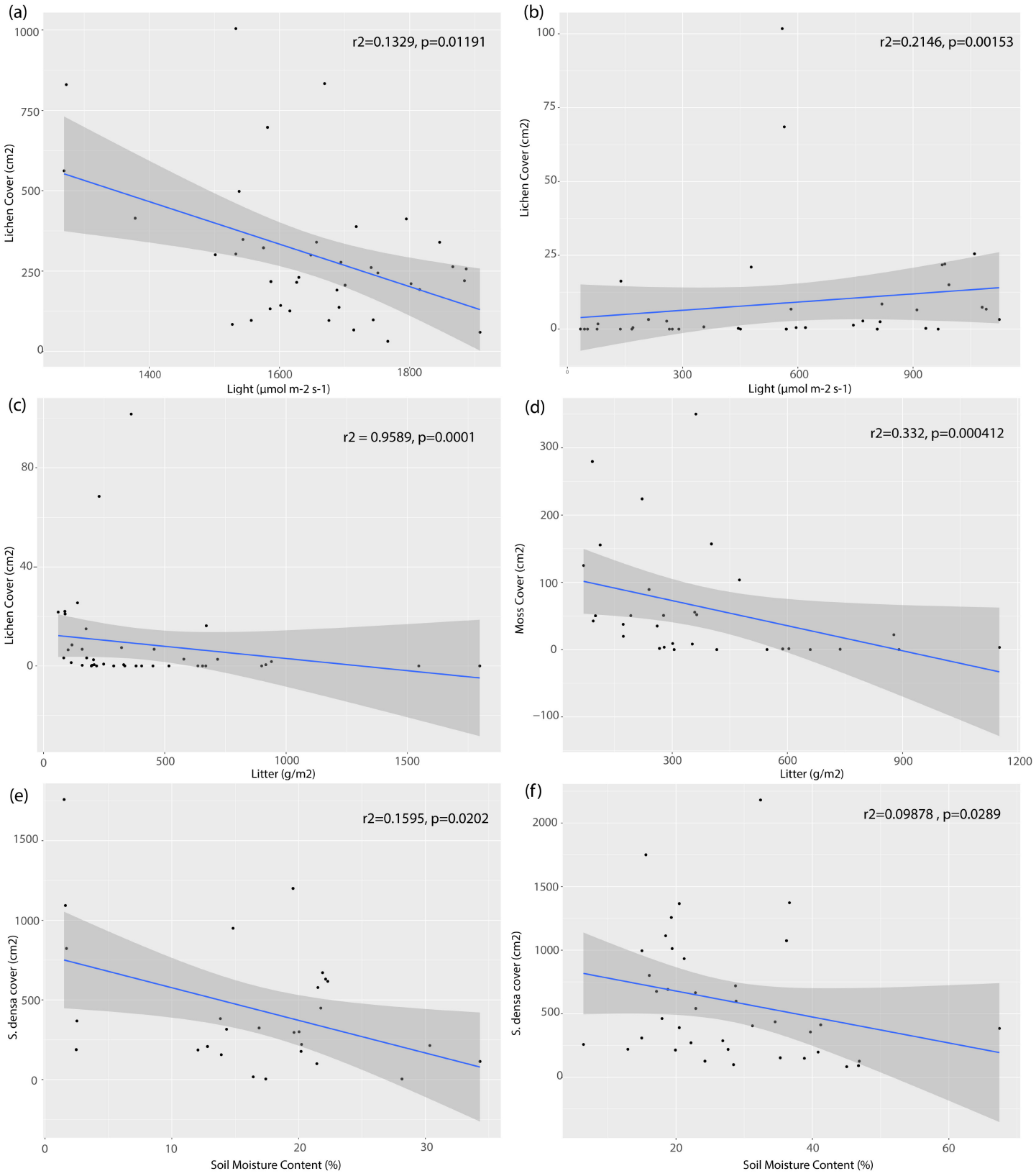

Figure 3.8. The relationship between abiotic factors and cover of biocrust constituent cover in all plots: (a) light at ground surface (µmol m^{-2} s^{-1}) and lichen cover (cm^2) at Mattheis, (b) light at ground surface (µmol m^{-2} s^{-1}) and lichen cover (cm^2) at Kinsella, (c) litter quantity (g/m^2) and lichen cover (cm^2) at Kinsella, (d) litter quantity (g/m^2) and moss cover (cm^2) at Sangudo, (e) soil moisture content (%) and *S. densa* cover (cm^2) at Twin River, and (f) soil moisture (%) and *S. densa* cover (cm^2) at Mattheis.

Chapter 4.

Synthesis

4.1 Introduction

Biocrusts, or biological soil crusts (BSCs), are important but often overlooked contributors to biodiversity and ecosystem function in the Canadian Prairies. Despite their importance, they are relatively understudied in parts of the world where they exist, and even less so in the northern Great Plains (Looman, 1964a, 1964b; Warren et al., 2021). Sadly, many rangeland managers appear to suffer from "biocrust blindness", often failing to note the presence of BSC on the landscape (Condon & Pyke, 2018) despite BSC's critical roles in soil stabilization, the hydrologic cycle, seed establishment and germination, and carbon and nitrogen cycling (Belnap & Büdel, 2016; Chamizo et al., 2016; Li et al., 2005; Porada et al., 2014). Land use intensification and climate change threaten to alter BSC communities and ecosystem function in grasslands (Ferrenberg et al., 2015). Furthermore, taxonomic ambiguity exists in many biocrust species groups, hindering our ability to detect and study changes in biodiversity. The goals of this thesis were to address taxonomic uncertainty in a particular group of lichen present in Alberta's grassland biocrust, the *Cladonia cariosa* group, and then study responses to biocrust community responses to drought and defoliation across an environmental gradient.

4.2 Hidden diversity in the *C. cariosa* group

This study identified two putative new species in the *C. cariosa* group, for now termed Clade E and Clade F. Both putative species are morphologically similar to *Cladonia symphycarpa*. Clade E had significantly longer and wider squamules than *C. symphycarpa, C. acuminata,* and *C.*

cariosa, longer squamule incisions than *C. cariosa* and *C. acuminata*, thicker squamule cortex layers than *C. cariosa* and *C. acuminata*, thicker squamules and shorter podetia than *C. acuminata*, and thinner hymenium layers in cross-section than *C. cariosa* and Clade F. Clade F specimens had significantly longer, wider squamules with longer incisions and thicker cortex than *C. cariosa* and C. Clade F had significantly thicker hymenium layers than *C. symphycarpa* and Clade E. Although Clade F was largely indistinguishable morphologically and chemically from Clade E and *C. symphycarpa*, its distribution was unique in that it was not as widespread as the other species, and found almost exclusively in the Mediterranean Region. Despite statistically significant differences in morphometrics, the range of morphological measurements of traits often overlapped between species. As such, we consider these species to be near-cryptic additions to the *C. cariosa* group.

Cryptic species, although not always readily or easily identified in the field, are still important contributors to biodiversity. Cryptic species have been identified in many groups of lichen forming fungi (Kraichak et al., 2015; Leavitt et al., 2016; Pardo-De la Hoz et al., 2018), especially since the advent and advancement of molecular sequencing technology. Cryptic species contribute to biodiversity, which provides an insurance effect on the resilience of ecosystem function (Yachi & Loreau, 1999). Higher biodiversity within a community increases the likelihood that ecosystem services can be maintained in the face of environmental perturbations through redundancy in contribution to ecosystem processes and maintenance of interaction networks between species (Oliver et al., 2015). Cryptic species in the *C. cariosa* group contribute to biodiversity in BSCs in the northern Great Plains. Maintenance of

biodiversity across our grassland landscapes is becoming increasingly important with the ongoing threat of climate change.

4.3 Implications of Phylogenetic Study for Alberta Grasslands

This research has greatly added to our understanding of the *C. cariosa* group in Alberta's grasslands. In our study, *C. cariosa*, *C. symphycarpa*, Clade E, and *C. acuminata* were represented in Alberta grasslands. The addition of cryptic Clade E increases our previous estimations of biodiversity in Alberta BSC. Clade E was likely previously misidentified as *C. symphycarpa* due to morphologic similarity between the two species and a shared range. Despite *C. dahliana* (Kristinsson, 1974) being no longer recognized as a distinct species (Ahti & Hammer, 2002; Burgaz et al., 2020; Pino-Bodas et al., 2012; Pino-Bodas & Stenroos, 2021), we found many psoromic acid-containing herbaria specimens still labelled as *C. dahliana*. These specimens grouped out with *C. symphycarpa*, Clade E, and Clade F, but did not appear in *C. cariosa* specimens in our study, indicating that its presence is still a useful discriminator between *C. cariosa* and the *C. symphycarpa* - Clade E - Clade F complex.

In addition to the *C. dahliana* herbaria specimens, widely-used foundational reference texts for lichen identification in western Canada (Goward, 1999) that were written prior to phylogenetic work within the group refer to *C. dahliana*'s periodic treatment as a separate species. The persistence of the *C. dahliana* name, despite multiple studies refuting its existence, signals a gap between published research and field reference materials. Furthermore, there is currently no reference text explicitly for Alberta lichen. Lack of field reference material increases lichen taxonomy's barrier to entry. Many experienced private sector biophysical assessors conducting baseline detailed vegetation inventories (DVI) and rare plant surveys for industrial projects in

Alberta rely on mailing field collections to out-of-province specialists for lichen identification. The field collections consist of periodic grab-samples, and are often not representative of the diversity of lichen present. This is hugely problematic, as DVIs and rare plant surveys in Alberta are often initiated as part of regulatory requirements to mitigate impacts of industrial projects, like oilsands operations, by avoiding rare species and creating vegetation inventories for reclamation planning. But because lichen identification is often considered specialized knowledge, their degree of inclusion in botanical surveys is often subject to budget. We believe the creation of Alberta-specific field identification materials is a critical need in Alberta, and that such materials will improve vegetation inventory datasets.

An interesting area of future work might involve a more intensive study on morphological and chemical characters of the *C. cariosa* group in Alberta's grasslands, especially in regard to discriminating Clade E from *C. symphycarpa*. In our relatively small sample size, we found Alberta grasslands Clade E specimens (n=2) contained atranorin alone or atranorin and rangiformic acid and *C. symphycarpa* grasslands specimens (n=4) that contained atranorin alone, atranorin and psoromic, and atranorin and an unknown terpene. Norstictic acid appeared to be more common in Clade E and *C. symphycarpa* in mountainous or northern regions in our dataset. In Alberta grasslands, Clade E tended to have shorter podetia with smaller apothecia than *C. symphycarpa*. There is certainly a possibility of discriminatory morphology and chemistry between the two when just looking at Alberta grasslands samples, but it's difficult to make strong conclusions with the sample size of our current dataset.

One question that arose during this study was whether morphological and chemical characters were indicative of taxonomy or a result of environmental conditions. As a consequence of using mainly herbaria specimens collected by others as opposed to our own fresh collections, many of

our specimens did not have consistently adequate habitat description to assess this question at depth. We had observed, for example, herbaria specimens with extremely small squamules (<2 mm) collected with underlying sandy substrates across taxonomic groupings, but we weren't confident in using this observation in the study having not seen the collection site. Additionally, if herbaria specimens did have habitat data, it was often described at the scale experienced by the specimen collector versus the microtopographic scale experienced by the specimen. For the *C. cariosa* group, it is not uncommon to find squamules in the same squamulose mat with differing chemistries, despite *Cladonia* in general not producing many sexual spores (Ahti, 2000). If squamules within one squamulose mat are clonal, it is possible that differences in chemistry might be differences in gene expression driven by environmental factors, in particular the experiences of lichens at the micro scale, especially as lichen metabolites have been shown to perform important and different biological roles in lichen (Lawrey, 1986; Molnár and Farkas, 2010). In future studies, we would endeavor to include a description of the microhabitat of the lichen, describing vegetation directly adjacent, soil grain size and chemistry, a description of shade cast by overlying vascular vegetation, and perhaps a measurement of humidity at ground level.

Our study also suggests that future phylogenetic work within the *C. cariosa* group should probably use next-generation sequencing moving forward, as multi-gene phylogenies have not been able to discern all species within the group. Schoch et al. (2012) proposed ITS rDNA as the standard barcode marker for fungi; however, some studies have shown that ITS rDNA provides poor resolution for species delimitation in *Cladonia* (Kotelko & Piercey-Normore, 2010; Pino-Bodas et al., 2011), lacks a barcode gap, and has a high failure percentage when searching in BLAST (Kelly et al., 2011). Other loci identified as potential candidates to use in combination

with ITS rDNA in *Cladonia* included *cox1* and *rpb2*, although only *cox1* showed a barcoding gap (Pino-Bodas et al., 2013). The multi-gene phylogeny completed by Pino-Bodas et al. (2012) using ITS rDNA, *rpb2,* and *ef1a* had better resolution than the ITS rDNA and *rpb2* constructed here, but seemed unable to discriminate at least one of the two new species identified in the GBS dataset. However, it is also important to recognize the limitations of using genome-scale data from approaches like GBS and RAD-seq, one of the major being that multi-species coalescent approaches to species delimitation can delimit population structure instead of species-level divergence, and thus overestimate the number of species present (Leaché et al., 2019; Sukumaran & Knowles, 2017). Genomic based results should, as best practice, be validated with other data types, such as morphologic and ecologic information.

