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A Comparison of Fruticose Ground Lichen Composition, Morphology, and Photosynthetic
Properties from Open and Closed *Pinus Rigida* Stands in the New Jersey Pine Barrens

A Thesis

Presented to the Faculty of the

Department of Biology

West Chester University

West Chester, Pennsylvania

In Partial Fulfillment of the Requirements

for

the Degree of

Master of Science

By

April H. Wallace

May 2022

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Abstract

Ground lichens colonize disturbed soils as pioneer biota, often due to plant removal, but can be replaced successionally by woody plants, like shrubs. Succession may lead to abiotic and biotic changes that impact ground lichens and facilitate their decline as ground cover through effects on morphology, photosynthetic activity, and reproduction as they are overtopped by plants. To understand how the presence of shrubs may influence the community structure of terricolous fruticose lichens in the New Jersey Pine Barrens, a field study was carried out to assess species composition and differences in microclimate abiotic variables in shrub covered and open canopy microsites. Abiotic variables did not differ significantly between microsites. *Cladonia subtenuis* (Dixie Reindeer Lichen) was found to be the most common species among all sites and was used in a second study to compare morphology, reproduction, and photosynthetic properties between sites. Dry biomass, number of apothecia, and chlorophyll content were compared between *C. subtenuis* collected from shrub covered and open canopy microsites. Results from the second study revealed a significant difference between dry biomass, number of apothecia, and chlorophyll content of *C. subtenuis* collected from adjacent microsites.

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Chapter 1. Introduction/Literature Review

Lichens

- Morphological growth forms: crustose, foliose, and fruticose

Lichens are symbiotic organisms composed of a fungal partner in association with one or more photosynthetic partners (Bhattacharyya et al., 2016). It has been estimated that there are 18,500 species of lichens (Molnára & Farkas, 2010). About 98% of fungal partners belong to the fungal group Ascomycota, with others belonging to the Basidiomycota and anamorphic fungi (Molnára & Farkas, 2010). About 21% of all fungi can act as a mycobiont and form a symbiotic relationship with a photobiont (Molnára & Farkas, 2010). About 40 genera are known to be involved with lichen as photobionts: 25 are algae and 15 are cyanobacteria. The photobiont in lichens is rarely known at the species level (Molnára & Farkas, 2010). The relationship is a mutualism (Molnára & Farkas, 2010).

Lichens are found in a wide range of habitats, from arctic to tropical ecosystems and from plains to high mountains (Molnára & Farkas, 2010). They are also found in a wide range of climates, from aquatic to xeric conditions (Molnára & Farkas, 2010). Most lichens are terrestrial, but some have been found to occur in freshwater streams or other marine intertidal zones (Molnára & Farkas, 2010).

Lichens are generally divided into three major morphological groups: crustose, foliose, and fruticose. Crustose lichens are tightly adhered to their substrate with their lower surface and cannot be removed without destruction. Crustose lichens are commonly found on tree bark (i.e., corticolous) or rock (i.e., saxicolous) surfaces, and occasionally on evergreen tree leaves in moist forests (Asplund & Wardle, 2017). In these lichens, a vegetative thallus is composed of mycobiont a cortex, medulla, and adhesion hyphae. The cortex is exposed to the surface, and

there is no lower cortex. Below the cortex is the photobiont layer, composed of either algae or cyanobacteria depending on the genus (Asplund & Wardle, 2017). The medulla lies below the photobiont layer. Hyphae adhere to and anchor lichens directly to its substrate. These lichens are very thin with low biomass (Asplund & Wardle, 2017). The form is tolerant of extreme environments, such as sun exposed rock and can experience extreme temperature variation, drastic changes in water availability, and nutrient depletion (Nash, 2010). Water loss generally occurs in the upper, exposed surface only.

The complexity of the thallus structure differs among genera of crustose lichen. Powder crust lichens of the genus *Lepraria* lack an organized thallus (Nash, 2010). In this genus, there is no distinct fungal or algal layer. Instead, fungal hyphae encircle clusters of photobiont cells (Nash, 2010). Many crustose lichens are endolithic, growing inside rock substrates. Here an upper cortex is developed and forms a dense layer called the lithocortex. Medullary hyphae may extend 2mm into sandstone in species such as *Lecidea* aff. *sarcogynoides*. This feature can contribute to the weathering of sandstone at a rate of 9.6 mm per 100 years (Nash, 2010). Crustose lichens can also contribute to the weathering of building material and historical monuments. Saxicolous lichen community distributions may be influenced by altitude, exposure, slope, nutrient enrichment from birds, geochemical rock composition, snow cover or microtopography of rock surface. Some crustose lichens can live for thousands of years (Nash, 2010).

Foliose lichens are flat and leaf-like. Lichens of this form are only partially attached to their substrate from the underside of the thallus and can be removed without destruction (Nash, 2010). Foliose lichens have distinct upper and lower cortexes, and the lower cortex is typically darker in color compared to the upper cortex. The thallus is often divided into lobes. Foliose lichens are typically lacinate and are lobate (Nash, 2010). The lobes can be radially arranged, like in

Parmelia species, or overlapping, like in *Peltigera* species. The lower cortex is covered in rhizinae, cilia or tomentum, and may anchor the thallus to the substrate (Nash, 2010). Umbilicate lichens have circular thalli with one unbranched lobe. These types of lichens are attached to their substrate through one single central umbilicus from the lower surface. This structure lacks photobiont cells and is found in groups such as *Parmeliaceae* and *Umbilicariaceae* (Nash, 2010).

The thallus of fruticose lichens is composed of hair-like, or shrubby lobes that may be flattened or cylindrical. A single cortex envelopes the entire thallus, and there is no distinct upper and lower cortex (Brodo, 2001). Fruticose lichens always stand upright from their substrate. Some fruticose lichen species have dorsiventrally arranged thalli, while others display radial symmetric thalli, such as species of *Usnea* (Nash, 2010). Branching patterns of lobes and overall size varies greatly among different groups of fruticose lichens, with some species of *Usnea* growing several meters in length. Fruticose lichens are found in many different types of climates, from desert to rainforest. They are also able to grow on many different substrates, including but not limited to sandy soils, tree bark, and rocky surfaces (Nash, 2010). Fruticose lichen thalli are often highly branched, and have a high surface area to volume ratio. This feature leads to more rapid drying and wetting patterns compared to foliose or crustose lichens (Nash, 2010).

- Cell Structure

The appearance of the lichen thallus is largely determined by the fungal partner, or mycobiont. The photosynthetic partner, or photobiont, rarely determines the appearance and habit of the thallus (Nash, 2010). The mycobiont is typically either an ascomycete or basidiomycete, however most are ascomycete. The photobiont is either a green algae or

cyanobacteria. *Treboxia* are common algae found associated with lichen, and *Nostoc* is among the common cyanobacteria found associated with lichen (Nash, 2010).

The presence of photobionts in lichens has prompted the production of new metabolites, or secondary compounds, as a result of the symbiosis (Bačkorová et al., 2010). Over 1000 unique secondary compounds have been documented in lichens. These secondary compounds can have a wide range of biological properties, including anti-bacterial, anti-fungal, anti-cancer and anti-prozoan (Ahman et al., 2017). In addition to these properties, lichen secondary compounds also have ecological and biological roles, including allelopathy, regulation of cell division of photobionts, antiherbivory, chelation of heavy metals and light screening (Bačkorová et al., 2010). Secondary compounds are secreted by the mycobiont and deposited on the surface of the thallus (Bačkorová et al., 2010). Secondary compounds generally account for 0.1-5.0% of the thallus dry weight (Bačkorová et al., 2010).

- Reproduction

The relationship between mycobionts and photobionts is obligate for the mycobiont and facultative for the photobiont. The green algae and cyanobacteria species may sometimes be found in natural habitats outside of the lichen symbiosis (Honegger, 1991). The lichen symbiosis is generally considered to be mutualistic where both partners benefit from the relationship (Honegger, 1991). The photobiont is either green algae, cyanobacteria, or in some rare cases containing both types. The intimate associations between partners can lead to co-evolution of both partners involved in the symbiosis (Steinová et al., 2019). This is often conditioned by the ecological preferences of one or both partners, and by the degree of partner specificity. Possibilities of relationships can range from generalist associations for one or both partners, to a high degree of specificity. Lichen species distribution can depend on abiotic conditions such as

substrate, light availability, habitat quality and climate. Species distribution may also be correlated with ecological specialization and physiological responses of photobionts (Steinová et al., 2019).

The mycobiont produces fruiting bodies. Once lichenized, the photobiont does not produce reproductive structures, nor do they reproduce sexually (Brodo, 2001). Algal cells reproduce asexually by sporulation, or by cell division inside lichen thallus (Nash, 2010). Nearly all lichen fungi are Ascomycetes, and produce fruiting bodies called ascomata. These can usually be produced in the form of either apothecia or perithecia (Brodo, 2001). Apothecia are disk or cup shaped fruiting structures and have an exposed spore-producing layer located on the upper surface. Perithecia are flask shaped and enclose a spore-producing layer and opening beneath a pore at the top of the structure (Brodo, 2001). Ascomata contain a layer or cluster of sac-like cells called asci. Sexual fusion of cell nuclei occurs inside of asci, and lead to the production of ascospores. At maturity, each ascus contains eight ascospores on average. However, there are instances where these asci may contain as few as one or up to several hundred. Ascospores can be diverse in shape as well as size, and they can be composed of one or many cells (Brodo, 2001).

Different methods of reproduction can offer different evolutionary advantages. Asexual propagules co-disperse both the mycobiont and the photobiont. One form of asexual reproduction is soredia. These are tiny, powdery propagules composed of fungal hyphae wrapped around photobiont cells (Brodo, 2001). Due to their small size, soredia may be able to be dispersed at much greater distances from the parent thallus. Isidia, granules and microsquamules are tiny outgrowths from the thallus that contain the photobiont enclosed in a cortex of fungal hyphae. Asexual reproduction can be advantageous in environments with low symbiont

availability. However, this mode of reproduction may lead to high co-evolutionary rates of the symbionts involved. This could also decrease genetic diversity of the partners (Brodo, 2001).

Many lichen-forming ascomycetes have the ability to reproduce sexually and disperse via ascospores. These ascospores must then relichenize after coming in contact with an appropriate photobiont. However, sexually reproducing lichen may also have the ability to reproduce asexually (Brodo, 2001). Despite the potential environmental challenges of sexual reproduction, this mode of reproduction may have advantages. Sexual reproduction increases genotypic diversity and successful long-range dispersal. Sexually reproduction may also allow for escape from old or parasitized thalli (Brodo, 2001).

- Ecology and Ecosystem Services

Lichens can play an important, and often overlooked role in many different types of ecosystems and ecological processes. More than 18,000 species of lichens have been described. These organisms are generally long-lived, slow-growing, and stress-tolerant. They are present in most terrestrial ecosystems. While lichens have low biomass contributions in many ecosystems, they can account for a large portion of the ground-layer biomass in some forest, grassland, and tundra ecosystems (Asplund & Wardle, 2017). Lichens may be abundant in nutrient-poor, dry, or cold habitats where it is difficult for vascular plants to obtain full ground cover. Lichens dominate eight percent of Earth's land surface. This is especially true in xeric high-latitude and high-elevation ecosystems (Asplund & Wardle, 2017).

There is a high degree of variability in the physical structure of lichens. This variability leads to differences in functional characteristics (Asplund & Wardle, 2017). Endolithic crustose lichens induce rock weathering over long periods of time through both physical and chemical

processes. Hyphae penetrate the substrate, and the thalli expand and contract in response to water. Crustose lichens also excrete a variety of organic acids. In contrast, foliose lichens may be loosely attached to their substrates. Lobes of foliose lichens may overlap and generate more favorable microclimate conditions for invertebrates (Asplund & Wardle, 2017). Shrubby, fruticose lichen may hang down from the canopy, such as species in *Usnea* (Asplund & Wardle, 2017).

Lichenized fungi's associations with different photobionts can also have important ecosystem-level implications (Asplund & Wardle, 2017). Lichens that have cyanobacteria as their photobiont, or cyanolichens, are commonly able to fix N_2 and typically have higher levels of N concentrations. Lichens that have green algae as their photobiont, or chlorolichens, can readily activate photosynthesis in equilibrium with high humidity. Some chlorolichens even prefer habitats with low liquid water. In contrast, cyanolichens require liquid water to activate photosynthesis. Cyanolichens are thus more abundant in rainforests and sites that experience heavy dewfall (Asplund & Wardle, 2017).

Lichen's ability to take in and retain resources may influence their effects on ecological processes. These features may include thallus nutrient concentration, secondary compounds, water-holding capacity, and specific thallus mass (Asplund & Wardle, 2017). Their varying abilities have significant ecological implications. Thin or pendulous lichens quickly take up water from humid air but have limited ability to retain moisture (Asplund & Wardle, 2017). Some foliose, cyanolichens have the ability to retain moisture for much greater lengths of time. Epiphytic lichens, those that grow on plants such as trees, also play an important role in partitioning water that has been derived from precipitation. This feature contributes to the humidity of the forest interior. Epiphytic lichen communities in habitats with low rainfall may be

important in absorbing moisture from fog. They can supply underlying soils water, and thus enhance the availability of soil moisture. This can promote tree and other plant growth in the surrounding areas (Asplund & Wardle, 2017). Fruticose hair-like lichens are especially effective at capturing dew and fog compared to other lichen forms (Asplund & Wardle, 2017).