4.4 BSC Characterization

BSC in our study sites in the grasslands of Alberta, Canada consisted mainly of chlorolichens growing on soil, interspersed by *Selaginella densa* mats, often with BSC organisms growing on failing or expired *S. densa. S. densa* is sometimes excluded from traditional definitions of BSC, but it is acknowledged that boundaries between club moss mats and soil crusts can be fluid (Belnap, Prasse, et al., 2001), which seems to be the case in our study area. Mosses were relatively rare and scattered at dry sites, but became more prolific at sites with higher annual precipitation. As BSC gave way to vascular vegetative cover, BSC changed from continuous mats to scattered individuals dispersed at ground level under a canopy of vascular vegetation. One site with particularly high litter and ANPP (Stavely) had no discernable cryptogamic cover. Characterization of BSC in Alberta is particularly important because BSC in this region is relatively unknown. The last studies of general BSC composition in the Canadian Prairies were

completed nearly 60 years ago (Looman, 1964a, 1964b). Hopefully this work can bring awareness to the uniqueness and importance of BSC in this region, and encourage future study.

4.5 Effects of drought and defoliation on BSC

A significant takeaway from the drought and defoliation BSC study was that effects are highly dependent on site. Sites were very different ecologically, and had different BSC composition. Across sites, BSC moss and lichen cover appeared to not be significantly impacted by drought, and *S. densa* appeared to have a neutral drought effect (experiencing an increase in cover with ambient plots, but no change in drought plots), despite drier than typical conditions at most sites in most study years (Batbaatar et al., 2021), indicating that BSC in our region may be largely resistant to the effects of drought alone. Biocrust species richness appeared to also not be effected by drought and defoliation treatments. Interestingly, many ecosysystem properties were relatively resistant to several years of imposed drought, including above-ground net primary productivity, litter quantity, root biomass, and species richness, although changes were observed in plant species composition (Batbaatar et al., 2021). The observed resilence of the community to drought conditions may be due to adaptations obtained from a history of drought in southern Alberta (Bonsal et al., 2011). Relative stability in the above-ground plant community likely impacted the stability observed in the underlying biocrust community.

The above-ground plant community generally influences BSC through canopy shade, litter, and root activity (Y. Zhang et al., 2016), with litter deposition as the most significant mechanism (Boeken & Orenstein, 2001). Canopy shade provides intermediate shade that is favorable to mosses but detrimental to lichen, whereas plant litter can provide photoprotection at low levels but inhibits BSC at high levels (Y. Zhang et al., 2016). Lichen cover at Mattheis was highest in

undefoliated plots, and also had a negative relationship with light levels, indicating that vascular vegetation may be providing photo-protection for lichen at sites with intense light levels. Additionally, Mattheis had relatively low ANPP and litter compared to wetter sites, so the vegetation here was likely not limiting the biocrust community. The inverse was true at Kinsella where lichen cover had a positive relationship with light levels and a negative association with litter. Kinsella had lower light levels and more litter than Mattheis, indicating that light was likely limiting at Kinsella. Moss cover at Sangudo was significantly higher in plots subjected to spring+ fall and fall defoliation, and lowest in undefoliated and spring-defoliated plots, but increased with all defoliation treatments. Moss was found to be negatively associated with litter at Sangudo, indicating there was likely a light-limitation. Other studies in the northern Great Plains have found similar results. Sites that had been protected from grazing for several years resulted in increases in the density and size of vascular vegetation and accumulation of litter, and consequently, a decrease in lichen cover, although moss cover appeared to not be negatively impacted (Looman, 1964a). These results point to the evolutionary history of grazing in our region, and that complete grazing exclusion is not the answer to biodiversity conservation in these areas, even when taking biocrust communities into consideration. When considering vascular vegetation, the ecological functions provided by grazing in the Great Plains are well known, and include creating heterogeneous landscapes, altering vegetation composition, soils, nutrient cycles, fire regimes, and promoting grassland species that associate with open, intensively-grazed habitat (Fuhlendorf & Engle, 2001). Loss of grazing disturbance leads to landscape homogenization in the vascular community and, ultimately, loss of biodiversity (Augustine et al., 2021), and our results suggest that this process is likely critical for BSC communities as well. However, future study is needed.

While we modelled defoliation treatments in this study to mimic common grazing practices, our study is limited in that it only addresses the impacts of vegetation removal on BSC communities. Grazers were excluded from our study, so we caution using these study results to make explicit management reccomendations for cattle producers. It is widely recognized that trampling by grazing animals is detrimental to BSC communities in arid regions (Warren & Eldridge, 2001), but that trampling intensity (Rogers & Lange, 1971; Hodgins & Rogers, 1997) and timing of livestock impact (Marble & Harper, 1989) is related to the degree of damage on BSC. The Great Plains of North America has a long evolutionary history of grazing by large herds of herbivores including bison, elk, deer, and pronghorn (Samson et al., 2004b) that is accepted by grassland ecologists as a keystone process in these systems (Knapp et al., 1999; Milchunas et al., 1988). Livestock can continue to provide the ecological functions and contributions to landscape heterogeneity that large herds of wild ungulates once did, albeit at a more constrained spatial scale and pending appropriate management that circumvents degradation to habitat and ecological processes (Allred et al., 2011). Given this evolutionary history, it would make sense that at least some level of livestock impact would be tolerable to BSC. Studying impacts of cattle grazing on BSC would be an interesting area of future study.

We did not see significant differences in biocrust community composition between treatments, although drought alone at Kinsella, spring+fall defoliation or fall defoliation at Sangudo, and drought in combination with spring+fall defoliation at Mattheis and Twin River caused greater shifts than other treatments in communities over time, depending on the site. At Mattheis, beta-diversity, i.e. species diversity within a treatment, was significantly lower in spring+fall defoliation treatments than other treatments, and highest beta-diversity was observed in undefoliated treatments. We believe this is due to extreme defoliation treatments excluding all

but disturbance-tolerant BSC constituents, resulting in more similar communities, in contrast with undefoliated treatments that leave microsites for shade-tolerant BSC constituents while also hosting disturbance-tolerant BSC constituents. In general, when trampling from cattle is taken into account, intense grazing tends to cause changes in biocrust communities by decreasing species diversity in biocrust (Kleiner and Harper, 1972). In contrast, when areas have been excluded from grazing for longer periods of time (7 years) in the Canadian Prairies, species diversity in biocrust also decreased as a result of being shaded out by the overlying vascular community and associated litter (Looman, 1964a). Lack of differences in species composition and richness between treatments may have been due to not applying changes for a long enough time, and the communities themselves being relatively resilient to changes in moisture and light. Longer term studies on changes in BSC composition as a result of drought and defoliation should be conducted to determine impacts of these stressors.

Selaginella densa was a prolific component of biocrust systems at our dry, southern sites: Twin River, Onefour and Mattheis. *S. densa* fills open xeric habitats, has fine fibrous roots in the upper 4 cm of soil, acts as an increaser species and is generally not grazed by cattle, is desiccation tolerant, protects soil from surface erosion, and increases infiltration and decreases runoff of water during storms (Van Dyne & Vogel, 1967). In addition to performing biocrust ecosystem services, *S. densa* also serves as a growing medium for typically-accepted biocrust constituents like lichen. *S. densa* preferred ambient conditions at Onefour, our driest site, but had a negative association with soil moisture at the other two semi-arid sites (Twin River and Mattheis), indicating perhaps an intolerance to wetter conditions, or decreased competitiveness in habitats with higher soil moisture contents. *S. densa* cover was resilient to high levels of defoliation (spring+fall) under ambient conditions, but under drought conditions, had higher cover under

undefoliated treatments and low cover with high defoliation levels, demonstrating that *S. densa* is sensitive to the interactive effects of drought and defoliation, and may not be as resilient as other BSC constituents when subjected to extreme water-limiting conditions imposed by drought and the removal of shade provided by overlying vascular vegetation.

BSC communities are significant in drylands because of the valuable ecosystem functions they provide. However, most studies on BSC function have occurred in arid deserts, and due to differences in species composition between desert BSC and Great Plains BSC, ecosystem services provided by each are likely different. BSC functionality in the Great Plains, while outside the scope of this study, would be an excellent opportunity for future work.

4.6 Conclusion

Landscape management decisions that promote biodiversity and ecosystem resilience are becoming increasingly important with the growing urgency of climate change. This research has helped to untangle some of the taxonomic questions in the BSC of the northern Great Plains, has added to the body of literature characterizing BSC in this region, and has examined the impacts of defoliation and climate-induced drought on BSC. We found that BSC communities likely have hidden diversity, as found in the *C. cariosa* group of lichen, and that BSC was largely adapted to a reduction in precipitation and defoliation, but may experience sensitivity to extremes in light intensity or litter quantity, which is promising in terms of biodiversity and resilience to environmental disruption. The effects of drought and defoliation on BSC communities were largely dependent on the ecosystems in which the BSC was found and the particular composition of the BSC. Successful land management strategies should strive to incorporate ecological

knowledge of the particular ecosystem when making decisions. The presence of cryptic species is indicative of evolutionary processes, such as recent speciation or morphological stasis in long-diverged species (Struck et al., 2018), which may be interesting to explore in future work. Our work explored BSC response across a wide climate gradient, but in future studies it might be interesting to examine more sites in a narrower climate gradient and to examine physiological responses (like photosynthetic rates) to drought and defoliation effects. While this study added to the precious few studies concerning BSC of the northern Great Plains, more taxonomic and ecologic work is needed to characterize underexplored areas, and to heighten awareness of these important communities.

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Appendix A. Chapter 2

Appendix A.1 - Single Gene Alignments of rpb2 and ITS regions.

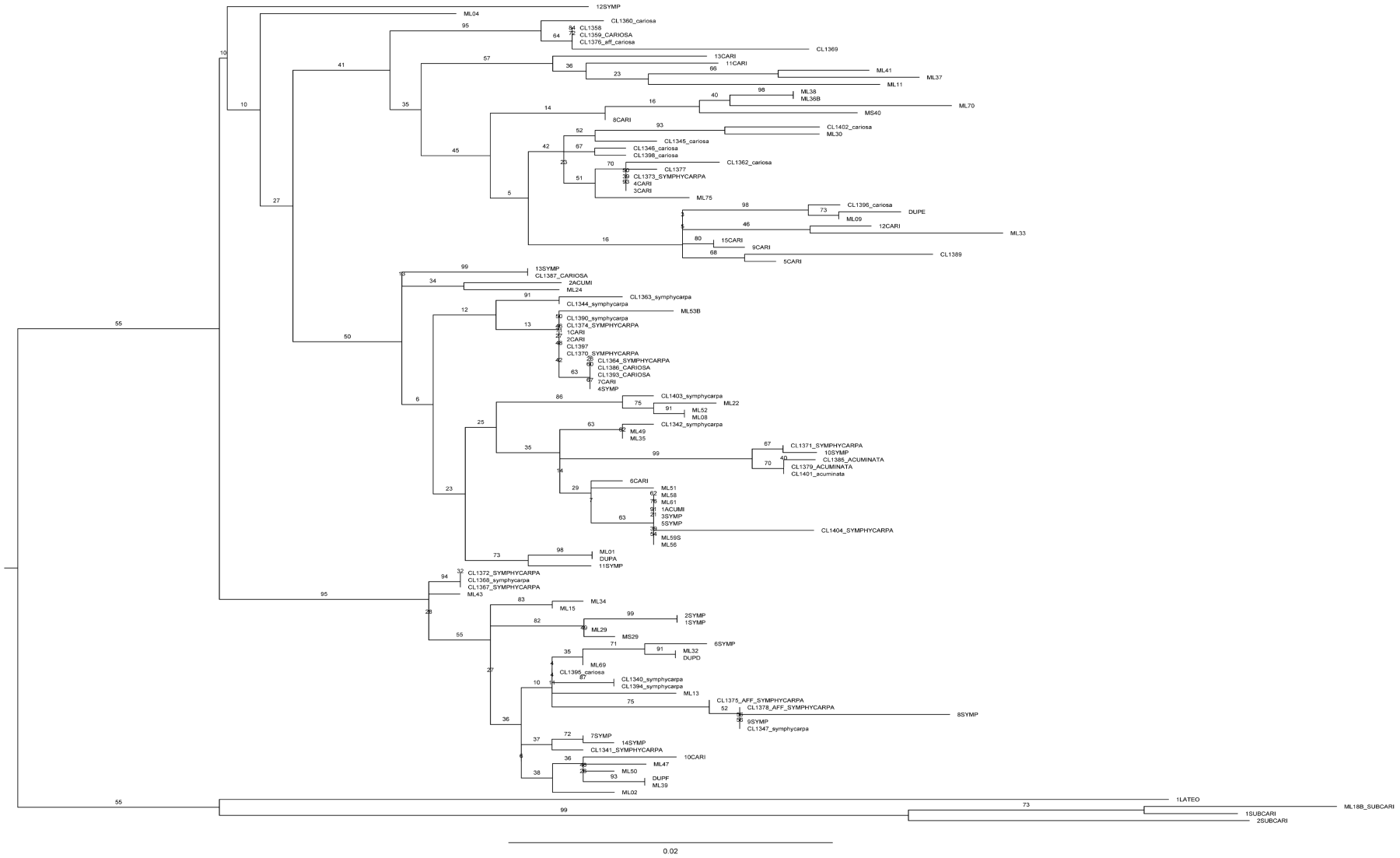


Figure A.1.1. Maximum likelihood tree of ITS sequences of members of the *Cladonia cariosa* group, aligned with ambiguous positions removed by GBlocks (LnL= -3066.50).