The pigmentations of lichens vary greatly, from nearly white to black in some species. This feature can lead to large differences in thallus surface temperatures. In cold environments, lichen with dark thalli may elevate temperatures and induce snow to melt and allow them to utilize water from the melted snow. Varying colors may also lead to differences in microclimates at the soil surface (Asplund & Wardle, 2017).

More than 800 carbon-based secondary compounds have been described in lichens. Many of these are weak phenolic acid derivatives. All secondary compounds in lichens are produced by fungal partners. The majority of these secondary compounds are unique to lichens, and few are found outside of non-lichenized fungi. Secondary compounds have evolved to function as protection from different stressors, such as light damage or predation. They may also play a role in lichen ecosystem processes, and community assembly through allelopathic effects on other plants from secondary compounds, limiting establishment or growth of seedlings. Lichens may also inhibit or promote seed germination. Lichen secondary compounds may have allelopathic effects on seedling germination and may inhibit mycorrhizal growth. Thick mats may also physically prevent seeds from establishing, as seeds may not be able to penetrate the mats (Sedia & Ehrenfeld, 2003). However, lichen mats may also conserve soil moisture, thus promoting seedling germination in some instances (Sedia & Ehrenfeld, 2003). Secondary compounds typically occupy only a small amount of the thallus, generally ranging from 1-5% in thallus dry mass. In *Hypogymnia physodes*, however concentrations can be as high as 20% or

more (Asplund & Wardle, 2017). There can be great variation among and even within species, depending on environmental factors. Secondary compounds may also serve as defense against lichenivores or abiotic stressors. Secondary compounds that are restricted to the cortex, such as atranorin, parietin and usnic acid, generally protect against excessive solar radiation (Asplund & Wardle, 2017).

Lichen-dominated ecosystems obtain a large portion of their nutrients from outside the ecosystem. Lichens, lacking roots, uptake nutrients from dry and wet depositions originate largely from outside the ecosystem. Their large surface area to biomass ratio and lack of cuticles and stomata allows for this (Asplund & Wardle, 2017). Dew and fog contain more nutrients than rain, and fruticose lichen are more effective at capturing dew and fog from the environment. Fruticose lichens are generally better than other forms at capturing nutrients due to their large surface area. However, foliose lichens are usually richer in elements such as nitrogen (N), phosphorous (P), and calcium (Ca) than fruticose lichens. A large portion of the total nutrients present in an ecosystem can reside in lichens. In *Picea mariana* woodland in northern Québec, terricolous lichen may cover 97% of the ground in some areas. In these mats, 25% of N and 12% of P in the ecosystem resided within the lichens. Lichen mats in this ecosystem account for just 20% of the total biomass (Asplund & Wardle, 2017).

Epiphytic lichens are important in the forest water-cycle as well as the forest nutrient cycle. In temperate oak savannah, lichen epiphytes increase canopy interception of precipitation by 50% (Ellis, 2012). Epiphytic cyanolichens may also sequester nitrogen from atmospheric sources. Sequestered nutrients can become available to the forest system as the lichen epiphytes litterfall, decompose, or as consumed by animals (Asplund & Wardle, 2017).

Roughly 10% of lichen species contain N₂-fixing cyanobacteria. These organisms can greatly increase the N inputs to the ecosystem, especially in nutrient poor ecosystems that lichens commonly reside. *Lobaria oregana* can contribute up to 50% of the total ecosystem N input in *Pseudotsuga menzeisii* forests in Oregon (Asplund & Wardle, 2017). Lichens from the genera *Peltigera*, *Stereocaulon* and *Nephroma* have been reported to be major contributors of nitrogen fixation in subarctic and arctic regions (Crittenden, 2000).

Lichens can also be important in forest food-webs. Birds often use lichens to construct nests. Some animals rely on lichen as their food source, such as caribou or lichenivorous insects. Caribou often consume lichen from the genera *Cladonia*, *Bryoria*, *Alectoria* and *Stereocaulon* (Asplund & Wardle, 2017). Terricolous lichen typically account for 60% of winter food intake by reindeer and caribou (Crittenden, 2000). Lichen consumption by these animals greatly declines in the summer months when other food sources become available (Crittenden, 2000). There are also insects that use lichen for camouflage. Symbiotic relationships between invertebrates and lichens have been observed. A positive relationship has been found between lichen biomass and the abundance of invertebrates (Ellis, 2012). Lichens provide a habitat for many invertebrates (Asplund & Wardle, 2017). Many crustose lichens are consumed by invertebrates. These organisms can include gastropods, mites, beetles, moth larvae, woodlice and springtails.

Lichens are often used as bioindicators and biomonitors since many are sensitive to air pollution and can accumulate pollutants (Crittenden, 2000). Mat-forming lichens can be particularly useful as indicators as they usually grow in open habitats where they intercept atmospheric deposition directly. They may also be partially isolated from chemical conditions of the soil below the mats due to underlying layers of dead thallus. Mat-forming lichens are also

efficient at scavenging inorganic N from precipitation (Crittenden, 2000). The impact of acid rain has been of special concern in northern latitudes for many decades as mat-forming lichens have been found to be sensitive to nitrates and acid deposition (Crittenden, 2000). *Cladonia portentosa*, for example, has been used in British heathlands as a model indicator based since the ratio of K^+ to Mg^{2+} in the thallus correlates with H^+ concentrations from precipitation and thallus N concentrations relates to total N deposition (Crittenden, 2000).

- Secondary Compounds

Roughly 1050 secondary compounds have been identified in lichens to date (Molnára & Farkas, 2010). This number includes substances identified from cultures (Molnára & Farkas, 2010). These secondary compounds display a wide variety of biological activities, including antiherbivory, protection of photobiont from excess light, antimicrobial activity, and resistance to microbial infection in lichens (Asplund & Wardle, 2013) (Bhattacharyya et al., 2016). These compounds are often aliphatic or aromatic with a low molecular weight (Bhattacharyya et al., 2016). Secondary compounds are produced by the mycobiont and accumulate in the cortex or the medulla layer in the form of extracellular crystals (Bhattacharyya et al., 2016). The location of these products within the thallus is correlated with the different functions of the compounds (Molnára & Farkas, 2010). For example, it has been reported that foliose lichen, including *Hypogymnia physodes*, *Vulpicida pinastri*, and *Xanthoria parietina* display higher concentrations of secondary compounds in sexual reproductive structures, as well as in asexual reproductive structures in *Hypogymnia physodes* and *Vulpicida pinastri* when compared to vegetative portions (Molnára & Farkas, 2010). Light-screening secondary compounds such as parietin, usnic acid

and vulpinic acid located in the upper cortex increase its opacity and protect the underlying photobiont from intense radiation (Molnára & Farkas, 2010).

Secondary compounds are not required for the survival and growth of lichens; however, their presence may have advantages. The function of these compounds in the lichen symbiosis remains poorly understood (Molnára & Farkas, 2010). They may offer advantages, such as protection from herbivory, pathogens and protection from high UV irradiation (Molnára & Farkas, 2010). Light-screening pigments include parietin, usnic acid, and vulpinic acid. These compounds absorb incident light and protect the photobiont from damage from excessive light (Molnára & Farkas, 2010). UV-B light may inhibit photosynthesis and damage DNA. Compounds including atranorin, calycin, pinastric acid, pulvinic acid, rhizocarpic acid, usnic acid, and vulpinic acid have been found to have strong UV absorption abilities and could filter excessive UV-B irradiation (Molnára & Farkas, 2010).

Secondary compounds in the thallus may function as a chemical defense. Usnic acid is a yellow compound and is found in a large number of lichen genera, including *Cladonia*. Additionally, usnic acid is one of the more potent secondary compounds in terms of antimicrobial activity (Crittenden, 2000). Most lichen secondary compounds are only slightly soluble in water. Trace quantities may be released into the soil from thalli during rainfall, or from decomposing thalli. These trace amounts may inhibit the growth of surrounding vascular plants (Crittenden, 2000). Species of *Cladonia* have been found to impede growth and development of mycorrhizae in boreal forest trees (Crittenden, 2000).

Lichen secondary compounds may have allelopathic effects on surrounding organisms. They could affect development or growth of other lichens, vascular plants, or mosses (Molnára & Farkas, 2010). Lichen compounds released into the surrounding environment have potential to

disrupt photosynthesis, respiration, transpiration, protein and nucleic acid synthesis, and permeability of other organisms (Molnára & Farkas, 2010). Secondary compounds may be involved in competition between different lichen species competing for space and light (Molnára & Farkas, 2010). Compounds such as vulpinic and evernic acids have been found to severely inhibit ascospore germination of crustose lichens, *Graphis scripta* and *Caloplaca citrina* (Molnára & Farkas, 2010). Mosses often occur together with lichens on rocks, trees, and soils. The presence of lichen compounds may impact competition interactions within cryptogam communities (Molnára & Farkas, 2010).

Ground lichens

- Gross Morphology

The podetium in fruticose *Cladonia* species is an erect, lichenized portion of the thallus. In some species of this genus, the podetia terminate in a closed, cuplike structure called a scyphus. The podetia could also terminate in scyphus-like openings. The podetium in *Cladonia* is considered to be a reproductive structure (Hammer, 1995).

The podetia of *Cladonia* is made up of several layers of fungal tissue, which are directly or indirectly derived from meristematic tissue. There are three types of tissue that podetial development can be described as (Hammer, 1995). The first is primary tissue. This type of tissue is exclusively made up of fungal tissue. A second type is called the inner medullary layer. This layer is also entirely comprised of fungal tissue. The third and final type is called the outer medullary layer, which is composed of both fungal tissue and algal cells. Synchronized development of these three types of tissue is responsible for the macroscopic feature of the lichen thallus (Hammer, 1995).

The genus *Cladonia* is a group of lichenized fungi that is distributed world-wide and is one of the most species-rich and morphologically distinctive genera of lichenized fungi (Steinová et al., 2019). Over 400 species have been described. *Cladonia* species are also known to have a high degree of specificity towards the green algal photobiont genus *Asterochloris* (Steinová et al., 2019).

Terricolous fruticose lichens have a thallus that is erect without a clearly distinguishable upper and lower surface. Lichens of this growth form can be highly branched and shrubby. A single cortex envelopes the entire thallus, and the photobiont layer lies just below the cortex. Below this layer lies the medulla, which can have several different forms depending on the genera. In species of *Cladonia* and *Thamnolia*, the central core area is hollow, whereas in species of *Evernia* and *Stereocaulon*, the central core is composed of loosely or densely packed fungal hyphae (Brodo, 2001). *Cladonia* have maculae, or sections where the photobiont layer is visible through the cortex. This feature is common among genera of stratified foliose and fruticose lichens (Brodo, 2001).

Cladonia subtenuis is commonly found in pitch pine-scrub oak communities. It tolerates a wide range of ecological conditions, and is known to establish on organic substrate including dead leaves, wood, and twigs. This species generally reproduces asexually through thallus fragmentation (Hammer, 1997).

- Cell structure

The thallus grows acropetally and vertically upwards in mat-forming fruticose lichen. In mature mats, the thallus base dies, leaving the upper living parts of the mats to be supported by a deep layer of dead, structurally intact thallus (Crittenden, 2000).

It is believed that these types of lichen receive most of their N and P from precipitation and dry deposition from dust particles and gases (Crittenden, 2000). In thick mats, areas of depletion may develop in lower levels of the mat due to interception of light and nutrients from the surface layer (Crittenden, 2000). Thus, sections of thallus below the light compensation depth create a respiratory burden and are expendable. This has been noted in *Cladonia stellaris* in the northern hemisphere (Crittenden, 2000). It has been suggested that N and P from senescent basal portions of the thallus may be translocated to the living, growing apices (Crittenden, 2000). This would be advantageous, as it would allow for a higher growth rate at the apices that could be supported by atmospheric supplies alone.

- Photosynthetic physiology

Lichens are poikilohydric organisms, meaning that they have no way to prevent desiccation. Lichens are subject to desiccation given that they are unable to regulate their water intake and are at the mercy of their environment. These organisms can use utilize dew, rain, humidity, fog, and melting snow and frost as water sources to power photosynthesis. Lichens can survive long periods of desiccation and resume active metabolism almost immediately after water becomes available (Cho et al., 2020). Their poikilohydric nature allows lichen to survive in desiccated states for long periods of time. Photosynthesis resumes in a few minutes upon rehydration. This feature allows for lichens to survive in extreme environments, such as deserts or the Arctic. These types of organisms display rapid repair after desiccation, restoring respiration and photosynthesis rapidly after rehydration (Cho et al., 2020).

Reactive oxygen species (ROS) are formed in the chloroplasts due to desiccation-rehydration cycles. ROS species that may form include superoxide ($O_2^{\bullet-}$), the hydroxyl radical ($\bullet OH$), singlet oxygen (1O_2), or hydrogen peroxide (H_2O_2) (Kranner et al., 2008). These can be

damaging to the tissue, so photobionts must develop some form of photoprotection (Cho et al., 2020). Thermal dissipation, known as non-photochemical quenching, during desiccation is one mode of protection employed by these organisms to protect from damage. During thermal dissipation, excess light energy is dissipated as heat. Heat dissipation occurs faster than light is utilized to power photosynthesis. This can prevent the formation of ROS. Different carotenoids and the reversible xanthophyll cycle are used to perform thermal dissipation (Cho et al., 2020). Heat dissipation can be regulated by solar radiation and extent of desiccation. This may be an adaptation to the environment; variation in heat dissipation has been observed in different lichen species (Cho et al., 2020).

Usnic acid, a phenol, has been observed to increase linearly in the thallus of *Cladonia subtenuis* with increasing sunlight exposure. This accumulation of phenol reduces the opacity of the outer medullary layer, resulting in a lower amount of radiation reaching the algal layer in these lichens. Shade populations then display higher levels of photosynthetic assimilation. More radiation is able to penetrate to the algal layer in shade populations. Some species of *Cladonia*, such as *C. verticillaris*, show color differences between shade and sun populations. Sun populations appear brown in color, while shade populations appear green. Shrubs filter out a larger portion of incoming radiation. This phenomenon could indicate that phenols can accumulate in the thallus in response to high levels of radiation (Legaz et al., 1978).

Chlorophylls may also be photo-oxidized if not protected by these phenols (Legaz et al., 1978). Green algae, the dominant photobiont in lichens, contain several different pigments. These include chlorophyll *a* and *b*, as well as carotenoids and xanthophylls. Chlorophyll *a* is the primary pigment of photosynthesis, while chlorophyll *b* functions to expand the absorptions spectrum of photosynthetic organisms. Carotenoids protect chlorophyll from photodamage, and

xanthophylls absorb blue light for use in photosynthesis (Voytsekhovich & Kashevarov, 2010). In a study done by Legaz et al., in 1978, it was found that there were more chlorophylls and carotenes in shade population of *C. subtenius* than in sun populations. Phenol production appears to be greatest in younger portions of the lichen thalli, such as the apical zones. These areas experience the highest amount of metabolic activity. The production of these compounds may be related to CO₂ fixation, as these areas also display the highest amounts of CO₂ fixation (Legaz et al., 1978). Legaz found that shaded populations may synthesize more (or have less destroyed) chlorophyll in younger portions of the thallus. This may allow for the lichens to maintain a high efficiency in low light conditions. A larger number of carotenoids were also found in shade populations. This may enhance photosynthetic ability. These features may allow for the lichens to maximize use of low light conditions (Legaz et al., 1978).

Lichens often reside in environments that have harsh conditions, such as mountain tops or polar regions. They must have mechanisms that allow for them to survive such unfavorable conditions. Lichens must possess the ability to tolerate high light during active metabolism as well as when desiccated (Beckett et al., 2021). Exposure to prior stress generally increases stress tolerance in lichens. Lichens may acclimate to their environments through phenotypic plasticity. These organisms display plasticity in their response to high light stress; there appears to be seasonal variation in photosynthetic capacity (Beckett et al., 2021). Lichens also show sun and shade forms, and lichens from shade populations often display lower light saturation and compensation points than those exposed to high light environments. Shade populations may also have less cortical pigments. There may be less obvious differences in photosynthetic capacity and chlorophyll content between sun and shade lichen forms. Pigments

in the fungal upper cortex may provide lichen photobionts with stronger light screening than what is observed in plant leaves (Beckett et al., 2021).

The photobiont is protected from high light by screening in the upper cortex of the thalli. Transmittance is reduced in the desiccated state. Secondary compounds located in the cortex can absorb UV radiation and some also absorb visible radiation. Many however do not absorb visible light. Secondary compounds often exist as crystals outside the fungal hyphae. While secondary compounds protect from visible light, they may however reduce photosynthetic efficiency (Beckett et al., 2021).

- Reproduction

In the genus *Cladonia*, both asexual and sexual reproducing taxa have been observed. In some species, both vegetative propagules and apothecia are present on the same thallus. Different methods of reproduction and dispersal can have varying evolutionary advantages and disadvantages. Asexual propagules or thallus fragments contain clonal diaspores. In this method of reproduction, both the mycobiont and its algal cells are co-dispersed (Steinová et al., 2019). Soredia are tiny, powdery propagules that are composed of fungal hyphae wrapped around photobiont cells. These structures are able to detach easily from the thallus and may travel greater distances from the parent thallus (Steinová et al., 2019). Isidia, granules, and microsquamules also contain fungal hyphae wrapped around photobiont cells. These structures are larger outgrowths of the thallus (Steinová et al., 2019).

- Ecology of Fruticose Lichens

Terricolous, mat-forming lichen are important in many ecosystems. This growth form is uncommon in lichens, making up less than 0.5% of 13,500 known species. They are often found

in ecosystems with at least well-drained, oligotrophic sandy or podzolic soils (Crittenden, 2000). These organisms grow on substrata that are deficient in nitrogen and phosphorous (Crittenden, 2000). Species of the genera *Cladonia*, *Cetraria*, *Stereocalauon*, and *Alectoria* are among the most ecologically successful mat-forming lichen. Species from these genera can form extensive ground cover in subarctic lichen woodland, forest tundra and tundra heathlands (Crittenden, 2000).

The development of mat-forming lichens appears restricted to well-drained surfaces, and areas in which vascular plant growth is limited due to poor soil fertility or are at early successional stages following a fire (Crittenden, 2000). Precipitation does not seem to be a factor that influences the development of lichen mats in an area, and mat-forming lichens are found in a wide range of areas that have vastly different annual precipitations (Crittenden, 2000). However, the importance of precipitation may vary between species.

- NJ Pine Barrens Pitch pine plant and ground lichen communities

The New Jersey pine barrens are an ecoregion dominated by pitch-pine (*Pinus rigida*) as a result of frequent fires (Moul & Buell, 1955). The New Jersey Pine Barrens are characterized as an Atlantic Coastal Pine Barrens ecosystem. The Atlantic coastal pine barrens ecoregion spans over roughly 16,000 square kilometer as of 2012, located primarily in southern New Jersey, as well as sections of New York and Massachusetts (Sohl & Sohl, 2012). This ecosystem comprises several unique habitats, including pitch pine and shrub oak, cedar swamps, coastal plain salt ponds, as well as maritime grasslands (Sohl & Sohl, 2012). Upland areas contain mostly pine trees of the species *Pinus rigida*, as well as several different species of oak including *Quercus velutina*, *Q. alba*, *Q. ilicifolia*, *Q. rubra*, and *Q. marilandica*. Pine and oak

trees can vary in abundance and dominance in these areas depending on history burning (Lendemer, 2006). Swamp forests are dominated by Atlantic white cedar (*Chamaecyparis thyoides*) and red maple (*Acer rubrum*) (Lendemer, 2006). Rainfall averages roughly 122 centimeters per year (Sohl & Sohl, 2012) Soil of the pine barrens is sandy, highly porous and drains quickly (Sohl & Sohl, 2012). The soils generally remain dry, with the exception of where the Cohansey-Kirwood Aquifer meets the surface, leading to bogs and swamps (Sohl & Sohl, 2012). Topography and soil moisture availability define the Pine Barren community types (Matlack et al., 1993). Much of the Pine Barrens lie below 30 m above sea level (Ledig et al., 2013).

Fire is a major source of natural disturbance and greatly influences the composition of vegetation in this ecoregion (Sohl & Sohl, 2012). In the absence of fire or other disturbance, vegetation would likely transition from pine dominated to oak and other hardwoods (Sohl, 2012). The fire return interval in the Pine Barrens is 16 to 26 years (Ledig et al., 2013). These forests may have stands of all ages due to fires that occur in the region (Buell & Cantlon, 1950). Species that have the ability to survive after fire have advantage in this environment. Pitch-pine is the dominate pine in this ecoregion, as it able to regenerate quickly after fire. Pines are favored in this environment because they are less susceptible to fire damage than broad-leaf trees and the mineral soil after burning is more favorable to the establishment of pine seedlings (Moul & Buell, 1955). Shrubs that are present in this environment are also able to sprout readily from the base after fire (Moul & Buell, 1955). The herbaceous layer, consisting of lichens, liverworts and mosses increases in cover as a result of fire (Moul & Buell, 1955). Bracken fern is another common species in this ecoregion due to its ability to regenerate from root stocks (Sohl & Sohl, 2012).

The Pine Barrens support a wide distribution of ericaceous shrubs in the majority of its biological communities. Twenty-three species of Ericaceae have been identified as native to the Pine Barrens, many of which belong to the Vacciniodeae subfamily (Matlack et al., 1993). Within sites that these shrubs are present, ericaceous shrubs frequently form dense subcanopies (Matlack et al., 1993). *Gaylussacia frondosa* is found to be dominant among lowland shrub communities (Ehrenfeld, 1986). *Gaylussacia* species are found to produce berries, have broad, deciduous leaves, and recover rapidly after fire (Matlack et al., 1993).

While no information was able to be found on the lichen species present at Brendan T. Byrne State Forest, the lichen flora of Wharton State Forest was surveyed by Lendemer in 2006 and found 190 species present (Lendemer, 2006). Wharton State Forest serves as a good representation of the New Jersey Pine Barrens, as it contains nearly all habitat and vegetation types that are characteristic of the pine barrens, similar to Brendan T Byrne State Forest (Lendemer, 2006). This area encompasses roughly 110,000 acres and is the largest state forest of New Jersey (Lendemer, 2006). Within the pine barrens, there are several smaller subtypes with lichen flora that differ due to elevation, proximity to water, understory composition, and dominant canopy species (Lendemer, 2006).

The New Jersey Pine Barrens have a long history of human use and habitation (Scheller et al., 2008). Parts of this ecoregion contain some the most well-preserved habitats in the Eastern United States. However, other sections are among the most developed lands in the country (Sohl & Sohl, 2012). After European settlement, upland forests were logged for fuel wood in iron and glass production and were harvested for timber (Scheller et al., 2008). The pine barrens experienced large wildfires in the nineteenth century due to logging and an increase in human population density (Scheller et al., 2008). Heavy rural development occurred in the twentieth

century around the periphery of the pine barrens (Scheller et al., 2008). Many of these areas regenerated naturally, and later became state forests.

In modern day, the Pine Barrens have been fragmented by agriculture, development, and roads (Scheller et al., 2008). Poor soils in this ecoregion restrict many agricultural activities. Blueberries and cranberries are often the only crops grown in this region. However, parts of the ecoregion have richer soils and are used to support fruit, vegetables, as well as other crops (Sohl & Sohl, 2012).

Human activity has impacted modern day fire regimes in these areas. Development in ecoregion has led to fire suppression (Sohl & Sohl, 2012). This has led to a change in the composition of many of the ecoregion's plant communities (Sohl & Sohl, 2012). Prolonged fire suppression leads to the replacement of pine barrens by oak forest (Sohl & Sohl, 2012). Prescribed burning has been introduced as a management practice. As a result, many fires in the region have been relatively cool, and spread at ground-level instead of reaching the tree canopy (Matlack et al., 1993).

- Purpose & Goals

The purpose of this thesis project is to compare composition and morphology of ground lichens in adjacent open canopy and shrub dominated sites located in *Pinus rigida* dominated forest stands at the Brendan Byrne State Forest in the NJ Pine Barrens. Ground lichens colonize disturbed soils as pioneer biota, often due to plant removal, but can be replaced successional by woody plants, like shrubs. Succession usually leads to abiotic and biotic changes that impact ground lichens and facilitate their decline in ground cover through effects on morphology, photosynthetic activity, and reproduction as they are overtopped by plants. By comparing the composition, community structure, morphology, and photosynthetic properties of a dominant

ground lichen found in open sun sites adjacent to shaded shrub dominated sites, the study could answer questions related to how successional change affects these rare and important lichen communities in the Pine Barrens.

To address these questions, several goals will be central to this study. Ground lichen species composition and community structure will be compared between open canopy and shrub dominated sites. In this study, composition refers to the collection of ground lichen species present and prevalence of growth (crustose, foliose, or fruticose) and reproductive (apothecial, isidial, or soredial) forms. Community structure refers to species richness and ground cover, measures of diversity and abundance, respectively. Gross morphology of the dominant ground lichen species will be compared between open canopy and shrub covered sites. Gross morphology will include thallus biomass dimensions as well as presence and amounts of reproductive structures present. Photosynthetic properties of the dominant species will be compared between open canopy and shrub covered sites. Photosynthetic properties refer to chlorophyll content.

Here, *Cladonia subtenuis*, a fruticose terricolous macrolichen belonging to the *Cladonia* genus, was used to assess the effects of microclimate on photosynthetic properties, reproduction, and phenotypic plasticity in relation to successional changes in the New Jersey Pine Barrens.

Chapter 2. Study Area & Experimental Design

- Brendan T. Byrne State Forest

Brendan T. Byrne State Forest was selected as a study site for this thesis project. This forest lies within the Pine Barrens ecoregion (Kelly et al., 2022) and is composed of roughly 38,000 acres of *Pinus rigida* dominated woodlands with some meadow and wetland communities. The Pine Barrens are characterized by nutrient-poor, acidic soil. Frequent fires play a large role in the forests' ecology, especially by maintaining the composition of dominant vegetation (Sohl & Sohl, 2012).

Previously known as Lebanon State Forest, Brendan Byrne is a popular location for many outdoor activities, including hiking, camping, mountain biking, fishing, and hunting. The state Forest has more than 25 miles of marked trails snaking through the forest, many of which are flanked by ground lichen communities like those studied in this thesis project. (Figure 1)

- Experimental Design

Seven miles of the Mt. Misery and Cranberry trails in Brenden T Byrne State Forest were searched in order to place study plots in areas that contained exposed lichen mats adjacent to lichen mats covered by a shrub layer. (Figure 2) At least 50% of a plot must have been covered by shrubs to be considered a shrub covered site. Plots selected were dominated by *Pinus rigida* in the overstory and huckleberry (*Gaylussacia* species) shrubs in the understory. All other woody and herbaceous species were noted in each site. *Quercus marilandica*, *Quercus rubra*, *Nyssa sylvatica*, bayberry (*Morella* species), and blueberry (*Vaccinium* species) shrubs were also present within sites (Table 1). Fifteen study plots (1m x 1m) were placed within *Pinus rigida* stands along 7 miles of the Mt. Misery and Cranberry trails in Brenden T Byrne State Forest. To

prevent pseudoreplication, plots were placed no closer than 50 m from another plot. This distance was chosen because thallus fragments generally travel less than 25cm (Heinken, 1999).