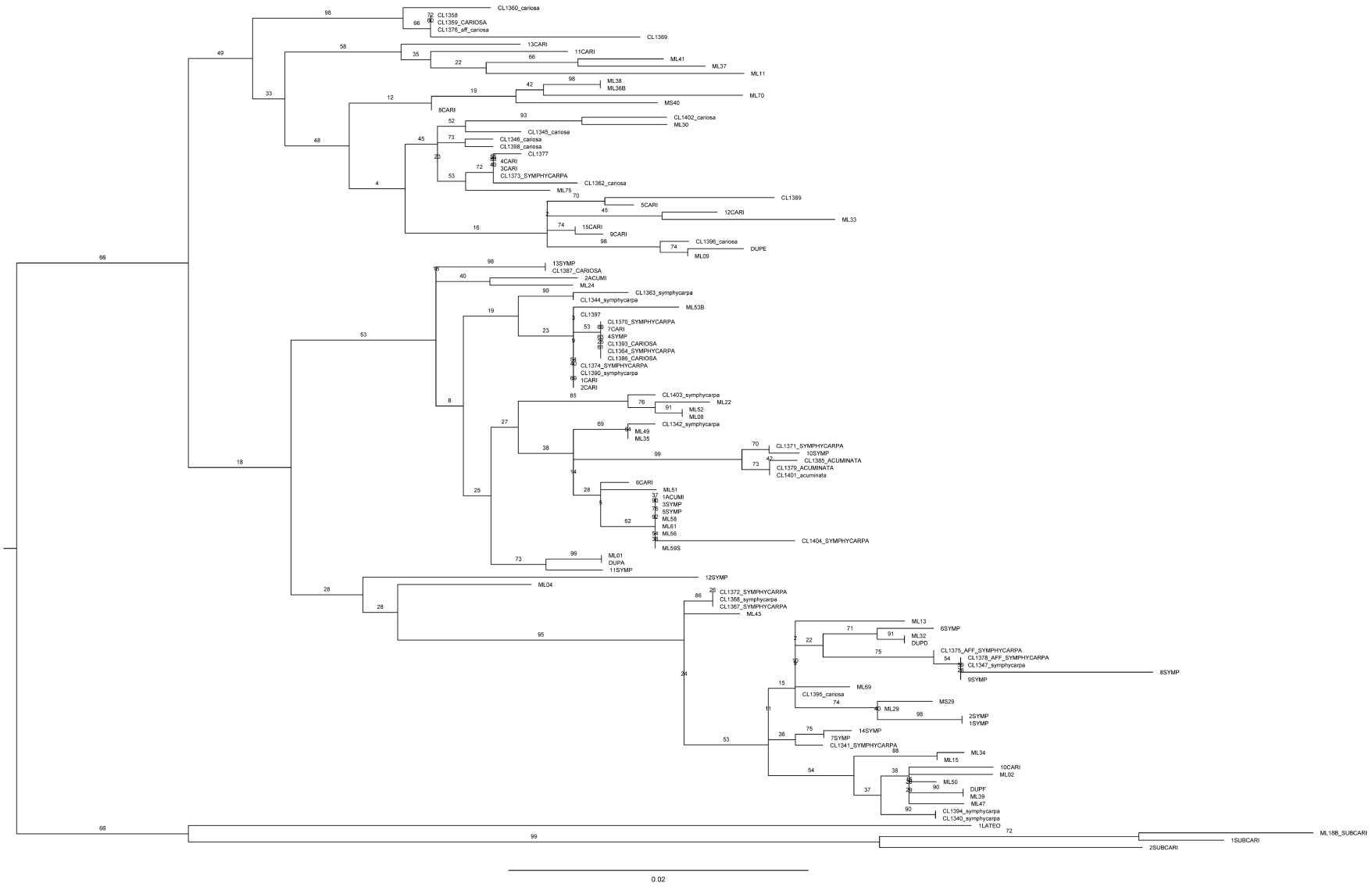


Figure A.1.2. Maximum likelihood tree of ITS sequences of members of the *C. cariosa* group, aligned with Mafft (LnL= -3280.26).

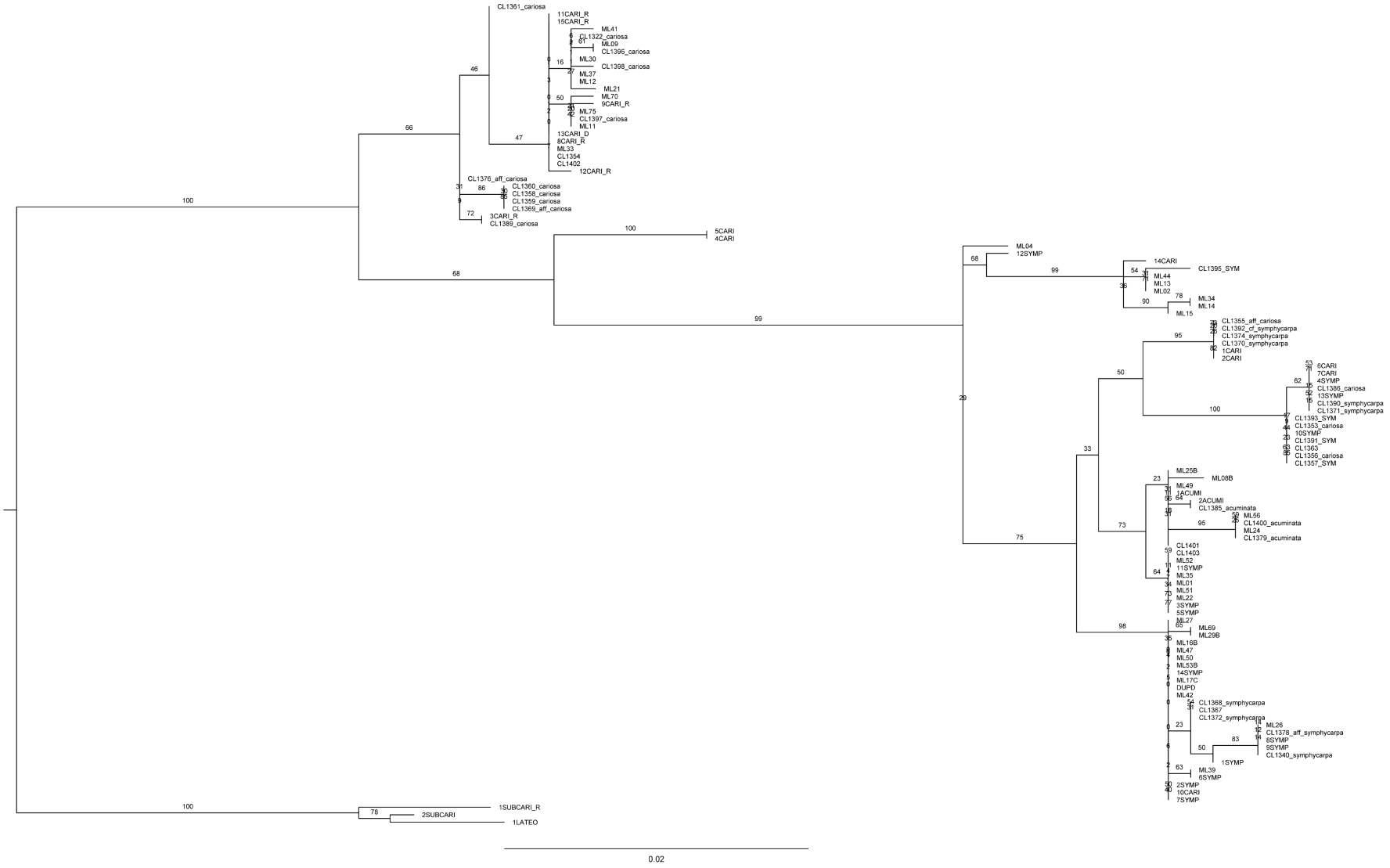


Figure A.1.3. Maximum likelihood tree of *rpb2* sequences of members of the *C. cariosa* group (LnL = -1836.09).

Appendix A.2. - Hybridization Analysis

Methods

Prior to validating species, we wanted to check for hybridization occurring within the group. An unlinked SNP dataset filtered using VCFtools was used for this analysis. Individuals missing more than 50% data were removed, a bi-allelic filter was applied, indels were removed, maximum amount of allowable missing data was 10%, and data were thinned to 300bp to ensure SNPs were unlinked. Singletons that did not fall into a clade in the RAxML or SVDQuartets analysis were removed. Concordance factors were calculated using the SNPs2CF v1.41 (Olave and Meyer, 2020). The concordance table was then processed through the TICR pipeline to produce a tree. The concordance table and tree were fed into PhyloNetworks v0.14.0 (Solís-Lemus et al., 2017) implemented in SNaQ v0.14.0 (Solís-Lemus & Ané, 2016). Multiple runs were completed with different maximum allowable values for hybrid nodes (hmax= 0 to 5), and the best network was selected using the pseudo-deviance value (multiple of the negative log-likelihood) of the network.

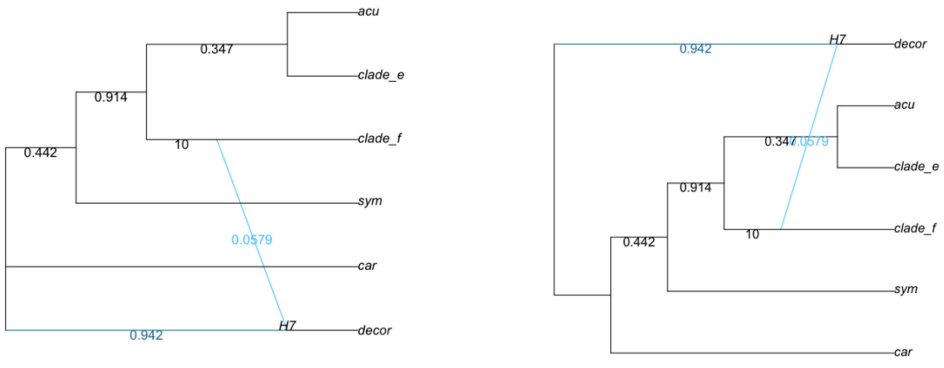


Figure A.2.1. PhyloNetworks hybridization/introgression analysis of unrooted (left) and rooted (right) species trees.

Appendix A.3. - Summary of previous work in the *C. cariosa* group.

Table A.3.1. Chemical races of members of the *Cladonia cariosa* group from Polish collections and specimens from other regions in the world. X indicates the presence of a metoabolite in the chemotype, and +/- indicates that a metabolite may be present or absent. (adapted from Ahti, 2000; Goward, 1999; Hansen and Ahti, 2011; Osyczka and Skubała, 2011; Pino-Bodas et al., 2012).

* Genetic analysis of European *Cladonia symphycarpa* specimens containing atranorin and psoromic acid (sometimes taxonomically differentiated as *Cladonia dahliana*) were found to not form a monophyletic clade, consistent with a taxonomic concept that *C. dahliana* is a chemotype of *C. symphycarpa* (Pino-Bodas et al. 2012).
** C. cariosa specimens containing homosekikaic acid are thought to belong to a separate species, *Cladonia scotteri* (Hansen and Ahti 2011).

Species	*Chemotype*	*Atranorin*	*Rangiformic/ Norrangiformic Acid*	*Norstictic/Connorstictic Acid*	*Fumarprotocetraric acid*	*Psoromic/Conpsoromic Acid*	*Homosekikaic Acid*	*Bourgeanic Acid*	*Stictic acid*	*Perlatolic acid*	*Zeorin*	*Morphological Characteristics*
C. acuminata	**1**	x		x								- Squamules similar in size to *C. symphycarpa* s.str. - Sorediate, mostly unbranched podetia (rarely dichotomously branched near tips) - Found on calcareous substrata - Cortex homogenous, no epinecral layer (Pino-Bodas et al., 2012)
	2	x				x						
	3	x			x				x			
	4	x										
	5					x						
C. cariosa	**1**	x	x									Smaller primary thallus than *C. symphycarpa* s.str and Clade D. Branched podetia with many lengthwise fissures. Squamules entire or incisions up to 30% of squamule length. Found on calcareous substrata. Thick epinecral layer above the cortex in the primary thallus (Pino-Bodas et al., 2012). Squamules shorter, less-wide, thinner than *C. symphycarpa* (Ahti, 2000)
	2	x										
	3	x	x		x							
	4	x	x	x								
	5	x		x								
	6**	x					x					
	7	x			x							
	8	x				x						
	9**	x	x				x					
C. dahliana*	**1**	x				x						Terricolous, podetia usually absent. Primary squamules 1-4 mm broad, 2-6 mm long, more or less incised, with lobate margins, ascending to erect, involute, pale green to greyish green above, the surface generally finely rugose or reticulately cracked, frequently faintly brownish at the margin,