Transects were run through the largest continuous section of shrub covered and open lichen mats in each site. One square meter plots were then randomly selected from the transect using a random number generator. In plots with little area of open and shrub covered lichen mats, 1 m² plots were placed non-randomly. A total of 30 microsites were established: 15 in shrub cover sites and 15 in exposed sites.

Lichen species were surveyed, collected in brown paper bags, and brought back to the lab for identification using methods described in Brodo 2016 prior to performing the field study. It was concluded that the dominant species in each site was *Cladonia subtenuis*. This species was then collected from each site for further studies.



Figure 1: Trail map of Brenden T Byrne State Forest. Field sites were established along portions of the red and white trails.



Figure 2: A field site showing open canopy and shrub covered lichen mats.

Chapter 3. Studies

Study 1: Comparison of abiotic factors

An initial study was conducted to assess abiotic variables between shrub covered and exposed microsites. In this study, light, humidity, temperature, and canopy cover were assessed in all sites in both shrub covered and exposed conditions. It was predicted that light availability will be greater in exposed microsites compared to shrub covered sites. Humidity is predicted to be greater in shrub covered microsites compared to exposed microsites. Temperature is expected to be greater in exposed microsites compared to shrub covered microsites. It has also been predicted that canopy cover will be greater in shrub covered compared to exposed microsites.

- **Methods**

Abiotic variables, including temperature, light level, canopy cover, and humidity were measured once for each microsite. Measurements were taken 1 meter above lichen mats in open microsites, and midway in the shrub layer of shrub covered microsites. Canopy cover was calculated using a densiometer in both open and shrub covered microsites by taking measurements at each cardinal side of the microsite. Light readings were obtained using an Extech Light Meter. Readings were taken when clouds were not directly covering the sun. Temperature and humidity were obtained using an Extech Hygro-Thermometer with Infrared Thermometer. Data were collected on September 11th and September 18th between 10am and 6pm.

- **Statistical tests of hypotheses**

Data were analyzed using paired T-tests for light and canopy cover. Canopy cover data were transformed using a square root transformation when normal distribution of residuals could not be met. Several outliers were recognized in the light data and were removed before performing a

paired T-test. Seven total outliers were removed from these data; four from the open canopy and three from the shrub covered. A paired T-test was then performed using unequal group sizes. Temperature and humidity data were assessed using Nonparametric Sign or Wilcoxon tests, respectively, in place of paired T-tests when transformations could not yield normal distribution for residuals. All statistical tests were performed using Statistical Package for Social Sciences (SPSS).

- Results

Light differed significantly between open and covered microsites ($P=0.011$) (Figure 5). Light levels were significantly greater in open microsites compared to shrub covered microsites. However, no significant differences were found between temperature, humidity, or canopy cover in open vs shrub covered microsites ($P > 0.05$) (Figure 4, 6, 7) (Table 3).

- Discussion

Light was the only abiotic variable found to differ significantly between open and shrub covered microsites. These findings were predicted and unsurprising. All other abiotic variables, including temperature, humidity, or canopy cover did not differ significantly between exposed and shrub covered microsites in the pine barrens. The presence of shrub cover did not influence temperature or humidity in the herbaceous layer in the pine barrens. Canopy cover provided by *Pinus rigida* also did not differ significantly in shrub covered or exposed microsites. These findings were opposite of what was predicted. Field sites may not be as different in their abiotic variables as what had been predicted.

Microsites surveyed in this study were located very close to one another, in that shrub covered and open canopy microsites were selected from the same study sites. Additionally, the criteria outlined in this study for a microsite to be considered 'covered', it must have 50% shrub cover or greater. Shrub covered areas that were accessible and had lichen mats growing beneath them did not appear as densely covered as much of the surrounding forest. The close proximity of the shrub covered microsites to the open canopy may have accounted for the lack of significant differences in temperature and humidity. In addition, the pine barrens of New Jersey are described as highly disturbed forest (Collins & Good, 1987). Terricolous lichen in this region appear to colonize disturbed areas. The areas that were surveyed in this study may be somewhat recently disturbed, and shrubs and pine trees in these sites may not have had enough time to fully cover these disturbed sites. Sites that were selected may have been relatively young, which may be deduced based on the fact that these areas appeared more open than the surrounding area in the forest. Sites were located adjacent to hiking trails, possible service roads or had been used for camping. Old campfire remains and tire tracks were observed near several sites. The shrub layer in these sites may not provide as much humidity or temperature differences to the herbaceous layer beneath as what had been originally predicted.

Light levels differed significantly between open canopy and shrub covered microsites. These findings were predicted. The shrub layer provided much more shade to the lichen mats beneath than what was found in the open canopy sites. The presence of the shrub layer significantly influenced the amount of light that reaches lichen mats in shrub covered and open microsites.

Edge effects from the adjacent open canopy area may have influenced the lack of differences found in the temperature and humidity in the shrub covered microsites. While there was no significant difference between temperature, humidity, or canopy cover, this experiment had limitations that may have influenced these results. All abiotic measurements were only recorded once, roughly in the middle of the day, during this study. Equipment that monitors these variables constantly were not available for use in this project.

Study 2. Comparison of ground lichen composition and community structure

An additional study was conducted to assess differences in ground lichen species composition and community structure between shrub covered and exposed microsites. This study was conducted to assess the effects of successional changes on species presence and biotic variables related to the most dominant terricolous lichen species, *Cladonia subtenuis*.

Ground lichen species richness is predicted to be greater in open canopy compared to shrub dominated microsites. I also predict that ground cover by all species will be greater in open canopy compared to shrub dominated microsites. *Cladonia subtenuis* height is predicted to be taller in shrub covered microsites.

- Methods

Prior to establishing field sites, roughly seven miles of the trails in Brenden T. Byrne State Forest were searched to assess terricolous lichen species that may be present in the area. Areas that were searched included sections of the Mt. Misery Trail (white) and the Cranberry Trail (red) (Figure 1). Vouchers of all terricolous species present were collected in brown paper bags and returned to the lab for identification. Chemical testing, microscopy, and reference guide

Brodo, 2016 were used to identify all species. Identification in the field was then able to be conducted based off knowledge gained from this preliminary work.

Field plots were established using methods described previously. A 1m x 1m square of PVC pipe was laid overtop the ground and all species within the square and all species in each microsite were identified visually based on prior identification. Vouchers of newly found species were collected to identify in the lab using methods described previously. Total ground cover for all species was visually estimated to the nearest %, and ground cover for each species present was estimated in the same way. Ground cover was visually estimated for all lichen species that covered 1% or more to the nearest whole number in each microsite.

Lichen height in microsities were measured by randomly selecting *Cladonia subtenuis* in each of four quadrants within the one m² microsities by measuring from the highest point in the randomly selected cluster. Four measurements were taken in each microsite, and only three were taken in microsities with low ground cover. The average of the three or four measurements were calculated for each microsite and used for statistical analysis.

- Statistical tests of hypotheses

Paired T-tests were used to assess differences between average *C. subtenuis* height, species richness and ground cover of open canopy and shrub dominated microsities. A square root transformation was used on the species richness data. Statistical tests were performed using SPSS.

- Results

It had been predicted that there would be greater species richness in the open canopy microsities. However, no significant difference was found. ($P = 0.184$) (Figure 10) It had also

been predicted that there would be greater total ground cover in the open canopy microsites. However, no significant difference was found. ($P = 0.29$) (Figure 9) *Cladonia subtenuis* height was predicted to be greater in shrub covered microsites, but no significant differences were found. ($P=0.081$) (Figure 8)

Cladonia subtenuis was found to be present and dominate in all 15 covered and open microsites. *Cladonia uncialis* (thorn cup lichen) was found to be the second most common species and was present in 9 covered sites and 14 open microsites. *Cladonia chlorophaea* (mealy pixie cup) was present in 4 covered sites and 7 open microsites. *Cladonia boryi* (fishnet lichen) was uncommon and was found in only 2 covered and open microsites. *Cladonia squamosa* (dragon cup lichen) was identified in 4 covered sites and 3 open microsites. *Cladonia cristatella* (British soldier lichens) was only found in 1 open microsite, and not found in any covered microsites. (Table 2)

- Discussion

Cladonia subtenuis height, lichen species richness and lichen ground cover were not affected by the presence of shrubs. The species found in open microsites were similar to species found in shrub covered microsites. These findings were unexpected and may be due to factors related to successional changes in the pine barrens. Previous work suggests that succession in the pine barrens does not occur rapidly (Sedia & Ehrenfeld, 2005), and lichen mats may persist for decades with very little vascular plant encroachment (Sedia & Ehrenfeld, 2003).

Within the pine barrens, lichen mats are commonly found in open areas within the forest matrix (Sedia & Ehrenfeld, 2005). These patches occur in areas that have experienced wildfires and may persist for decades or more (Sedia & Ehrenfeld, 2005). Lichens can play an important role in the nutrient dynamics in communities where they are abundant, and the effects that lichen

mats have on soil properties may affect the successional development on the Pinelands ecosystems (Sedia & Ehrenfeld, 2005). Lichen-dominated areas are found to maintain nutrient-poor conditions and have little organic matter accumulation (Sedia & Ehrenfeld, 2005). Low nutrient availability may then impede the establishment of vascular plant communities (Sedia & Ehrenfeld, 2005). The low organic matter content associated with lichen mats may contribute to the long-term persistence of lichen-dominated mats (Sedia & Ehrenfeld, 2005).

An experiment was conducted by Sedia and Ehrenfeld in 2005 to assess organic matter, soil moisture content, and N mineralization under lichen mats, grass patches, moss mats, and bare soil in the New Jersey pine barrens. The Mt. Misery Trail in Brendan T. Byrne State Forest (previously known as Lebanon State Forest) was used as a study site in this experiment and was noted to have experienced a wildfire that burned 28,000 ha in 1963 (Sedia & Ehrenfeld, 2005). Many of the sites used in the study presented here were located on this trail. Lichen mats were found to maintain soil characteristics similar to that of bare soils and were found to have a low organic matter content and low rates of net mineralization (Sedia & Ehrenfeld, 2005). In contrast, soil under mosses were similar to those beneath vascular plants with higher organic matter content and higher net mineralization (Sedia & Ehrenfeld, 2005). These findings suggest that cryptogam mats have an important effect on soil properties and development and are important in the dynamics of the ecosystems in which they are present (Sedia & Ehrenfeld, 2005).

Cladonia species increase in abundance following fires, and patches may show no sign of vegetation development even after thirty years post-fire (Sedia & Ehrenfeld, 2005). While several *Cladonia* species were observed in nearly every site, *C. subtenuis* was overwhelming dominant in all sites. A study was conducted in the Piedmont of North Carolina to assess lichen

successional changes by Robinson in 1959. In this study, *C. subtenuis* was found to begin to colonize an abandoned field after 12-15 years which occurred when young pine trees began to form dense, relatively closed stands (Robinson, 1959). From the time that they first appeared, *C. subtenuis* persisted in pine dominated areas for roughly 60 years (Robinson, 1959). It was also noted that *C. subtenuis* was present in a wide range of successional stages (Robinson, 1959).

Successional trends in Pine Barrens lichen flora may be similar to what occurs in other ecosystems that have similar characteristics. In Canadian boreal forests, terricolous lichens of the genus *Cladonia* are replaced successionally by mosses (Zouaoui et al., 2014). Successional changes are accompanied by closure of the canopy by woody plants, which results in reduced light levels at the ground and increased humidity in the understory (Zouaoui et al., 2014). As stands increase in age, there remain some terricolous lichen species that are described as late successional (Zouaoui et al., 2014). These species may appear later successionally due to limitations in dispersal or are associated with micro-habitats only found in older forests (Zouaoui et al., 2014). Mosses were not observed in many of the sites surveyed in this study and only in low amounts in sites where they were present. Humidity as well as canopy cover did not differ significantly between shrub covered and open canopy conditions in this study, suggesting that conditions for the establishment of late successional lichen species were not met in the sites assessed. The lack of differences in all abiotic variables, aside from light, may not yet favor the establishment and growth of late successional terricolous lichen species.

The time since last disturbance may not have been long enough to influence humidity and canopy cover in this study's microsites. While not directly assessed, the shrub cover in areas that had lichen mats were not as dense as surrounding understory woody plants in the area. The shrubs overtopping lichen mats may still be in earlier stages of succession, and not enough time

may have passed since the last disturbance to find microsite differences in these abiotic variables. The areas selected for this study appear disturbed, and were located next to hiking trails, possible old service roads, and may have been used as camp sites. These aspects may have contributed to the dominance of *C. subtenuis* in all of the study's sites. The lichen species found here may be present in early or many stages of succession, and conditions may not be ideal for late successional species to have been established.

In addition, studies suggest that terricolous fruticose lichens have slow growth and low mortality rates (Sedia & Ehrenfeld, 2005), features that may contribute to the lack of significant differences found between open canopy and shrub covered *C. subtenuis* height found in this study.

Study 3: Comparison of Cladonia subtenuis thallus gross morphology and reproduction

An additional study was performed to assess the effects of successional changes and shrub cover on thallus gross morphology. Metrics assessed in this study included thallus biomass, and presence and amounts of sexual reproductive structures (i.e., apothecia). *Cladonia subtenuis* was collected from each field site and stored fully desiccated in brown paper bags at room temperature until use. The methods described in this study were not found in the primary literature and were created for use in this study. It was predicted that both apothecia production and biomass would be greater in shrub covered compared to open canopy populations.