Species	*Chemotype*	*Atranorin*	*Rangiformic/ Norrangiformic Acid*	*Norstictic/Connorstictic Acid*	*Fumarprotocetraric acid*	*Psoromic/Conpsoromic Acid*	*Homosekikaic Acid*	*Bourgeanic Acid*	*Stictic acid*	*Perlatolic acid*	*Zeorin*	*Morphological Characteristics*
												white below. Cortex of squamules 30-90 um thick, colourless to faintly brownish; medulla 180-340 um thick, the algal layer rather discontinuous (Kristinsson, 1974)
C. decorticata	**1**									x		Primary squamules usually persistent; small, up to 4 mm by 2 mm, irregularly or palmately divided, crenate, usually becoming concave; upper side glaucescent or olive-green or whitish glaucescent; underside white, darkening toward the base; esorediate or sparingly granulose. Podetia from the upper side of the primary squamules, 10-40 mm tall, up to 2.5 mm in diameter, cylindrical, cupless, simple or subsimple, dichotomous to trichotomous; the tips which bear apothecia dilated, those which are sterile blunt or subulate; the sides entire or fissured, sparingly granulose sorediate; cortex verruculose, the verruculae dispersed, partly developing into small squamules; the upper part of the podetium with reflexed, squarrose squamules similar to the primary squamules, the lower part becoming entirely squamulose, the part between the squamules decorticate; dull and not translucent, white or ashy or ashy brown, the verruculae and squamules white or ashy or olive-glaucescent. **Apothecia** middle-sized to large or often small, confluent or lobate-fissured, dark brown or red-brown. **Pycnidia** on the upper side of the primary squamules and the base of the podetia. (Thompson 1984).
C. symphycarpa	**1**	x		x								Cracked, large, prostrate squamules. Podetia, if present, are corticate with areolate zones, slightly fissured. Found on calcareous substrata. Squamules larger and thicker than *C.cariosa.* Thick, homogenous cortex, no epinecral layer (Pino-Bodas et al., 2012). Longer, wider, thicker squamules than *C. cariosa* (Ahti, 2000).
	2	x										
	3	x			x							
	4*	x				x						
	5	x		x				x				
	6	x				x		x				
C. scotteri*	**1**	x	-/+									Primary thallus consisting of rather small, grey to brown, persistent squamules. Podetia sparingly produced, 0.5–2 cm tall, clavate to slender, basic colour grey but easily browned, at the very base blackening, ascyphous, always with apothecia; corticate throughout, cortex checkered, areolae flattish, smooth; podetial squamules confined to the basal parts, not abundant. Conidiomata on primary squamules, rarely seen, ampullaceous. Apothecia with numerous brown hymenial disks. Distinguished from C. symphycarpa by smaller, epruinose squamules, brownish smaller podetia. From *C. cariosa* it is distinguished by larger squamules, thicker, brownish podetia.(Hansen & Ahti, 2011).
	2	x	-/+				x					
	3		-/+				x					
	4		x									
Cladonia Clade D (Pino-Bodas et al. 2012)	**1**	x			x							Primary thallus consisting of large squamules, similar in size to those of *C. symphycarpa*, but with podetia similar to those of C. cariosa (with many fissures). An intermediate morphotype between C. cariosa and C. symphycarpa. Lacking an epinecral layer. Found on acid substrata, above 1000 m altitude (Pino-Bodas et al., 2012).
	2	x				x						
	3	x		x								
	4	x									x	

Appendix A.4. - Chi-square post-hoc testing

Table A.4.1. Post-hoc tests for chi-square contingency tables for specimen chemistry and qualitative features. Adjusted Agresti residuals were calculated following Sharpe (Sharpe, 2015), and then compared to a critical z-value of +/- 2.58 to determine which cells contributed most to the chi-squared statistic. The critical z-value was obtained by applying the Bonferroni correction on a p-value of 0.05 and 10 contrasts per test to obtain a new p-value of 0.005, and then finding the associated z-score for the new critical value (2.58). Post-hoc tests with critical z-values >|2.58| are considered significant. Positive values indicate likelihood of observing feature in the clade is higher in the group compared to others, negative values indicate that likelihood of observing feature is lower compared to others.

	CNST_Y	CNST_N	CNNRST_Y	CNNRST_N	FUM_Y	FUM_N	NST_Y	NST_N	PSO_Y	PSO_N	RANG_Y	RANG_N
Clade A	**-3.179**	**3.179**	-1.556	1.556	**5.22**	**-5.22**	**-3.952**	**3.952**	-2.148	2.148	**5.457**	**-5.457**
Clade B	-1.312	1.312	-1.324	1.324	-2.264	2.264	-2.148	2.148	2.287	-2.287	-0.367	0.367
Clade C	2.503	-2.503	**5.382**	**-5.382**	-1.699	1.699	**4.335**	**-4.335**	-1.372	1.372	-2.112	2.112
Clade E	-1.007	1.007	-0.893	0.893	-1.528	1.528	-0.448	0.448	1.932	-1.932	-1.781	1.781
Clade F	**4.013**	**-4.013**	-0.994	0.994	-0.861	0.861	**3.681**	**-3.681**	-0.394	0.394	**-2.771**	**2.771**

	SECONDARY_ PODBRANCH_YES	SECONDARY PODBRANCH_NO	LONGRIBS_YES	LONGRIBS_NO	SOREDIA_Y	SOREDIA_N	PODBRANCH_ WIDE	PODBRANCH_ NARROW
Clade A	2.507	-2.507	1.593	-1.593	-1.845	1.845	2.487	-2.487
Clade B	-1.156	1.156	1.288	-1.288	-1.583	1.583	2.004	-2.004
Clade C	-2.215	2.215	**-3.001**	**3.001**	**6.257**	**-6.257**	**-6.782**	**6.782**
Clade E	1.227	-1.227	0.893	-0.893	-1.112	1.112	1.384	-1.384
Clade F	-0.445	0.445	-0.997	0.997	-1.182	1.182	1.103	-1.103

Appendix B. Chapter 3

Appendix B.1. – Species List

Table B.1.1. Species list of biocrust constituents found at study sites.

KINSELLA	Study Code
Bryophytes	
Abietinella abietina (Hedwig) Fleischer	THUIDIA_F
Amblystegiaceae G. Roth	MOSS_GRP
Amblystegium serpens (Hedwig) Schimper	MOSS_GRP
Brachytheciaceae Schimper	MOSS_GRP
Bryaceae Schwagrichen	MOSS_GRP
Ceratodon purpureus (Hedwig) Bridel	MOSS_GRP
Hypnaceae Schimper	MOSS_GRP
Plagiomnium cuspidatum (Hedwig) T. Koponen	MOSS_GRP
Pylaisia polyantha (Hedwig) Schimper	MOSS_GRP
Sanionia uncinata (Hedwig) Loeske	MOSS_GRP
Syntrichia ruralis (Hedwig) F. Weber & D. Mohr	MOSS_GRP
Thuidiaceae Schimper	THUIDIA_F
Thuidium recognitum (Hedwig) Lindberg	THUIDIA_F
Unknown moss	MOSS
Lichen	
Candelariella sp. Müll. Arg.	CANDELA_SPP
Cladonia acuminata (Ach.) Norrlin	CL_ACU_REI_COR
Cladonia chlorophaea (Flörke ex Sommerf.) Sprengel	CL_CHL_FIM
Cladonia fimbriata (L.) Fr.	CL_CHL_FIM
Cladonia magyarica Vainio	CL_MAG_POC
Cladonia multiformis G. Merr.	CLADMUL
Cladonia pocillum (Ach.) O. J. Rich.	CL_MAG_POC
Cladonia rei Schaerer (Syrek & Kukwa 2008, Dolnik et al. 2010, Pino-Bodas et al. 2010)	CL_ACU_REI_COR
Cladonia robbinsii A. Evans	CLADROB
Cladonia sp. P. Browne	CLADSP
Cladonia symphycarpa (Flörke) Fr.	CLADSYM
Peltigera sp. Willd.	PELTIGERA
Phaeophyscia constipata (Norrlin & Nyl.) Moberg Syn.: Physcia constipata	PHAECON
Physconia muscigena (Ach.) Poelt Syn.: Physcia muscigena	PHYSMUS
Unknown black crust lichen	BLACKCRU
Unknown green crust lichen	GREENCRU

MATTHEIS	
Bryophytes	
Bryaceae Schwagrichen	MOSS_GRP
Ceratodon purpureus (Hedwig) Bridel	MOSS_GRP
Polytrichaceae Schwägrichen	POLYTRI_F
Polytrichum piliferum Hedwig	POLYTRI_F
Lichen	
Amandinea punctata (Hoffm.) Coppins & Scheid. Syn.: Buellia punctata, B. myriocarpa	BUELPUN
Caloplaca sp. Th. Fr.	CALO_SPP
Caloplaca stillicidiorum (Vahl) Lynge (Šoun et al. 2011, Arup et al. 2013)	CALOSTI
Candelariella vitellina (Hoffm.) Müll. Arg.	CANDVIT
Cladonia chlorophaea (Flörke ex Sommerf.) Sprengel	CL_CHL_FIM
Cladonia fimbriata (L.) Fr.	CL_CHL_FIM
Cladonia imbricarica Kristinsson	CLADIMB
Cladonia magyarica Vainio	CL_MAG_POC
Cladonia multiformis G. Merr.	CLADMUL
Cladonia pocillum (Ach.) O. J. Rich.	CL_MAG_POC
Cladonia rei Schaerer (Syrek & Kukwa 2008, Dolnik et al. 2010, Pino-Bodas et al. 2010)	CL_ACU_REI_COR
Cladonia robbinsii A. Evans	CLADROB
Cladonia sp. P. Browne	CLADSP
Cladonia symphycarpa (Flörke) Fr.	CLADSYM
Diploschistes muscorum (Scop.) R. Sant. subsp. muscorum	DIPLMUS
Ochrolechia turneri (Sm.) Hasselrot (Brodo & Lendemer 2012)	OCHRTUR
Phaeophyscia constipata (Norrlin & Nyl.) Moberg Syn.: Physcia constipata	PHAECON
Physconia muscigena (Ach.) Poelt Syn.: Physcia muscigena	PHYSMUS
Xanthoparmelia camtschadalis (Ach.) Hale Syn.: Parmelia camtschadalis	XANTCAM
Xanthoparmelia wyomingica (Gyelnik) Hale Syn.: Parmelia wyomingica	XANTWYO
Lycophytes	
Selaginella densa Rydberg	SELADEN

ONEFOUR	
Bryophytes	
Bryaceae Schwagrichen	MOSS_GRP
Unknown moss	MOSS
Lichen	
Amandinea punctata (Hoffm.) Coppins & Scheid. Syn.: Buellia punctata, B. myriocarpa	BUELPUN
Caloplaca stillicidiorum (Vahl) Lynge (Šoun et al. 2011, Arup et al. 2013)	CALOSTI
Candelariella vitellina (Hoffm.) Müll. Arg.	CANDVIT
Cladonia cariosa (Ach.) Sprengel	CLADCAR
Cladonia magyarica Vainio	CL_MAG_POC
Cladonia pocillum (Ach.) O. J. Rich.	CL_MAG_POC
Cladonia rei Schaerer (Syrek & Kukwa 2008, Dolnik et al. 2010, Pino-Bodas et al. 2010)	CL_ACU_REI_COR
Cladonia robbinsii A. Evans	CLADROB
Cladonia sp. P. Browne	CLADSP
Cladonia symphycarpa (Flörke) Fr.	CLADSYM
Cladonia tonnerii	CLADTON
Diploschistes muscorum (Scop.) R. Sant. subsp. muscorum	DIPLMUS
Lecidella wulfenii (Hepp) Körber Syns.: Lecidea heppii, L. wulfenii	LECIWUL
Lepraria sp. Ach.	LEPR_SPP
Phaeophyscia constipata (Norrlin & Nyl.) Moberg Syn.: Physcia constipata	PHAECON
Physconia muscigena (Ach.) Poelt Syn.: Physcia muscigena	PHYSMUS
Unknown grey crust lichen	GREYCRU
Xanthoparmelia camtschadalis (Ach.) Hale Syn.: Parmelia camtschadalis	XANTCAM
Xanthoparmelia chlorochroa (Tuck.) Hale Syn.: Parmelia chlorochroa	XANTCHL
Xanthoparmelia wyomingica (Gyelnik) Hale Syn.: Parmelia wyomingica	XANTWYO
Lycophytes	
Selaginella densa Rydberg	SELADEN

OYEN	
Bryophytes	
Amblystegiaceae G. Roth	MOSS_GRP
Amblystegium serpens (Hedwig) Schimper	MOSS_GRP
Brachytheciaceae Schimper	MOSS_GRP
Bryaceae Schwagrichen	MOSS_GRP
Ceratodon purpureus (Hedwig) Bridel	MOSS_GRP
Polytrichum juniperinum Hedwig	POLYJUN
Pottiaceae Schimper	MOSS_GRP
Syntrichia ruralis (Hedwig) F. Weber & D. Mohr	MOSS_GRP
Lichen	
Candelariella sp. Müll. Arg.	CANDELA_SPP
Cladonia cornuta (L.) Hoffm. subsp. cornuta	CL_ACU_REI_COR
Cladonia chlorophaea (Flörke ex Sommerf.) Sprengel	CL_CHL_FIM

Cladonia fimbriata (L.) Fr.	CL_CHL_FIM
Cladonia magyarica Vainio	CL_MAG_POC
Cladonia multiformis G. Merr.	CLADMUL
Cladonia pocillum (Ach.) O. J. Rich.	CL_MAG_POC
Cladonia rei Schaerer (Syrek & Kukwa 2008, Dolnik et al. 2010, Pino-Bodas et al. 2010)	CL_ACU_REI_COR
Cladonia sp. P. Browne	CLADSP
Unknown black crust lichen	BLACKCRU
Unknown green crust lichen	GREENCRU
Lycophytes	
Selaginella densa Rydberg	SELADEN

SANGUDO	
Bryophytes	
Amblystegiaceae G. Roth	MOSS_GRP
Barbula convoluta Hedwig	BARBUNG_CON
Barbula unguiculata Hedwig	BARBUNG_CON
Brachytheciaceae Schimper	MOSS_GRP
Bryaceae Schwagrichen	MOSS_GRP
Bryum argenteum Hedwig	MOSS_GRP
Campyliadelphus chrysophyllus (Bridel) Kanda	MOSS_GRP
Ceratodon purpureus (Hedwig) Bridel	MOSS_GRP
Eurhynchiastrum pulchellum (Hedwig) Ignatov & Huttunen	MOSS_GRP
Hygroamblystegium varium (Hedwig)	HYGRVAR
Leptobryum pyriforme (Hedwig) Wilson	MOSS_GRP
Unknown moss	MOSS

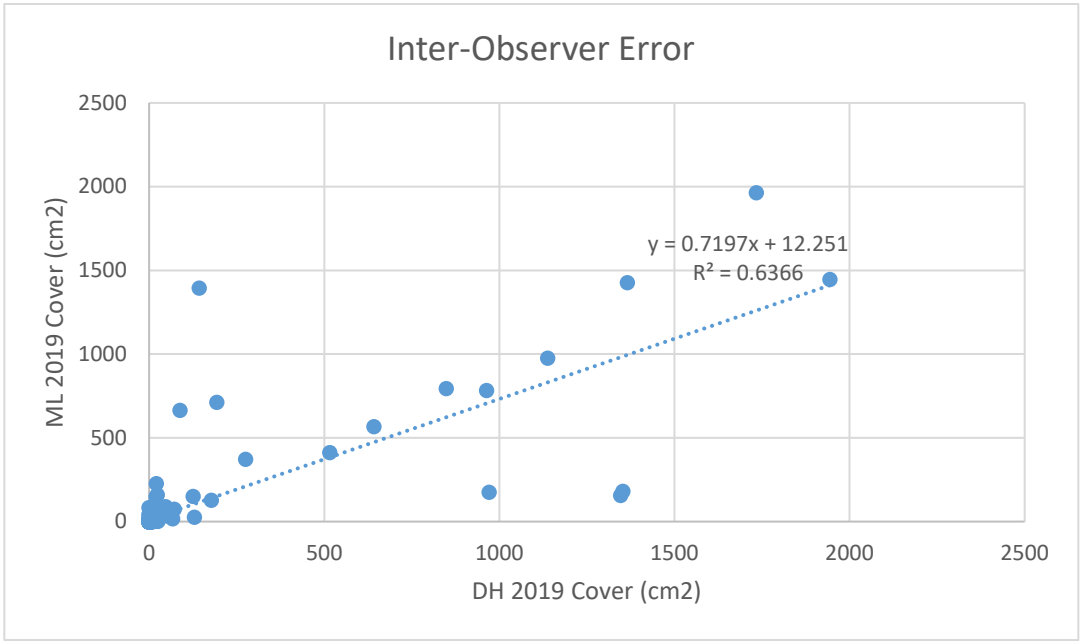
STAVELY	
Bryophytes	
Bryaceae Schwagrichen	MOSS_GRP
Unknown moss	MOSS

TWIN RIVER	
Bryophytes	
Brachytheciaceae Schimper	MOSS_GRP
Bryaceae Schwagrichen	MOSS_GRP
Bryum lanatum (P. Beauvois) Bridel	MOSS_GRP
Ceratodon purpureus (Hedwig) Bridel	MOSS_GRP
Polytrichum juniperinum Hedwig	POLYJUN
Syntrichia ruralis (Hedwig) F. Weber & D. Mohr	MOSS_GRP
Unknown moss	MOSS

Lichen	
Amandinea punctata (Hoffm.) Coppins & Scheid. Syn.: Buellia punctata, B. myriocarpa	BUELPUN
Caloplaca stillicidiorum (Vahl) Lynge (Šoun et al. 2011, Arup et al. 2013)	CALOSTI
Candelariella sp. Müll. Arg.	CANDELA_SPP
Cladonia chlorophaea (Flörke ex Sommerf.) Sprengel	CL_CHL_FIM
Cladonia coniocraea (Flörke) Sprengel	CLADCON
Cladonia fimbriata (L.) Fr.	CL_CHL_FIM
Cladonia sp. P. Browne	CLADSP
Cladonia symphycarpa (Flörke) Fr.	CLADSYM
Ochrolechia turneri (Sm.) Hasselrot (Brodo & Lendemer 2012)	OCHRTUR
Lycophytes	
Selaginella densa Rydberg	SELADEN

Appendix B.2. – Inter-observer Variability

(a)



(b)

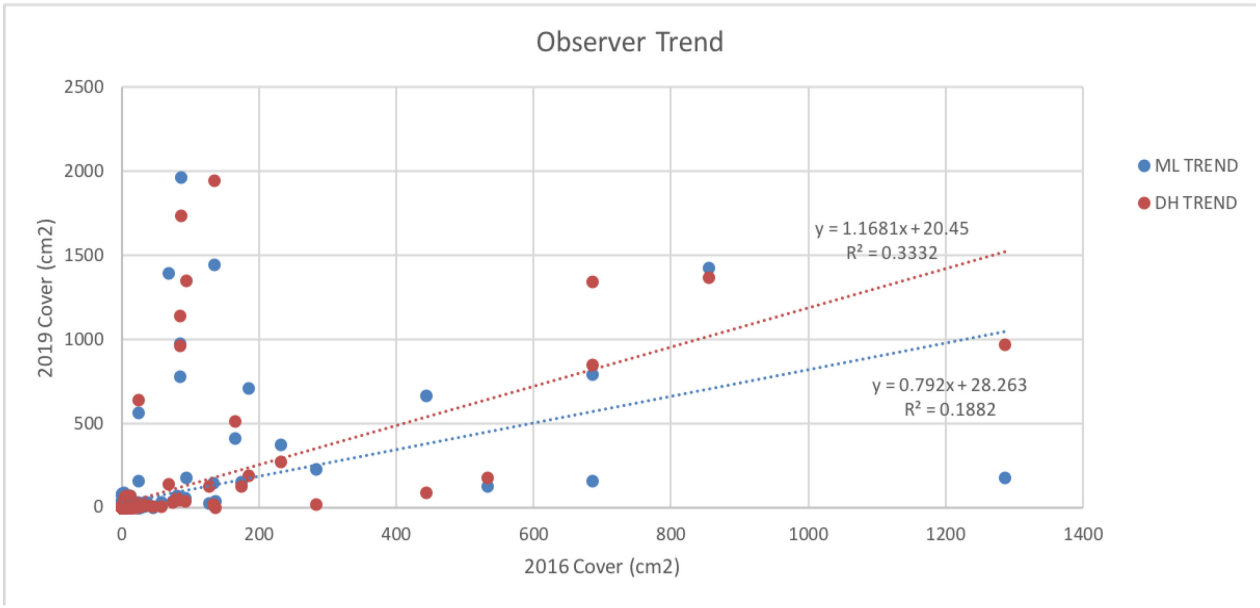


Figure B.2.1. Regression of error in BSC cover estimates between (a) Observer 1 (DH) and Observer 2 (ML) in 2019, and (b) trend in Observer 1's 2016 and 2019 cover estimates (red) and Observer 2's 2019 cover estimates vs. Observer 1's 2016 cover estimates.

Appendix B.3. All means graphs

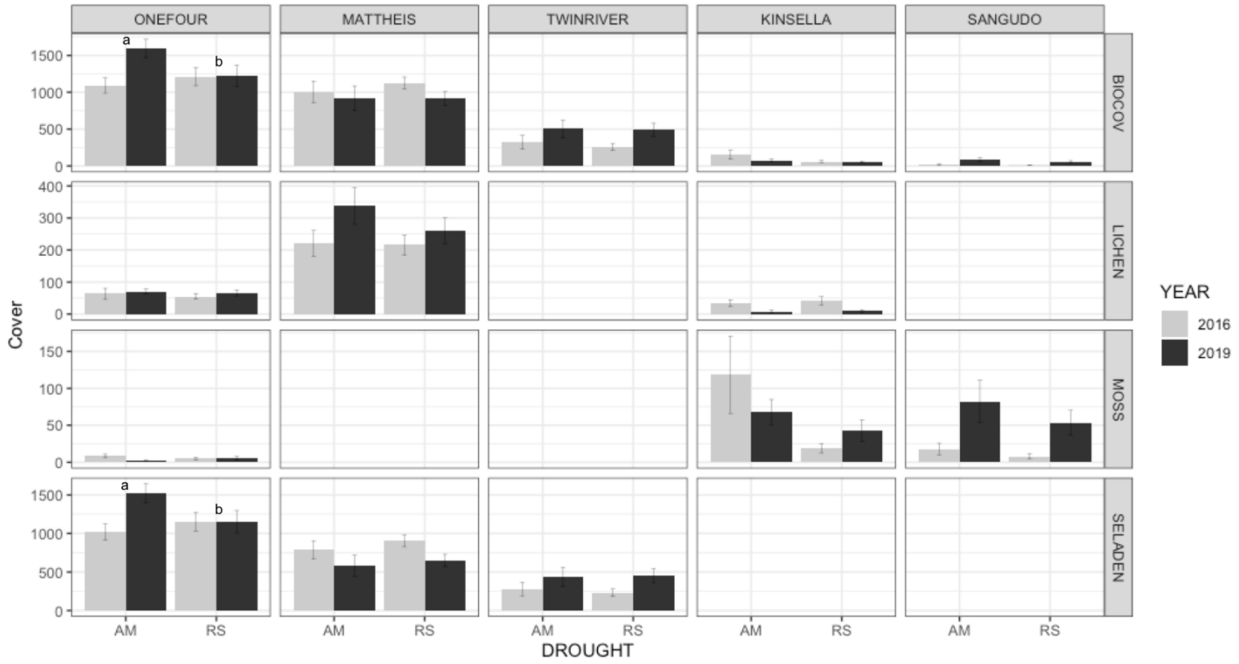


Figure B.3.1. Mean of biocrust constituent cover in drought treatments at study sites.

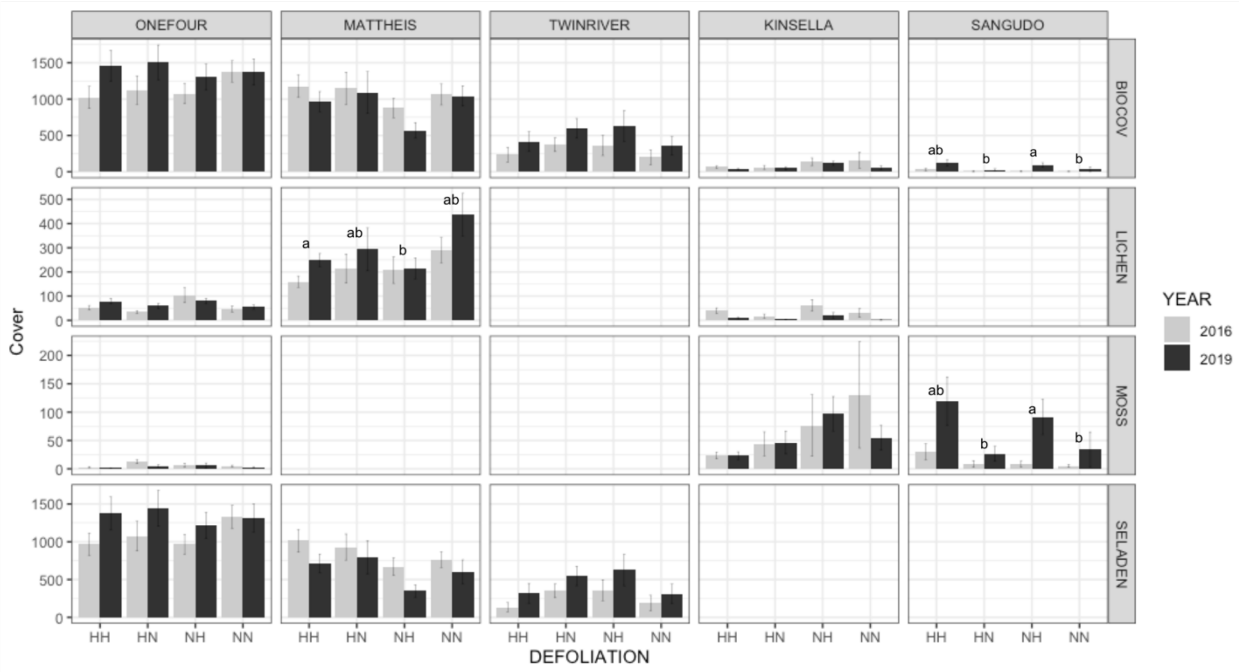


Figure B.3.2. Mean of biocrust constituent cover in defoliation treatments at study sites.

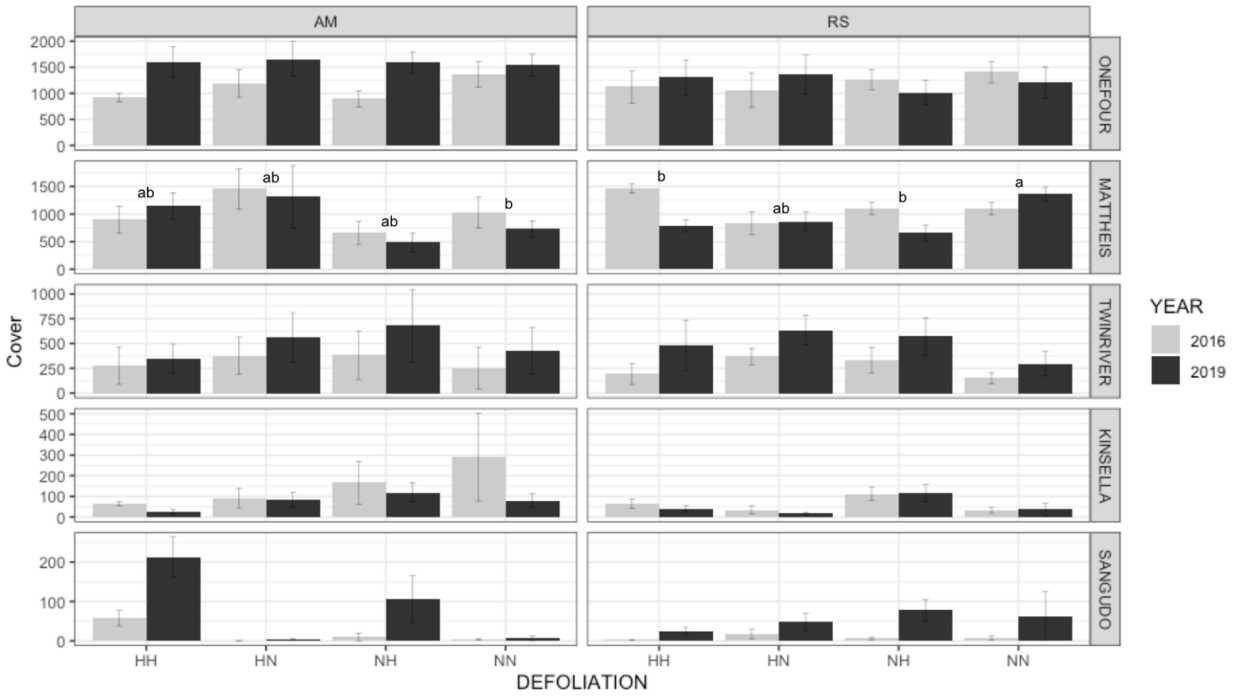


Figure B.3.3. Interactive effects of drought and defoliation on BSC cover at study sites