- Methods - Reproduction



Figure 3: Apothecial tip under a dissecting scope. Apothecia indicated by circle.

Lichens were randomly selected from collection bags and sprayed with water until fully hydrated. Debris (e.g., pine needles, leaves, etc.) were removed using tweezers. A 15 mm² cookie cutter was used to collect a plug from roughly the middle of each thallus. Plugs were then placed in a petri dish and carefully teased apart into small pieces using tweezers to expose apothecia. Apothecia were then counted using a cell counter under a dissecting microscope. The plugs were not turned over during counting to prevent double counting and were able to be viewed without turning them over. Plugs were removed immediately after apothecia were counted to prevent recounting. After counting, the plugs were placed in individual brown paper bags and allowed to dry fully at room temperature. Once fully dried, the plugs were weighed. Number of apothecia were divided by plug weight to obtain number of apothecia per gram of thallus.

- Statistical tests of hypotheses

Paired T-tests were intended to be used to assess apothecia/gram in open versus shrub covered microsites. However, normality of residuals could not be achieved after multiple data transformations, and so a non-parametric sign test was used in place of a paired T-test. The test was carried out using SPSS.

- Results

It was found that there is a significant difference between the number of apothecia per gram in shrub covered and open canopy microsites ($P=0.022$). There were a greater number of apothecia per gram produced in lichen collected from shrub covered microsites (Figure 14).

- Methods – Biomass

Lichens were randomly selected from collection bags and sprayed with water until hydrated. Debris were then carefully removed from the samples using tweezers. Fully hydrated lichens were measured across the longest axis in preparation for plug collection. The center, and midway between each end and the middle were selected for plug collection, totaling three plugs per sample. A 15mm x 15mm cookie cutter was pushed into the lichen sample and a plug was then removed. The in-tact plug was gently pushed out of the cookie cutter and measured in cm while hydrated. Each plug was placed into separate paper bags and allowed to dry at room temperature. After plugs were fully dried, they were weighed individually. Remaining debris was removed prior to weighing.

The height and weight of the three plugs from each sample were then averaged for statistical analyses. Average height was divided by the average weight from each sample to obtain the height per gram of thallus.

- Statistical tests of hypotheses

A paired T-test was used to assess differences between open canopy and shrub covered lichen biomass. A square root transformation was necessary for these data prior to performing the paired T-test.

- Results

The biomass per unit of height was significantly greater in *C. subtenuis* collected from the open canopy microsites ($P=0.011$) (Figure 13). Interestingly, there is no significant difference between biomass of plugs alone ($P=0.091$) or height of plugs alone ($P=0.418$) (Figure 11, 12).

- Discussion

Microsite differences influenced the production of apothecia in *C. subtenuis*. In addition, microsite influenced the biomass per unit height of *C. subtenuis*. More apothecia were produced in shaded conditions and thallus density was greater in open canopy conditions. The effects of biotic and abiotic factors on lichen morphology have been explored by other authors and yield interesting comparisons to this study and suggest that lichen functional traits, such as reproduction, growth, size, and development may be influenced by environmental conditions (Chuquimarca et al., 2019). Thallus morphology has been found to relate to light, temperature, and water requirements for photosynthesis and water storage. Reproductive strategy has been found to relate to dispersion ability and establishment (Chuquimarca et al., 2019).

Phenotypic plasticity may be important for slow growing, photosynthetic organisms such as lichens where response to environmental differences over small spatial scales may greatly influence growth and survival. Lichen morphology is important for water relations within the thallus (Sojo et al., 1997). In addition, light may be an important variable that influenced the results reported in this study. Sojo et al., in 1997 examined light effects on thallus morphology of *Catillaria corymbosa* from sun exposed and shaded populations and found that lichen in sheltered microsites displayed more compact vegetative morphology (e.g., reduced surface area to volume ratio) resulting in increased evaporative resistance. Such resistance in turn allowed lichens to retain water for longer periods and remain metabolically active for greater lengths of time. This is the opposite of what was found in this study, as *C. subtenuis* from sun exposed

microsites displayed greater thallus density. While the findings of Sojo et al., 1997 are opposite of what was reported in this study, something similar may be happening with sun exposed *C. subtenuis*. The habitats of *C. corymbosa* and *C. subtenuis* are quite different. *Catillaria corymbosa* is a fruticose maritime lichen that occurs on exposed rock and colonizes sheltered overhangs in which water is scarcely available (Sojo et al., 1997). Where *C. corymbosa* faces water scarcity in shaded overhangs, *C. subtenuis* may face water scarcity in sun exposed sites at the Pine Barrens and may experience rapid drying on the sandy soil that is characteristic of the ecoregion.

An additional study conducted by Snelgar and Green in 1981 assessed thallus density of *Pseudocyphellaria dissimilis*, a foliose lichen, in relation to different light environments. In particular, these authors found that denser thalli were found in sun exposed habitats of *P. dissimilis* compared to shaded populations, due to greater evaporative demands placed on lichens in sun exposed areas in the environment, which were similar to sun exposed areas of the Pine Barrens (Snelgar & Green, 1981) and corroborate the finding of this study.

Hydration is important for increasing metabolically active periods and repairing damage caused by excess light (Gauslaa et al., 2005) and evaporative demands in environments in which lichens are present may influence the development of more dense thalli to retain more water for potentially long lengths of time. Thus, an increase in thallus thickness can lead to slower water loss, allowing lichens to hold more moisture for longer periods of time, allowing lichens to remain metabolically active during long periods of drought or low precipitation. This may be especially important for sun exposed lichens. Lichens are poikilohydric and are, thus, not able to regulate the amount of water present in thalli. This makes them especially sensitive to environmental changes involving humidity and level of precipitation (Chuquimarca et al., 2019).

It may then be important to develop more dense thalli to retain water for longer periods of time, especially when exposed to greater evaporative stress in drier sunnier light environments.

Dispersal of thallus fragments is an important mode of reproduction in many fruticose lichen species, and may allow establishment in favorable microsite during succession (Heinken, 1999). Fragments disperse via wind, rain, and animals (Heinken, 1999). Lichens become brittle when dry and may then be dispersed through fragments when trampled by animals or humans. In addition, the amount of moisture in the thallus may influence fragments' ability to be dispersed through wind, as water-saturated thalli will be heavier than dry thalli (Heinken, 1999). However, dispersal of thallus fragments may be inefficient at colonizing isolated habitats. Colonization of these areas may then depend on chance dispersal by animals or rare weather events involving extreme wind (Heinken, 1999). Sexually reproducing species may then have an advantage in colonizing isolated microsities (Heinken, 1999). Lichen under shrub may also be hydrated for longer, and thus not break apart as easily as exposed lichen mats.

Apothecial production was also found to be significantly greater in shrub covered compared to open sun microsities. While no studies have specifically looked at apothecial production in response to light, some studies have found that lichen reproduction is influenced by environmental change (Chuquimarca et al., 2019) and microhabitats. The previously mentioned study conducted by Sojo et al., 1997 assessed the surface area of thallus occupied by soredia, an asexual reproductive structure, in the maritime lichen species, *Catillaria corymbos*, in different microhabitats. Specifically, they found that *C. corymbos* lichens growing in shaded microclimates developed more compact and dense thalli and had a greater density of soredia compared to *C. corymbos* lichens growing in sun exposed microclimates. This is the opposite of what was found in this study for apothecia. However, *C. corymbos* may differ from *C. subtenuis*

in that the open canopy sites in this study may have had more evaporative stress compared to the shaded sites, which is opposite of that from that study.

Several lichen species, including *Lasallia pustulata*, *Xanthoparmelia cumberlandia*, and *Umbilicaria spodochoa* have been found to produce apothecia only once a certain size threshold has been reached, (Jackson et al., 2006) suggesting that a specific mass must be reached in order to have enough energy to allocate to the production of reproductive structures (Jackson et al., 2006). However, these are foliose lichens, which can differ physiologically from fruticose *C. subenuis*. Another study conducted by Pringle et al., in 2003 found evidence suggesting a positive correlation with thallus size and number of apothecia produced in *Xanthoparmelia cumberlandia*, also foliose. However, these studies did not take light variability into account (Jackson et al., 2006).

Apothecia present on lichens are permanent once formed and can provide evidence of sexual effort of an individual lichen (Jackson et al., 2006). The presence of a significantly greater number of apothecia on shrub covered compared to sun exposed lichens may then indicate a greater need for sexual reproduction in shaded microsites, and less of a need among sun exposed lichens since fragments that can break from a mother thallus may be a sufficient means of reproductive dispersal in sun exposed lichens where plant cover is minimal (Jackson et al., 2006).

Apothecial production may be greater in shrub covered sites for other reasons as well. Chlorophyll *a* and *b* were found to be greater in shrub covered compared to sun exposed sites, suggesting that greater carbohydrate availability occurs in shrub covered lichens, which may support greater apothecial production costs relative to those produced in open sun conditions.

Given that light was found to be significantly greater in open sun sites and that soils in the Pine Barrens are very porous and subject to rapid draining, lichens in the open canopy sites may desiccate more rapidly. These lichens may then be metabolically active for shorter periods of time compared to shrub covered lichens. Lichens under shrub cover may also remain hydrated for longer periods of time. Together, these differences may result in the differences found in this study for apothecial density. Another aspect that may influence the production of apothecia is disturbance. There may be less disturbance under the shrub cover, given simple physical impediment. Animals such as deer and hikers may be less likely to step on the lichen mats found under shrub cover, and these lichens may then not be distributed via thallus fragmentation. Ultimately, a combination of these factors may induce greater apothecial production under shrub cover and help explain the results found in this study.

Study 4: Comparison of Cladonia subtenuis chlorophyll content

Green algae contain both chlorophyll *a* and *b*, and is an important component in the lichen thallus (Caesar et al., 2018). Lichens have the ability to physiologically adapt to different microhabitats (Pintado et al., 1997). The purpose of this study is to assess the effects of microhabitat on chlorophyll concentrations in the representative ground lichen species, *Cladonia subtenuis*. To address this question, chlorophyll content of *C. subtenuis* collected from open canopy and shrub covered microsites were assessed via chlorophyll extraction and spectrophotometry. Chlorophyll *a* and chlorophyll *b* were assessed.

It is hypothesized that lichen collected from the shadier microclimate will have greater chlorophyll content compared to lichen collected from the sunnier microclimate. It is further predicted that the ratio of chlorophyll *a/b* will be greater in shrub covered microsites. Previous studies have found that populations of lichens from shaded sites have higher chlorophyll content

compared to lichen from sunnier sites, similar to what is observed in vascular plants (Pintado et al., 1997). This increase in chlorophyll content may be a mechanism involved in maximizing photosynthetic utilization at low irradiances (Pintado et al., 1997).

- Background and other methods

Previous work has been performed by various authors to assess chlorophyll content in lichen. Solvents most used in lichen chlorophyll extraction methods include ethanol, acetone, *N,N*-Dimethylformamide, and dimethyl sulfoxide (DMSO) (Caesar et al., 2018). While acetone may give strong chlorophyll absorption peaks and is often used in chlorophyll extraction assays, it may be a poor solvent for green algae. Acetone is highly flammable and may be damaging to polystyrene and polymethylacrylates (Ritchie, 2008). Methanol is also highly efficient extractant for chlorophyll, but it is a toxic solvent and must be handled with caution (Ritchie, 2008). Ethanol is a much safer solvent compared to acetone and methanol. Ethanol does not damage polystyrene and polymethylacrylates, so plastic spectrophotometer cuvettes may be used in chlorophyll extraction assays using ethanol (Ritchie, 2008). Here, 100% ethanol has been used due to low cost and availability.

Various methods have been utilized throughout the literature to assess lichen chlorophyll *a* and *b* amounts. Biological soil crusts have been used to determine total chlorophyll content by Caesar et al., in 2018. When assessing biological soil crusts, it has been found that DMSO has been most effective at extracting chlorophyll *a* using a double extraction technique (Castle et al., 2011), In contrast, Lan et al., 2011 concluded that ethanol extractions were superior to DMSO when assessing chlorophyll *a* from biological soil crusts (Caesar et al., 2018). In a study conducted by Pintado et al., in 1997, the saxicolous fruticose lichen species *Ramalina capitata*

var. *protecta* were collected from different microclimates and chlorophyll content was assessed. In this experiment, each thallus was divided into three different zones and used for chlorophyll analysis. Chlorophyll content was determined following Barnes *et al.*, 1992. In this method, lichen samples were washed with CaCO₃-saturated 100% acetone and chlorophyll were then extracted using CaCO₃-saturated DMSO (Pintado *et al.*, 1997). In a study performed by Wakefield and Bhattacharjee in 2011, chlorophyll content of foliose lichens was used to assess the effects of air pollution on lichen thalli. Chlorophyll content was assessed via destructive sampling three times over a 12-month period. Thalli were removed from bark, air dried overnight, and distributed into 0.02g subsamples. These subsamples were then placed in 10 ml pf DMSO overnight in the dark. Optical densities were measured using a spectrophotometer at 645nm and 665nm (Wakefield & Bhattacharjee, 2011).

- Methods

Lichen material were randomly selected from collection bags and 0.5g of air-dried lichen material was weighed. Debris were removed using tweezers. Lichen material were thoroughly ground until homogenized using a cold mortar and pestle that had been removed from a freezer immediately prior to use. The mortar and pestle were cleaned with water and returned to the freezer between each use. 50mg of fully ground material was transferred to a brown, 1.5ml Eppendorf centrifuge tube. Remaining lichen material were transferred to a 15mL centrifuge tube, covered in tin foil, and stored in a freezer.