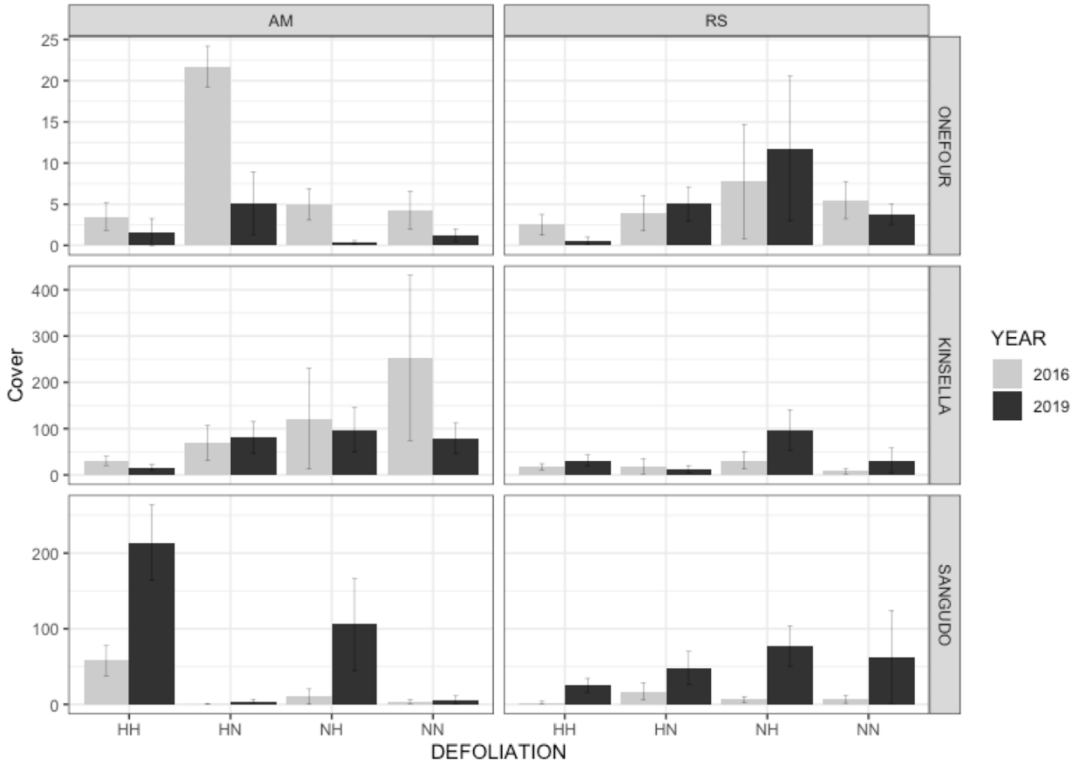


Figure B.3.4. Interactive effects of drought and defoliation on BSC moss cover at study sites

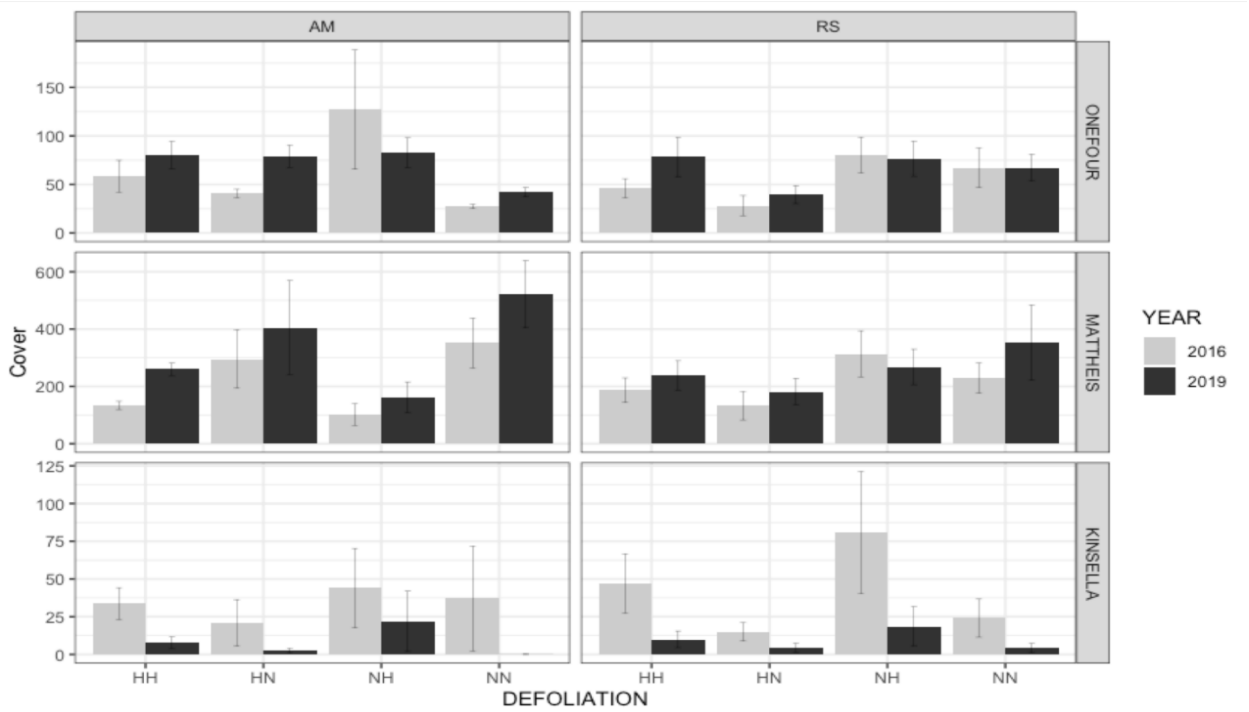


Figure B.3.5. Interactive effects of drought and defoliation on biocrust lichen cover at study sites.

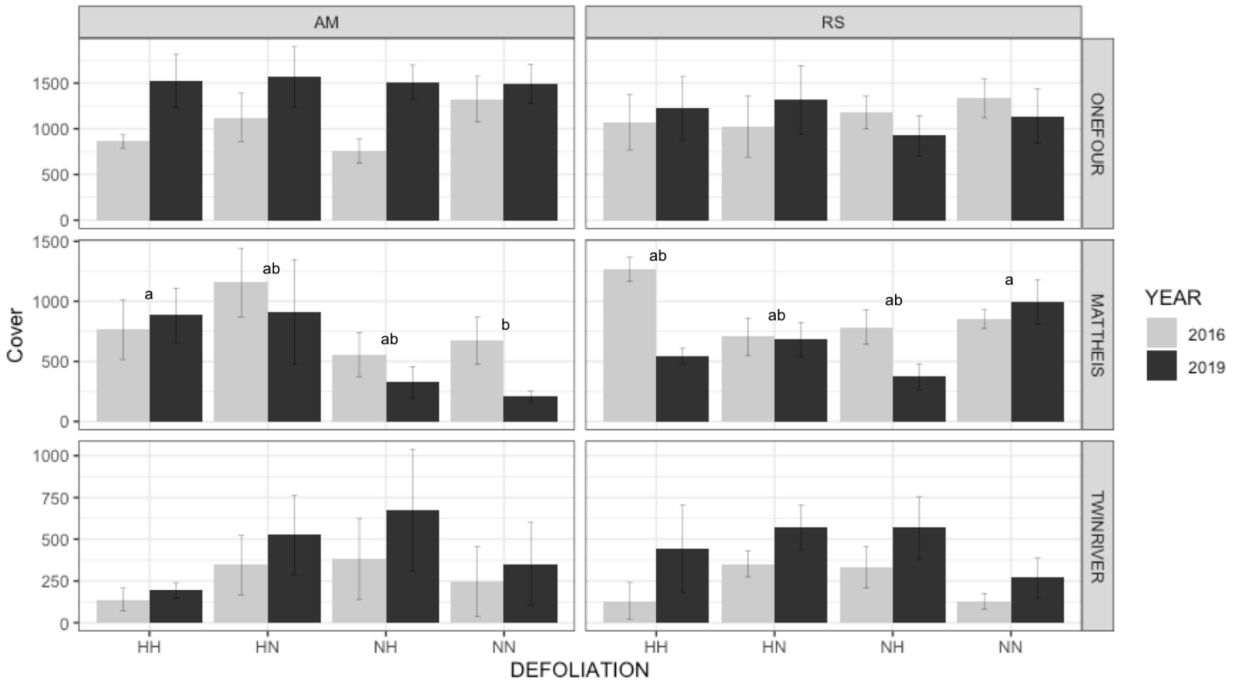


Figure B.3.6. Interactive effects of drought and defoliation on S. densa cover at study sites.

Appendix B.4. NMDS Ordinations for all sites

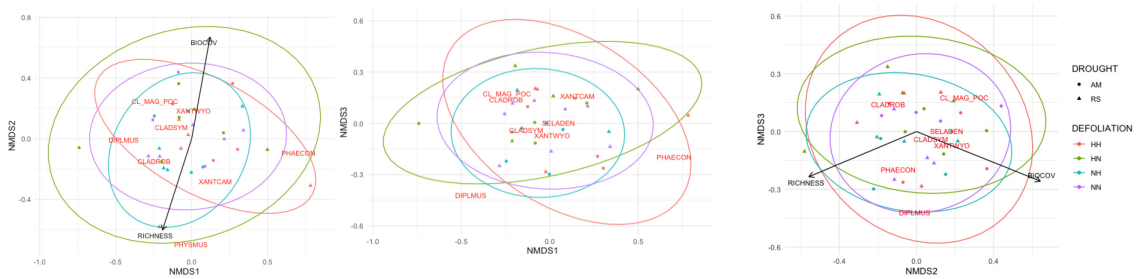


Figure B.4.1. NMDS ordinations for Onefour. Three-dimensional solution with a stress of 0.1457653. Data standardized with Wisconsin double standardization and were square-root transformed. Configuration rotated to maximize variance of points on first dimension, origin centered to the average of the axes, and halfchange scaling.

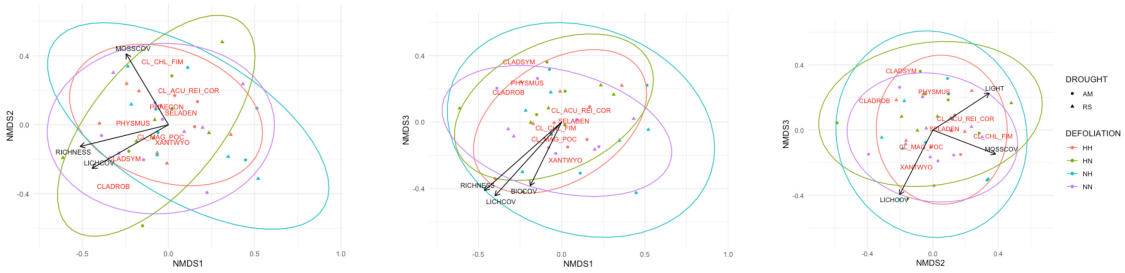


Figure B.4.2. NMDS ordinations for Mattheis. Three-dimensional solution with a stress of 0.1704126. Data standardized with Wisconsin double standardization and were square-root transformed. Configuration rotated to maximize variance of points on first dimension, origin centered to the average of the axes, and halfchange scaling.

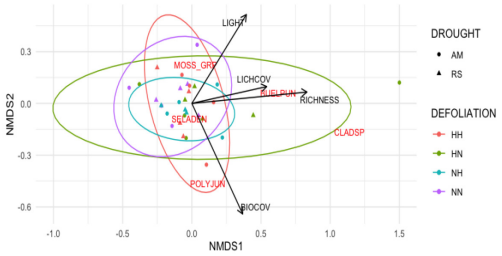


Figure B.4.3. NMDS ordinations for Twin River. Two-dimensional solution with a stress of 0.105076. Data standardized with Wisconsin double standardization and were square-root transformed. Configuration rotated to maximize variance of points on first dimension, origin centered to the average of the axes, and halfchange scaling.

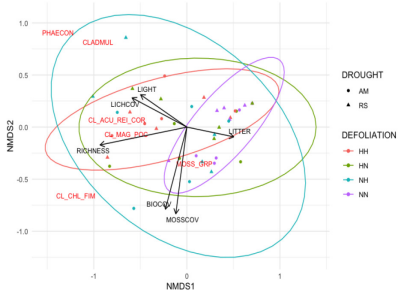


Figure B.4.4. NMDS ordinations for Kinsella. Two-dimensional solution with a stress of 0.1138806. Data standardized with Wisconsin double standardization and were square-root transformed. Configuration rotated to maximize variance of points on first dimension, origin centered to the average of the axes, and halfchange scaling.