A spatula tip of chalk (containing CaCO₃) was ground using sandpaper and added to each tube containing the ground lichen material. 600µl of 100% ethanol was added to the tube, and a small hole was poked into the cap of the tube using a syringe. In place of a water bath, a beaker

of water was placed on a hot plate and heated to 80°C. Temperature was frequently monitored using a glass thermometer. Tubes were added to the beaker, and ethanol inside tubes was brought to a boil. Once ethanol began to boil, tubes were held in water for 5 min.

Samples were removed from beaker and allowed to cool at room temperature for 10 minutes. Parafilm was then wrapped around the samples to prevent liquid from escaping from small hole in cap. Samples were securely taped to a vortex mixer, and vortexed at a moderate speed for 20 minutes. Samples were then centrifuged at 3000 x g and 15°C for 10 minutes. At this point, supernatants were clear green in color. 100% ethanol was pipetted into the cuvette as a blank. 100µl of supernatant was pipetted into cuvette of spectrophotometer. Readings were recorded at 665 and 649. The cuvette was cleaned using DI H₂O in between each use. This method has been adapted from the ethanol section of Caesar et al., 2018. Chlorophyll *a* and chlorophyll *b* were then calculated using equations 1 and 2, respectively (Ritchie, 2006).

$$1) \text{ Chlorophyll } a (\mu\text{g/mL}) = 13.70 \times (A_{665}) - 5.76 \times (A_{649})$$

$$2) \text{ Chlorophyll } b (\mu\text{g/mL}) = -7.60 \times (A_{665}) + 25.8 \times (A_{649})$$

- Results

There is a significant difference between the chlorophyll *a* and *b* content in *Cladonia subtenuis* from shrub covered and open canopy microsites. There is a greater amount of chlorophyll *a* in *C. subtenuis* populations from shrub covered microsites (P=0.003) (Figure 15). There is also a greater amount of chlorophyll *b* in *C. subtenuis* populations from shrub covered sites (p=0.002) (Figure 16). No significant difference was found between the ratio of chlorophyll *a/b* in shrub covered versus open canopy *C. subtenuis* (P>0.05) (Figure 17).

- Discussion

Microsite differences influenced chlorophyll *a* and *b* content in *C. subtenuis*. There are significantly greater amounts of both chlorophyll *a* and *b* in populations from shrub covered microsites compared to open microsites. These findings were as expected. However, there is no difference between the ratio of chlorophyll *a/b* in *C. subtenuis* of shrub covered and open canopy population. These findings were similar to what has been reported by other authors (Pintado et al., 1997).

Previous authors have found similar results when assessing lichen chlorophyll content in response to different light environments. Pintado et al., 1997 used *Ramalina capitata*, a fruticose saxicolous species, to study the effects of microclimate on phenotypic plasticity. It was found that lichen collected from the shaded microclimate had significantly higher chlorophyll contents when considering thallus dry weight than those collected from the sunny microclimate (Pintado et al., 1997). However, there was no significant difference found between chlorophyll *a/b* ratio from microclimates (Pintado et al., 1997). These results were similar to results reported here from *C. subtenuis*. In another study, Green et al., 1997 assessed chlorophyll content in response to light environment using several species of chlorolichen. It was found that chlorophyll content was greater among lichens from shaded environments compared to those from sunny environments. Sojo et al., 1997 found that lichen from differing microclimates had higher chlorophyll content in lichen from shaded sites compared to exposed sites. These findings suggest that lichen may improve light harvesting by increasing chlorophyll content (Sojo et al., 1997). All of these results were similar to what was observed in chlorophyll content of *C. subtenuis*.

Legaz et al., 1986 assessed chlorophyll concentrations of *Cladonia verticillaris*, a fruticose lichen species similar to *C. subtenuis*, in response to different light environments. These

authors reported a similar trend to *C. subtenuis* and found greater chlorophyll *a* and *b* concentrations in shade populations. However, total chlorophyll reported from this study was much lower compared to results reported here. Legaz et al., 2011 found 42.1 ± 9.3 $\mu\text{g/g}$ of chlorophyll *a* and 26.1 ± 2.3 $\mu\text{g/g}$ of chlorophyll *b* in shade lichen. In sun lichens, these authors reported 14.5 ± 2.1 $\mu\text{g/g}$ of chlorophyll *a* and 8.9 ± 1.0 $\mu\text{g/g}$ of chlorophyll *b*. These differences may be due to differences in extraction methods utilized in both studies, as these authors used acetone for chlorophyll extraction. Solvents may vary in their ability to extract chlorophyll, and acetone may be less efficient than ethanol for chlorophyll extraction in lichens (Castle et al., 2011).

Lobaria pulmonaria was used in a transplant study by Gauslaa et al., in 2005 to assess the effects of light environments on chlorophyll content and dry mass. Greater dry mass and lower chlorophyll content was found in lichen from sunny habitats, and lower dry mass and greater chlorophyll in shaded habitats. This is similar to the results reported here of *C. subtenuis*.

Light was found to differ significantly between shrub covered and open canopy microsites. This feature may have influenced the results found here. Many previous studies have found that populations of lichens from shaded sites have higher chlorophyll content compared to lichen from sunnier sites, similar to what is observed in vascular plants (Pintado et al., 1997). This increase in chlorophyll content may be a mechanism involved in maximizing photosynthetic utilization at low irradiances (Pintado et al., 1997). The coverage provided by shrubs in this study may have provided lichen mats with longer periods of shade, and thus longer periods of hydration compared to fully sun exposed sites. While direct sun may provide higher irradiance, this also comes with rapid drying in poikilohydric organisms. Greater irradiance may produce a rapid drying of poikilohydric organisms and decrease the period of metabolic activity (Pintado et

al., 1997). Lichens may then overcome short period of metabolic activity by increasing maximum rates of net photosynthesis (Pintado et al., 1997).

Lichens may overcome lower light availability and improve their light harvesting by utilizing a more efficient photobiont arrangement and increasing chlorophyll content (Sojo et al., 1997). Increased chlorophyll content may allow for greater photosynthetic productivity in low light environments (Green et al., 1997). This may be especially useful for lichen population under shrub cover which experience lower light levels. Additionally, excessive light during desiccated state has been found to be detrimental for some lichen species (Gauslaa et al., 2005). This may have attributed to the lower chlorophyll content found in open canopy *C. subtenuis*.

Chapter 4: Conclusions

Lichens are an important component in the ecosystems in which they are present. Lichen functional traits, such as reproduction, growth and development may be influenced by environmental factors (Chuquimarca et al., 2019). Here, the morphological and photosynthetic responses of *Cladonia subtenuis* were explored. This is the first use of *Cladonia subtenuis* in an eco-morphology study, and some results agreed with past finding of other lichen species. Morphological variability in populations of lichens has been found in many previous studies (Sojo et al., 1997).

Some species of lichens within the same population may differ in their physiology and morphological plasticity when in habitats with contrasting environmental conditions (Pintado et al., 1997). Previous authors have found eco-morphological patterns in several morphological features of lichen species they had studied. These include variations in the proportion of algal to fungal cells, chlorophyll content, thallus shape and size, as well as upper cortex and medulla thickness (Pintado et al., 1997).

Despite the fact that significant differences were not observed in the majority of the abiotic variables between shrub covered and exposed sites, noteworthy differences were found between morphological, reproductive, and photosynthetic features. Light was the only abiotic variable found to differ significantly between microsites and may have influenced the differences in morphology of *C. subtenuis* found here. The morphology, reproduction, and photosynthetic properties of *C. subtenuis* have been found to be influenced by the presence of shrub cover in *P. rigida* stands. Thallus density was found to be significantly greater in exposed sites compared to shrub covered sites. Apothecia was found to be greater in shrub covered sites. Chlorophyll *a* and *b* was found to be greater in shrub covered sites.

Phenotypic plasticity may be of great importance to the selective advantage of slow growing organisms such as lichens (Pintado et al., 1997). Lichens may adapt to evaporative stress in their environments by developing a denser thallus in order to retain moisture for longer periods of time. Chlorophyll content may be increased in an attempt to harvest more light in low light environments. These organisms may also put more effort into sexual reproduction in environments with lower disturbance. The research presented here provides evidence that environmental conditions may influence the morphology, reproductive habits, and chlorophyll content of *C. subtenuis* in an understudied environment.

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Tables

Table 1: Woody plants, grasses, cryptogams, and macrofungi present in field sites.

Site #	Woody plants						Grasses	Cryptogams	Macrofungi
	<i>Pinus rigida</i>	<i>Quercus marilandica</i>	<i>Gaylussacia</i>	<i>Quercus rubra</i>	<i>Nyssa sylvatica</i>	Bayberry	Grass	Moss	Earth Star/Mushrooms
1	X	X	X				X	X	
2	X	X	X				X	X	
3	X		X					X	X
4	X	X	X					X	
5	X	X	X				X		X
6	X	X	X					X	
7	X	X	X	X					X
8	X	X	X	X				X	
9	X	X	X	X				X	X
10	X	X	X			X		X	
11	X	X	X	X					
12	X		X	X				X	
13	X	X	X			X			
14	X	X	X		X		X		
15	X		X						

Table 2: Lichen species present in each field site

Site	Condition	<i>C. subtenuis</i>	<i>C. uncialis</i>	<i>C. boryi</i>	<i>C. chlorophaea</i>	<i>C. squamosa</i>	British soldier	<i>Cladonia</i> species unknown
1C	C	X	X					
2C	C	X	X					X
3C	C	X	X					X
4C	C	X	X					
5C	C	X						
6C	C	X	X	X		X		
7C	C	X	X		X	X		X
8C	C	X						
9C	C	X	X		X			X
10C	C	X	X		X			X
11C	C	X	X		X			
12C	C	X				X		
13C	C	X		X		X		
14C	C	X						
15C	C	X						
Total frequency		15	9	2	4	4	0	5

10	O	X	X		X	X	X	X
20	O	X	X					X
30	O	X	X					
40	O	X			X			
50	O	X	X					
60	O	X	X					
70	O	X	X					X
80	O	X	X					X
90	O	X	X		X	X		
100	O	X	X		X			
110	O	X	X	X		X		
120	O	X	X	X	X			
130	O	X	X					
140	O	X	X		X			
150	O	X	X		X			X
Total frequency		15	14	2	7	3	1	5

Table 3: Results of paired T-test of all abiotic field variables. Nonparametric Sign test was used for temperature, and nonparametric Wilcox test was used for humidity. Bolded p-values indicate significance. ($p < 0.05$)

Abiotic Variables	P-Value
Temperature (C°)	0.267
Light (kLum)	0.011
Humidity (%)	0.532
Canopy Cover (%)	0.631

Table 4: Results of paired T-test of all biotic variables from field and lab studies. Nonparametric Sign test was used for apothecia/gram. Bolded p-values indicate significance. ($p < 0.05$)

Biotic Variables	P-Value
Height in field (cm)	0.081
Ground Cover (%)	0.29
Species Richness (#)	0.184
Biomass/Height (g/cm)	0.011
Apothecia/gram ($\#/cm^3$)	0.022
Biomass – alone (g)	0.091
Biomass – height only (cm)	0.418
Chlorophyll A ($\mu g/mg$)	0.003
Chlorophyll B ($\mu g/mg$)	0.002
Chlorophyll A/B ($\mu g/mg$)	0.128

Figures

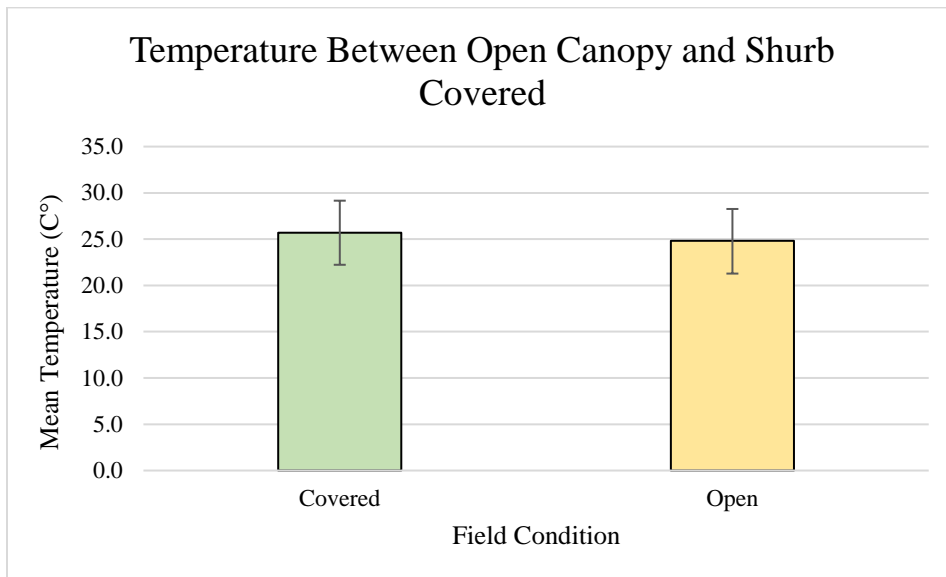


Figure 4: Bar graph of mean temperature (C°) from shrub covered and open canopy sites. Asterisks imply significant differences at $p < 0.05$