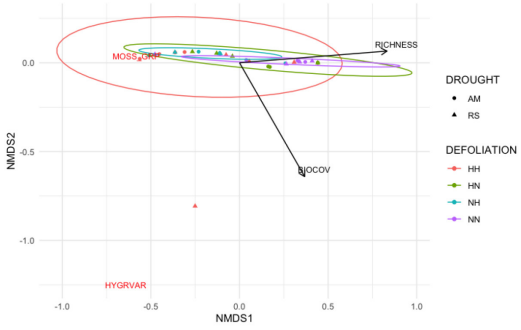


Figure B.4.5. NMDS ordinations for Sangudo. Two-dimensional solution with a stress of 0.0009866313. Data standardized with Wisconsin double standardization and were square-root transformed. Configuration rotated to maximize variance of points on first dimension, origin centered to the average of the axes, and halfchange scaling.

Appendix B.5. - Modelling Details

Table B.5.1. Model type, transformation, and identity variance structures used for cover and richness models.

Site	Response	Model	Transformation	Identity Variance Structures for Model Weights
Onefour	Biocrust cover	GLS	none	Drought, Defoliation
	Lichen Cover	GLS	none	Drought, Defoliation
	Moss Cover	GLS	log+1	Drought, Defoliation
	S. densa cover	GLS	none	Drought, Defoliation
	Biocrust Richness	GLS	sqrt	Drought, Defoliation
Mattheis	Biocrust cover	GLS	log+1	Drought, Defoliation
	Lichen Cover	GLS	log+1	none
	Moss Cover	n/a	n/a	n/a
	S. densa cover	GLS	log+1	Drought, Defoliation
	Biocrust Richness	GLS	log+1	Drought, Defoliation
Twin River	Biocrust cover	GLS	sqrt	Drought, Defoliation
	Lichen Cover	n/a	n/a	n/a
	Moss Cover	n/a	n/a	n/a
	S. densa cover	GLS	sqrt	Drought
	Biocrust Richness	GLS	log+1	Drought, Defoliation
Kinsella	Biocrust cover	GLM - Gamma	arcsine	n/a
	Lichen Cover	GLM - Gamma	arcsine	n/a
	Moss Cover	GLS	log+1	Defoliation
	S. densa cover	n/a	n/a	n/a
	Biocrust Richness	GLS	log+1	Drought, Defoliation
Sangudo	Biocrust cover	GLS	log+1	Drought, Defoliation
	Lichen Cover	n/a	n/a	n/a
	Moss Cover	GLS	log+1	Drought, Defoliation
	S. densa cover	n/a	n/a	n/a
	Biocrust Richness	n/a	n/a	n/a

Appendix B.6. – BSC community analysis results

Table B.6.1. Additional community composition results for PerMANOVA, Betadisper, and Bray-Curtis distance shift analyses.

Community Analyses		PerMANOVA						Betadisper						Bray-Curtis Distance Moved 2016 to 2019		
Site	**Test**	**Df**	**Sum Sqs**	**MeanSqs**	**F.Model**	**R2**	**Pr(>F)**	**Df**	**Sum sq**	**Mean sq**	**F**	**N.Perm**	**Pr(>F)**	**Df**	**Chisq**	**Pr (>Chisq)**
Onefour	DROUGHT	1	0.055	0.055	1.074	0.017	0.311	1	0.011	0.011	0.832	10000	0.367	1	1.210	0.271
	DEFOLIATION	3	0.121	0.040	0.789	0.038	0.541	3	0.054	0.018	1.165	10000	0.331	3	1.292	0.731
	YEAR	1	0.127	0.127	2.486	0.040	0.096	1	4.80E-04	4.78E-04	0.036	10000	0.847	-	-	-
	DROUGHT:DEFOLIATION	3	0.047	0.016	0.305	0.015	0.931	7	0.052	0.007	0.948	10000	0.477	3	4.755	0.191
	YEAR:DROUGHT	1	0.175	0.175	3.430	0.056	**0.050**	3	0.044	0.015	1.001	10000	0.403	-	-	-
	YEAR:DEFOLIATION	3	0.074	0.025	0.484	0.024	0.795	7	0.020	0.003	0.311	10000	0.960	-	-	-
	YEAR:DROUGHT:DEFOLATION	3	0.092	0.031	0.600	0.029	0.697	15	0.087	0.006	0.578	10000	0.891	-	-	-
	RESIDUALS	48	2.451	0.051		0.780		-	-	-	-	-	-	-	-	-
Mattheis	DROUGHT	1	0.565	0.565	5.167	0.055	**0.003**	1	0.221	0.221	14.900	10000	**4.00E-04**	1	0.238	0.626
	DEFOLIATION	3	0.813	0.271	2.481	0.080	**0.010**	3	0.092	0.031	1.700	10000	0.175	3	3.469	0.325
	YEAR	1	0.585	0.585	5.357	0.057	**0.002**	1	0.158	0.158	10.285	10000	**0.002**	-	-	-
	DROUGHT:DEFOLIATION	3	0.646	0.215	1.970	0.063	**0.039**	7	0.327	0.047	2.360	10000	**0.032**	3	8.533	**0.036**
	YEAR:DROUGHT	1	0.042	0.042	0.385	0.004	0.841	3	0.318	0.106	7.453	10000	**2.00E-04**	-	-	-
	YEAR:DEFOLIATION	3	0.149	0.050	0.455	0.015	0.944	7	0.291	0.042	2.911	10000	**0.009**	-	-	-
	YEAR:DROUGHT:DEFOLATION	3	0.419	0.140	1.279	0.041	0.223	15	0.147	0.010	1.268	10000	0.243	-	-	-
	RESIDUALS	64	6.992	0.109		0.685		-	-	-	-	-	-	-	-	-
Twin River	DROUGHT	1	0.214	0.214	1.023	0.016	0.368	1	0.131	0.131	2.974	10000	0.090	1	0.195	0.659
	DEFOLIATION	3	1.411	0.470	2.247	0.106	**0.010**	3	0.346	0.115	2.298	10000	0.085	3	6.209	0.102
	YEAR	1	0.429	0.429	2.050	0.032	0.079	1	0.068	0.068	1.509	10000	0.223	-	-	-
	DROUGHT:DEFOLIATION	3	0.943	0.314	1.502	0.071	0.115	7	0.812	0.116	3.205	10000	**0.007**	3	8.741	**0.033**
	YEAR:DROUGHT	1	0.030	0.030	0.143	0.002	0.990	3	0.176	0.059	1.177	10000	0.332	-	-	-

Community Analyses		PerMANOVA						Betadisper						Bray-Curtis Distance Moved 2016 to 2019		
Site	**Test**	**Df**	**Sum Sqs**	**MeanSqs**	**F.Model**	**R2**	**Pr(>F)**	**Df**	**Sum sq**	**Mean sq**	**F**	**N.Perm**	**Pr(>F)**	**Df**	**Chisq**	**Pr (>Chisq)**
	YEAR:DEFOLIATION	3	0.373	0.124	0.595	0.028	0.874	7	0.196	0.028	1.167	10000	0.337	-	-	-
	YEAR:DROUGHT:DEFOLATION	3	0.252	0.084	0.402	0.019	0.977	15	0.435	0.029	1.220	10000	0.278	-	-	-
	RESIDUALS	46	9.628	0.209		0.725		-	-	-	-	-	-	-	-	-
Kinsella	DROUGHT	1	0.413	0.413	1.531	0.019	0.155	1	0.006	0.006	0.835	10000	0.359	1	10.458	**0.001**
	DEFOLIATION	3	1.820	0.607	2.248	0.082	**0.009**	3	0.125	0.042	2.915	10000	**0.041**	3	2.882	0.410
	YEAR	1	0.644	0.644	2.387	0.029	**0.040**	1	0.033	0.033	3.528	10000	0.065	-	-	-
	DROUGHT:DEFOLIATION	3	1.179	0.393	1.456	0.053	0.121	7	0.139	0.020	0.854	10000	0.552	3	6.711	0.082
	YEAR:DROUGHT	1	0.133	0.133	0.492	0.006	0.820	3	0.038	0.013	1.394	10000	0.257	-	-	-
	YEAR:DEFOLIATION	3	0.481	0.160	0.594	0.022	0.891	7	0.228	0.033	1.718	10000	0.121	-	-	-
	YEAR:DROUGHT:DEFOLATION	3	0.365	0.122	0.450	0.016	0.975	15	0.364	0.024	0.744	10000	0.729	-	-	-
	RESIDUALS	64	17.276	0.270		0.774		-	-	-	-	-	-	-	-	-
Sangudo	DROUGHT	1	0.171	0.170	0.851	0.010	0.431	1	1.90E-04	1.88E-04	0.004	10000	0.947	1	1.001	0.317
	DEFOLIATION	3	1.737	0.579	2.890	0.102	**0.009**	3	0.112	0.037	0.675	10000	0.568	3	14.615	**0.002**
	YEAR	1	1.380	1.380	6.891	0.081	**0.002**	1	0.041	0.041	0.690	10000	0.405	-	-	-
	DROUGHT:DEFOLIATION	3	2.038	0.679	3.391	0.120	**0.003**	7	0.486	0.069	1.146	10000	0.347	3	0.309	0.958
	YEAR:DROUGHT	1	0.271	0.271	1.355	0.016	0.239	3	0.220	0.073	1.066	10000	0.374	-	-	-
	YEAR:DEFOLIATION	3	0.789	0.263	1.312	0.046	0.229	7	0.335	0.048	0.600	10000	0.754	-	-	-
	YEAR:DROUGHT:DEFOLATION	3	0.981	0.327	1.632	0.058	0.126	15	0.258	0.017	0.416	10000	0.972	-	-	-
	RESIDUALS	48	9.614	0.200		0.566		-	-	-	-	-	-	-	-	-

Table B.6.2. Results of indirect gradient analysis for environmental factors by site. Gradient analysis was completed using the envfit function in Vegan (Oksanen et al., 2019) with 1000 permutations.

Environmental Gradient	Onefour											
	NMDS1	NMDS2	r2	Pr(>r)	NMDS1	NMDS3	r2	Pr(>r)	NMDS2	NMDS3	r2	Pr(>r)
PPT	0	0	0	1	0	0	0	1	0	0	0	1
STEMP	-0.97912	0.20331	0.0207	0.806	-0.99739	0.07215	0.0203	0.821	0.99649	-0.08367	0.0009	0.987
SMC	-0.05183	-0.99866	0.0004	0.993	-0.04559	0.99896	0.094	0.355	0.03683	0.99932	0.0936	0.35
LIGHT	-0.53878	0.84244	0.0595	0.493	-0.73123	-0.68213	0.0381	0.646	0.86892	-0.49496	0.0407	0.626
GRASS	0.0623	-0.99806	0.1779	0.155	0.22543	-0.97426	0.0498	0.586	-0.82949	-0.55853	0.2403	0.053
FORB	-0.43996	0.89802	0.0508	0.552	-0.41207	0.91115	0.0587	0.525	0.64544	0.76381	0.0777	0.417
SHRUB	0	0	0	1	0	0	0	1	0	0	0	1
ANPP	0.00223	-1	0.1371	0.229	0.14771	-0.98903	0.0253	0.777	-0.86004	-0.51023	0.175	0.112
LITTER	-0.71453	-0.69961	0.1736	0.125	-0.94631	-0.32326	0.1208	0.251	-0.85261	-0.52255	0.0591	0.54
BIOCOV	0.17772	0.98408	0.4644	**0.002**	0.2843	-0.95873	0.1027	0.331	0.92832	-0.37178	0.4786	**0.004**
LICHCOV	-0.61282	-0.79023	0.0002	0.998	0.03035	-0.99954	0.1517	0.189	-0.10862	-0.99408	0.1538	0.164
MOSSCOV	-0.86151	-0.50774	0.2244	0.083	-0.96331	-0.26839	0.1965	0.116	-0.74788	-0.66384	0.0365	0.697
RICHNESS	-0.30316	-0.95294	0.3989	**0.004**	-0.70416	-0.71004	0.07	0.467	-0.92217	-0.3868	0.3672	**0.012**
Environmental Gradient	Mattheis											
	NMDS1	NMDS2	r2	Pr(>r)	NMDS1	NMDS3	r2	Pr(>r)	NMDS2	NMDS3	r2	Pr(>r)
PPT	0	0	0	1	0	0	0	1	0	0	0	1
STEMP	-0.90559	-0.42416	0.0139	0.783	-0.97915	0.20312	0.0124	0.802	-0.93051	0.36627	0.0021	0.972
SMC	0.48897	-0.8723	0.0335	0.596	0.86499	-0.50178	0.0125	0.807	-0.95343	-0.30161	0.0248	0.64
LIGHT	0.31984	0.94747	0.1465	0.062	0.45459	0.8907	0.0645	0.31	0.83609	0.54859	0.1702	**0.042**
GRASS	-0.04681	0.9989	0.1317	0.075	-0.95593	-0.29359	0.0004	0.991	0.99991	-0.01311	0.1313	0.074
FORB	-0.89686	-0.44231	0.0741	0.239	-0.99994	-0.01085	0.0636	0.342	-0.99765	-0.06847	0.0107	0.825
SHRUB	0	0	0	1	0	0	0	1	0	0	0	1
ANPP	-0.64292	0.76593	0.0902	0.174	-0.99945	-0.03304	0.0458	0.457	0.99901	-0.04447	0.0444	0.433
LITTER	0.06385	-0.99796	0.1298	0.083	0.32168	-0.94685	0.0046	0.919	-0.98205	-0.18862	0.1329	0.078
BIOCOV	-0.79326	0.60889	0.0799	0.205	-0.44213	-0.89695	0.1823	**0.031**	0.35681	-0.93418	0.1509	0.053
LICHCOV	-0.86857	-0.49557	0.2606	**0.001**	-0.66959	-0.74273	0.357	**0.001**	-0.45686	-0.88954	0.1975	**0.012**