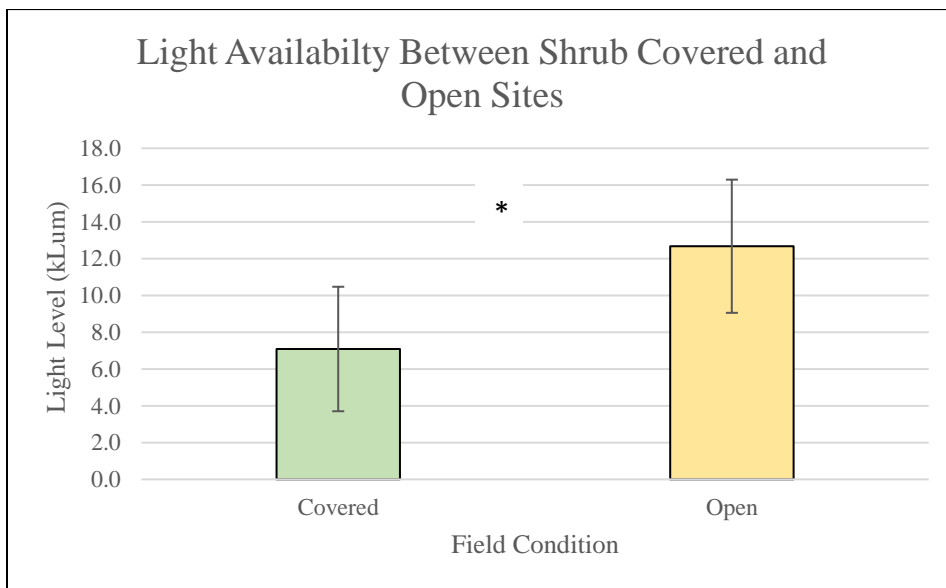


Figure 5: Bar graph of mean light level (kLum) from shrub covered and open canopy sites. Asterisks imply significant differences at $p < 0.05$

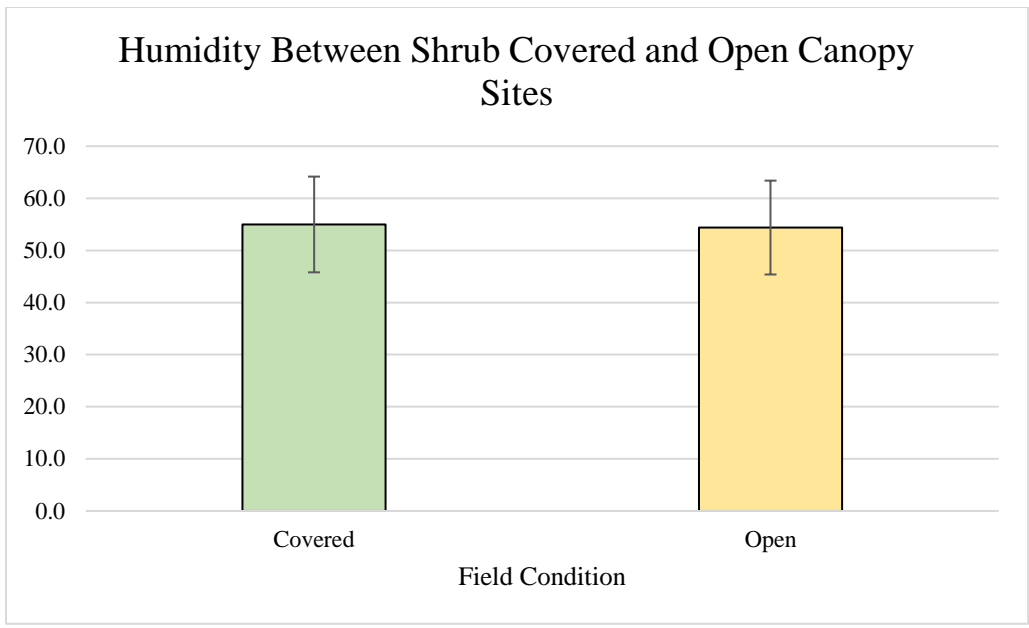


Figure 6: Bar graph of mean humidity (%) from shrub covered and open canopy sites. Asterisks imply significant differences at $p < 0.05$

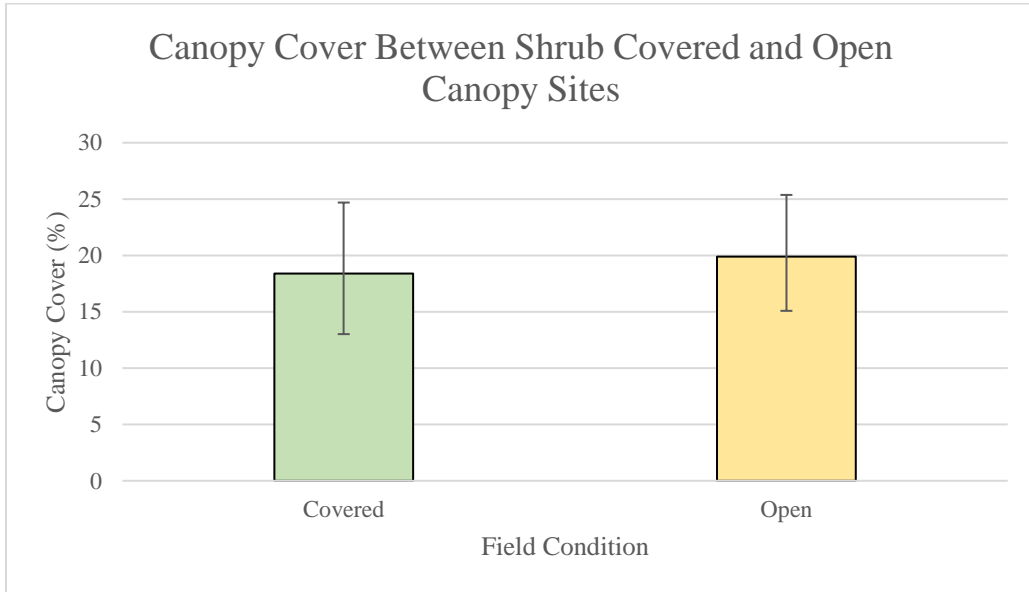


Figure 7: Mean canopy cover (%) from shrub covered and open canopy sites. Asterisks imply significant differences at $p < 0.05$

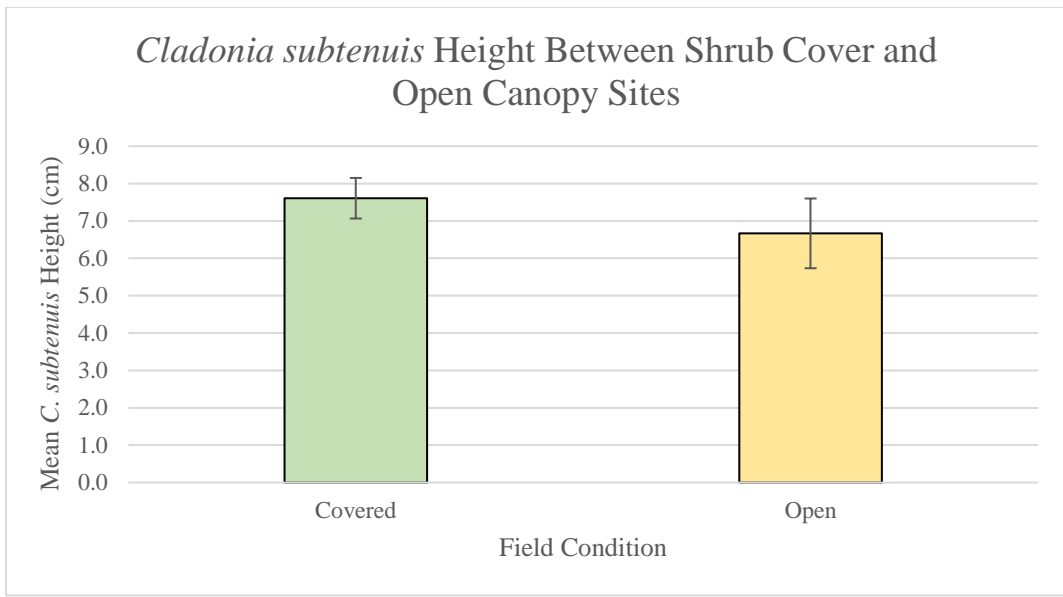


Figure 8: Mean *C. subtenuis* height (cm) in open canopy and shrub covered sites. Asterisks imply significant differences at $p < 0.05$

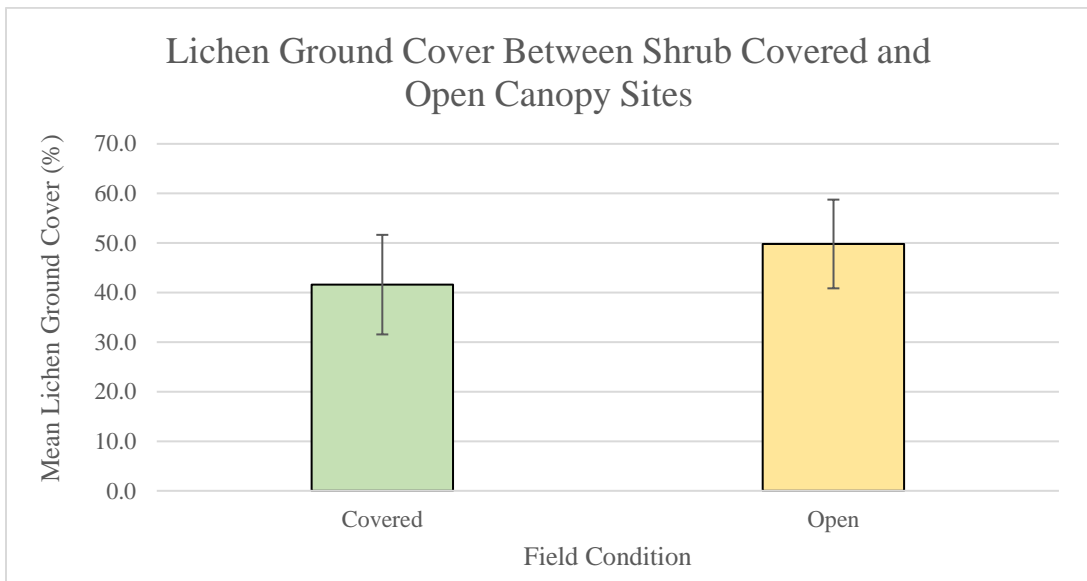


Figure 9: Mean lichen ground cover (%) in shrub covered and open canopy sites. Asterisks imply significant differences at $p < 0.05$

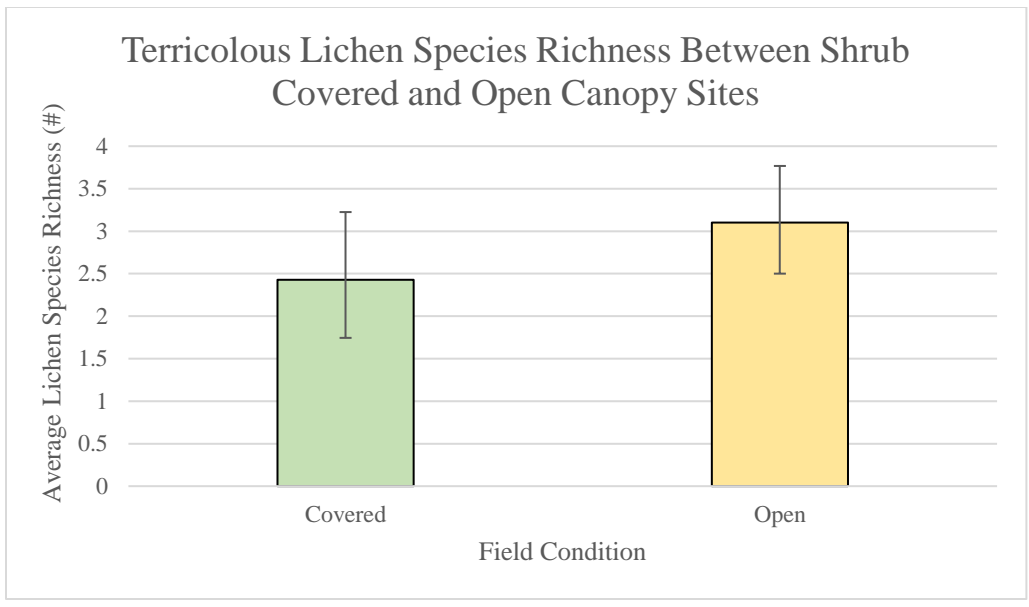


Figure 10: Average number of terricolous lichen species richness (#) from shrub covered and open canopy sites. Asterisks imply significant differences at $p < 0.05$

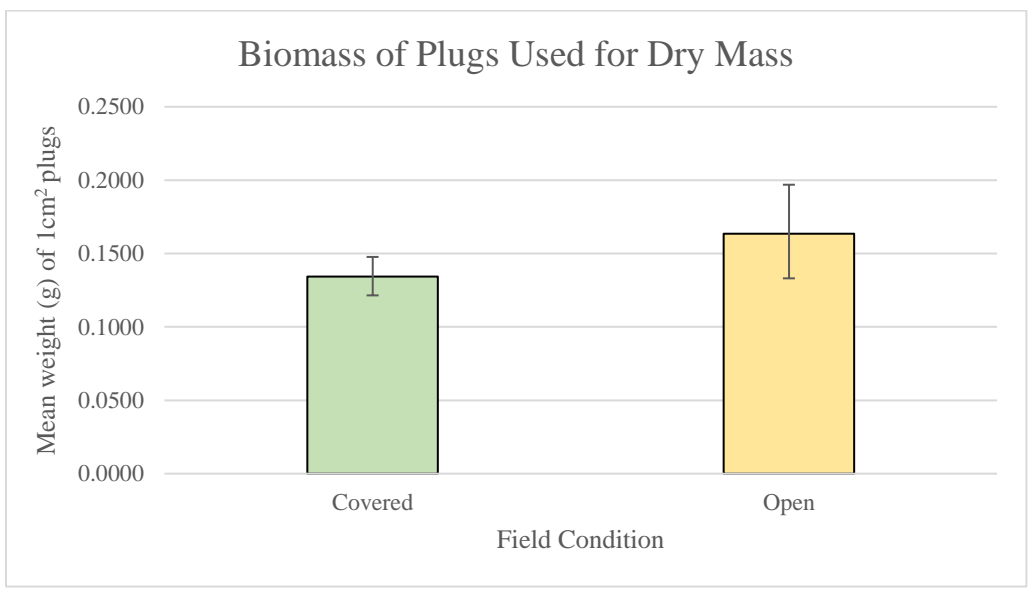


Figure 11: Average mass (g) of 1 cm² plugs used for dry mass measurements. Asterisks imply significant differences at $p < 0.05$