MOSSCOV	-0.51288	0.85846	0.2296	**0.01**	-0.84663	-0.53219	0.0958	0.158	0.93361	-0.35829	0.169	**0.033**
RICHNESS	-0.97098	-0.23916	0.2787	**0.004**	-0.74761	-0.66414	0.3839	**0.002**	-0.2668	-0.96375	0.1336	0.071
Environmental Gradient	Kinsella				Sangudo				Twin River			
	NMDS1	NMDS2	r2	Pr(>r)	NMDS1	NMDS2	r2	Pr(>r)	NMDS1	NMDS2	r2	Pr(>r)
PPT	0	0	0	1	0	0	0	1	0	0	0	1
STEMP	0.9985	0.05477	0.0417	0.469	-0.5677	-0.82323	0.0048	0.91	0.64154	0.76709	0.0038	0.946
SMC	-0.37916	0.92533	0.0241	0.64	-0.76066	-0.64915	0.0161	0.769	-0.98473	0.17407	0.0975	0.277
LIGHT	-0.84821	0.52966	0.345	**0.001**	-0.95304	-0.30285	0.0201	0.777	0.60806	0.79389	0.4211	**0.002**
GRASS	0.86742	-0.49758	0.0995	0.163	0.325	0.94571	0.0626	0.406	-0.97536	0.22061	0.0324	0.637
FORB	0.83303	0.55323	0.0323	0.56	0.99607	0.08852	0.0999	0.106	-0.57203	-0.82023	0.0686	0.406
SHRUB	0	0	0	1	-0.45723	0.88935	0.0086	0.879	0	0	0	1
ANPP	0.90747	-0.42012	0.108	0.137	0.63416	0.7732	0.0972	0.22	-0.9534	-0.30171	0.0514	0.513
LITTER	0.9833	-0.18197	0.2628	**0.005**	0.98462	0.17472	0.3321	**0.01**	-0.68071	0.73255	0.1427	0.122
BIOCOV	-0.28101	-0.9597	0.6745	**0.001**	-0.93321	0.35932	0.7334	**0.001**	0.49776	-0.86732	0.5459	**0.001**
LICHCOV	-0.90282	0.43002	0.4262	**0.001**	0	0	0	1	0.98412	0.1775	0.3026	**0.014**
MOSSCOV	-0.1413	-0.98997	0.7072	**0.001**	-0.93321	0.35932	0.7334	**0.001**	0.89337	-0.44933	0.1438	0.16
RICHNESS	-0.98355	-0.18063	0.9004	**0.001**	-0.51307	-0.85835	0.4892	**0.001**	0.99688	0.07898	0.6974	**0.001**

Table B.6.3. Pairwise permANOVA post-hoc testing for drought-year interaction effects at the Onefour site. Post-hoc testing was completed using the RVAideMemoire (Hervé, 2021) package, using Bray-Curtis distance with 10000 permutations.

	AM2016	**AM2019**	**RS2016**
AM2019	**0.0036**	-	-
RS2016	0.3931	0.0519	-
RS2019	0.4253	**0.0335**	0.8227

Appendix B.7. – Species cover changes

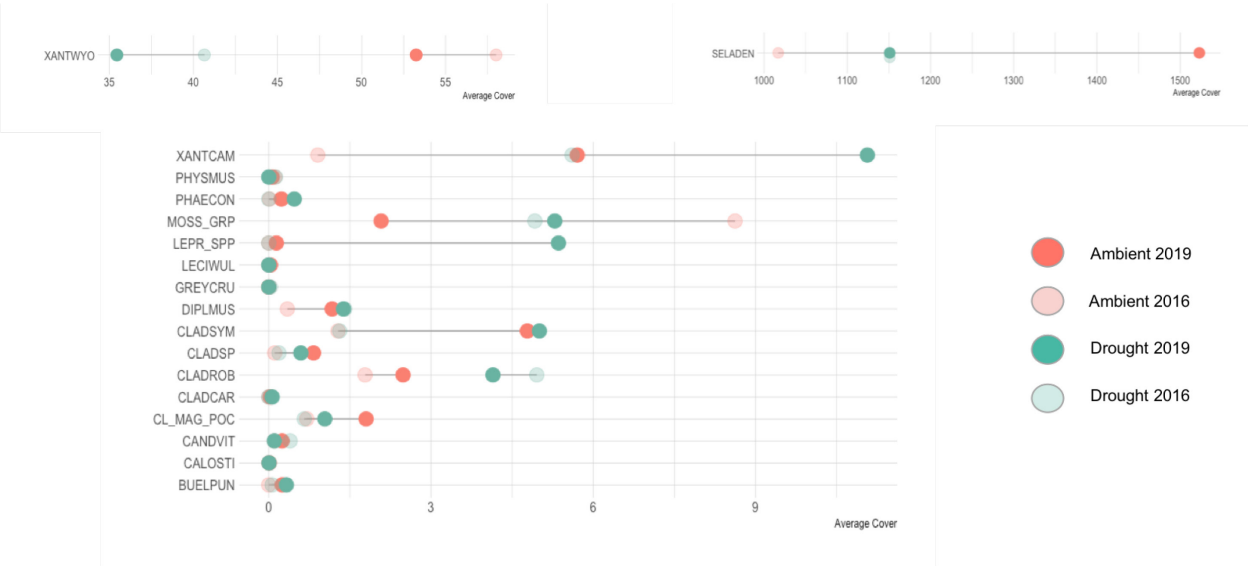


Figure B.7.1. Change in species cover of biocrust species by drought treatment from 2016 to 2019 at the Onefour study site.

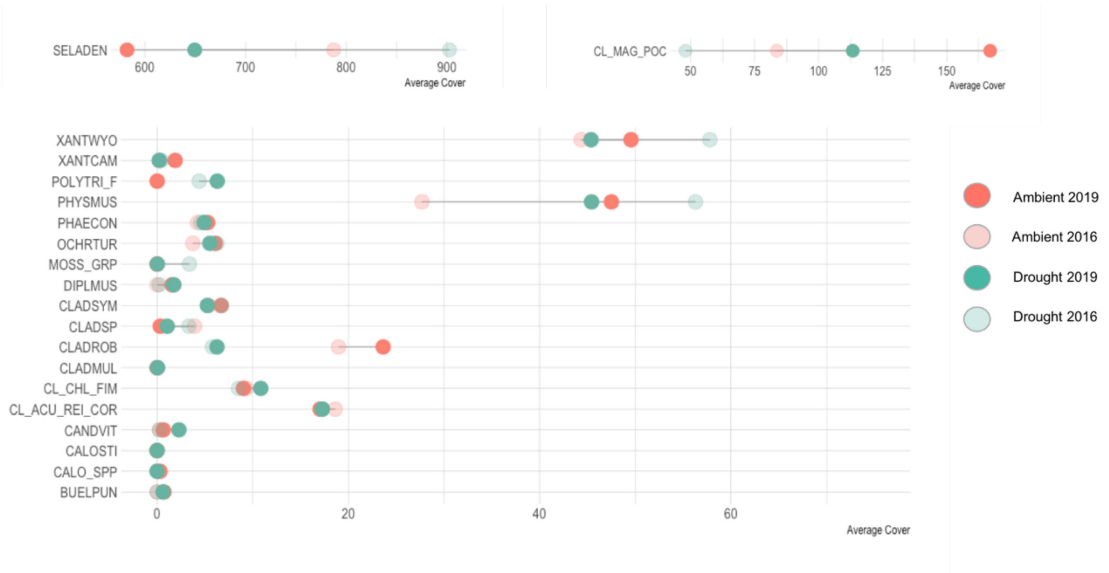


Figure B.7.2. Change in species cover of biocrust species by drought treatment from 2016 to 2019 at the Mattheis study site.

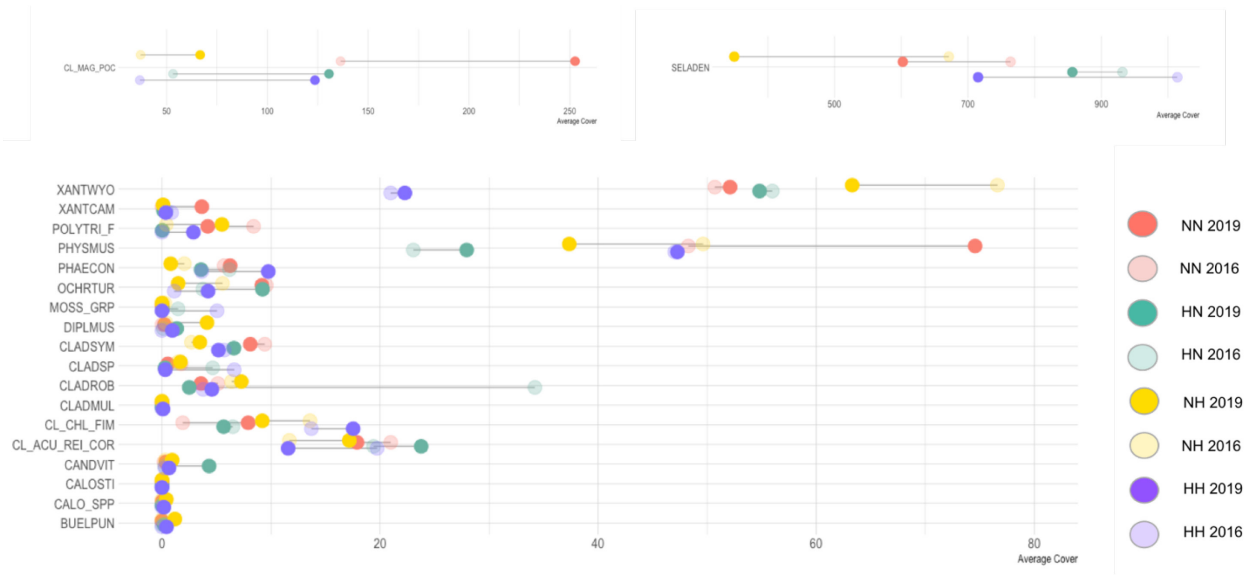


Figure B.7.3. Change in species cover of biocrust species by defoliation treatment from 2016 to 2019 at the Mattheis study site.

Appendix B.8 – Photo Plates of Study Sites

Plate B.8.1. Landscape view of the Onefour site (Photo: D. Haughland 2019).

Plate B.8.2. Onefour BSC (Photo: D. Haughland 2019).

Plate B.8.3. View of the landscape at the Mattheis Ranch site (Photo: D. Haughland 2019).

Plate B.8.4. View of BSC at the Mattheis site (Photo: D. Haughland 2019)

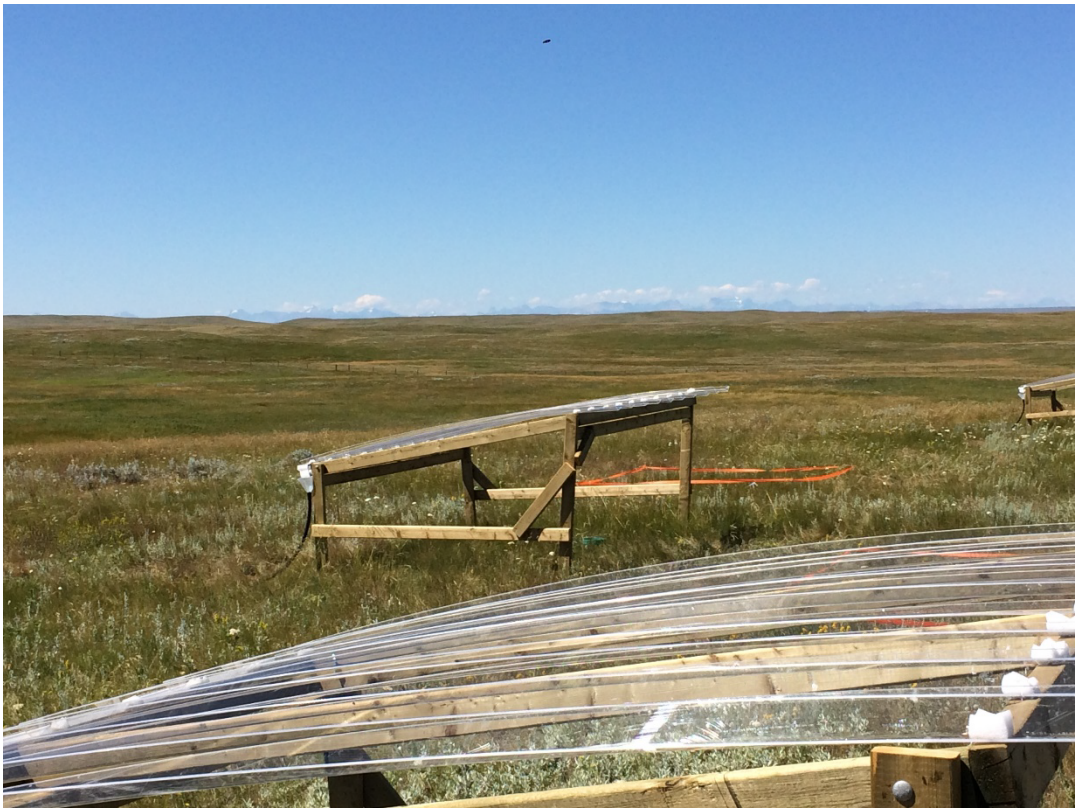


Plate B.8.5. Landscape view of the Twin River site (Photo: D. Haughland 2016).

Plate B.8.6. Landscape view of the Stavely site (Photo: B. Amgaa 2016)

Plate B.8.7. Landscape view of the Kinsella site (Photo: D. Haughland 2016).

Plate B.8.9. View of plot at the Kinsella site (Photo: D. Haughland 2016).

Plate B.8.10. Landscape view of the Sangudo research site (Photo: D. Haughland 2016).

Plate B.8.11. View of plot at the Sangudo site (Photo: D. Haughland 2016).