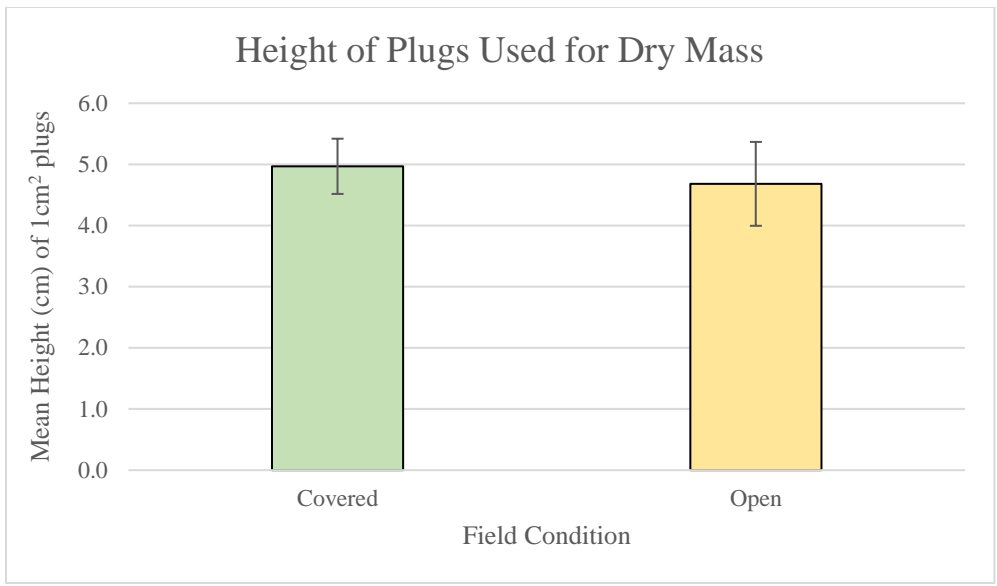


Figure 12: Mean height (cm) of 1 cm² plugs used for dry mass measurements. Asterisks imply significant differences at $p < 0.05$

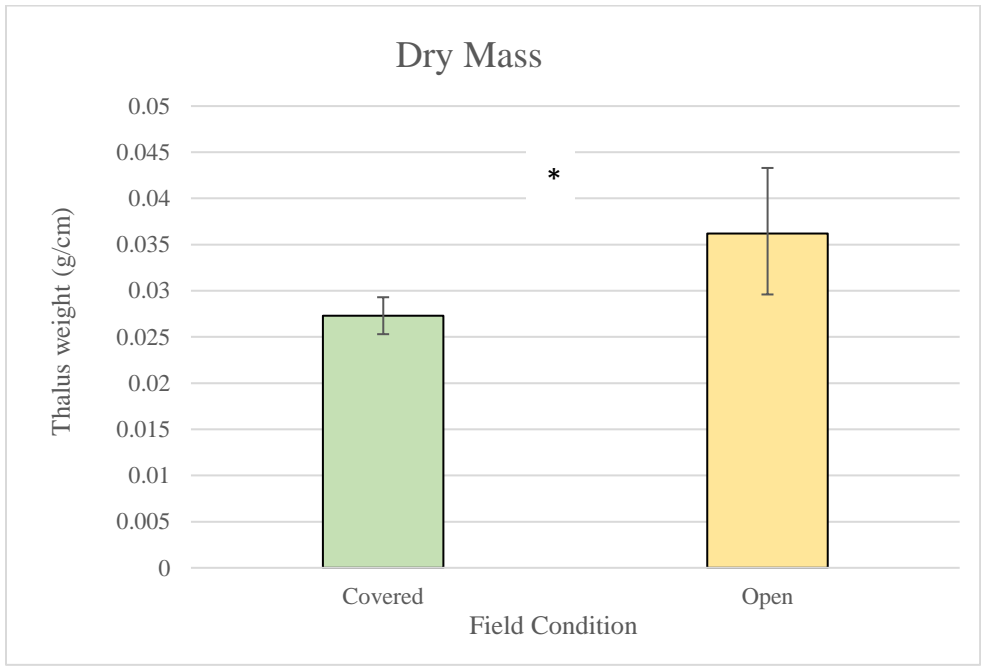


Figure 13: Mean *C. subtenuis* dry mass in open canopy and shrub covered sites. Asterisks imply significant differences at $p < 0.05$

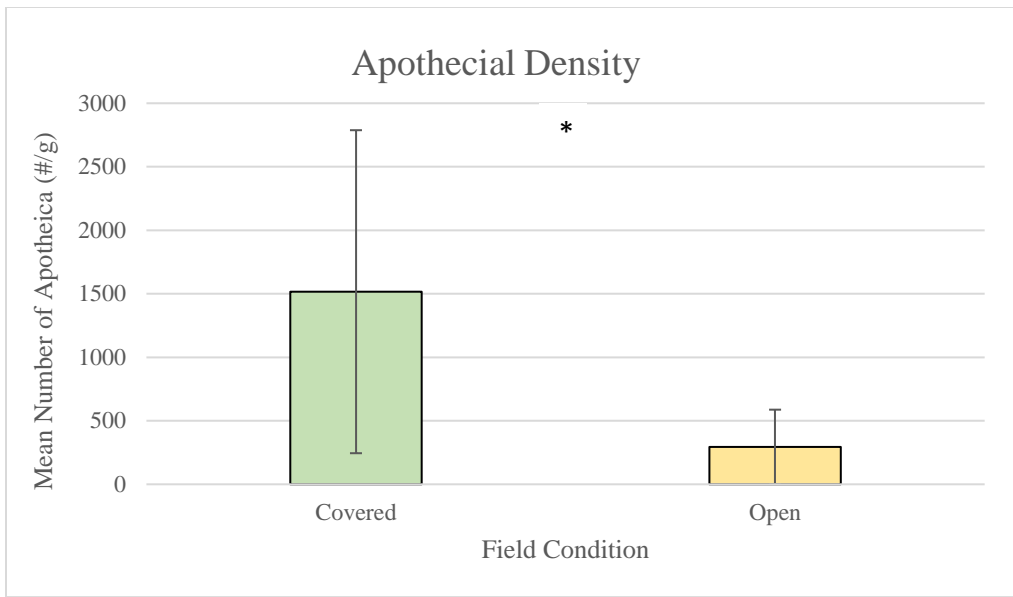


Figure 14: Mean apothecial density(#/g) of shrub covered and open canopy sites. Asterisks imply significant differences at $p < 0.05$

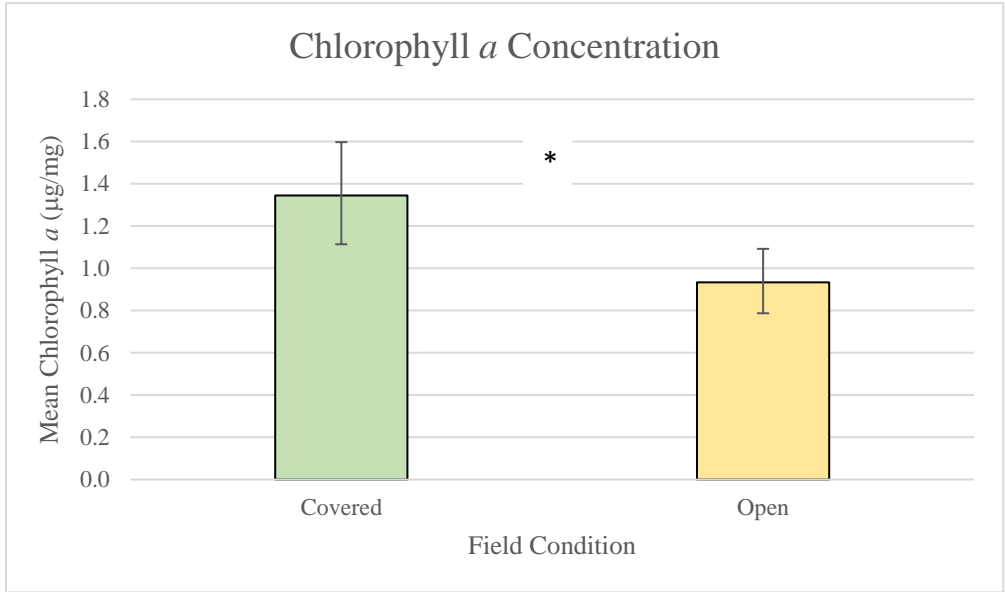


Figure 15: Mean Chlorophyll a concentration ($\mu\text{g}/\text{mg}$) in shrub covered and open canopy sites. Asterisks imply significant differences at $p < 0.05$

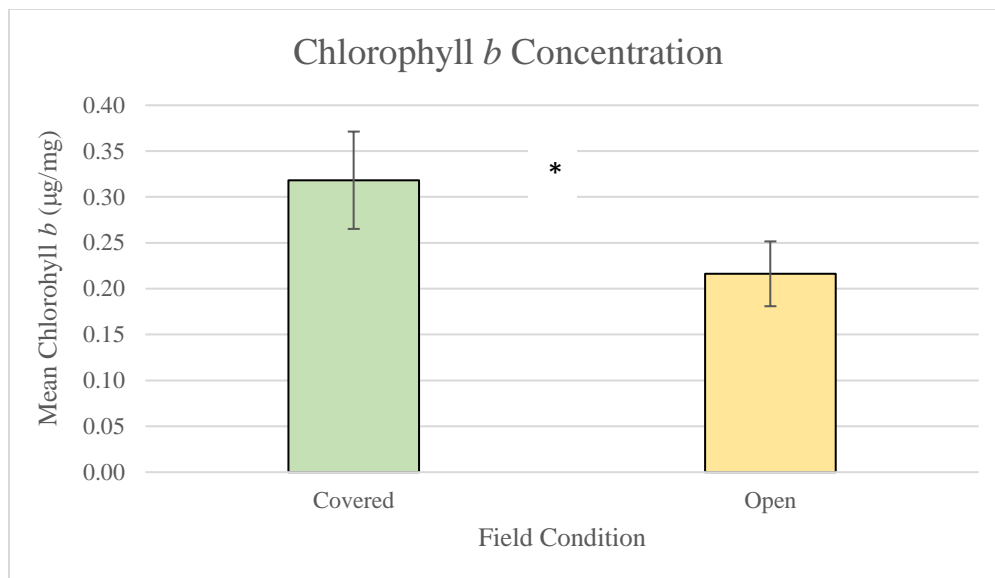


Figure 16: Mean Chlorophyll *b* concentration ($\mu\text{g}/\text{mg}$) in shrub covered and open canopy sites. Asterisks imply significant differences at $p < 0.05$

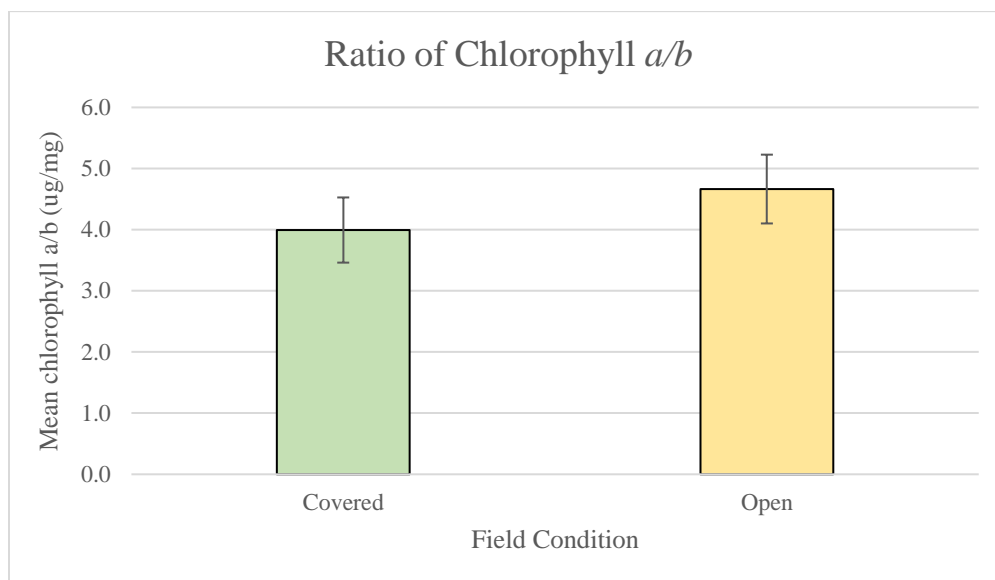


Figure 17: Mean ratio of chlorophyll *a/b* concentration ($\mu\text{g}/\text{mg}$) in shrub covered and open canopy sites. Asterisks imply significant differences at $p < 0.05$