Master's thesis

NTNU
Norwegian University of Science and Technology
Faculty of Natural Sciences
Department of Biology

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Photosynthetic activity in relation to water content in fruticose lichen species from Norway

Master's thesis in Biology: Plant Ecophysiology
Supervisor: Richard Strimbeck
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PREFACE

This thesis is a part of the Natural Science with Teacher Education study at the Norwegian University of Science and Technology. Incorporating lichen photosynthesis from the Norwegian mountains into the curriculum can enrich environmental literacy and promote learning.

Studying lichen photosynthesis contributes to several of the Sustainable Development Goals by highlighting the role of biodiversity during climate change, emphasizing the importance of terrestrial ecosystems, and promoting experimental learning. By integrating this topic, educators can empower students to address environmental challenges, become informed citizens, and work with species with which they are familiar. This topic can also give a clearer picture of the cultural and natural heritage of the Norwegian landscape and biodiversity.

ACKNOWLEDGMENTS

First, I extend my gratitude to my supervisor, Richard Strimbeck (NTNU), for his exceptional guidance, unwavering support, and insightful feedback throughout this journey. His expertise, encouragement, and mentorship have been invaluable, shaping both my research and personal growth.

I am immensely grateful to my dear friend and fellow master's student, Vibeke Alnes, whose steadfast support, collaborative approach, and shared enthusiasm have enriched this journey. Our time together during your fieldwork was not only productive but also filled with laughter, creating lasting memories that I will always cherish.

I owe a debt of gratitude to my loving boyfriend, whose support, patience, and encouragement have been a constant strength and inspiration. Your belief in me and willingness to stand by my side through every challenge have been remarkable.

I am deeply grateful to my family for their love, encouragement, and belief in my abilities. Your support and understanding have been the foundation of my whole academic journey, and I am profoundly grateful for everything you have done for me.

Jestem wdzięczna mojej rodzinie za ich miłość, nieustanną pomoc i wiarę w moje możliwości. Wasze wsparcie i zrozumienie stanowiły fundament mojej całej drogi edukacyjnej, i jestem wdzięczna za wszystko, co dla mnie zrobiliście.

Finally, I would like to thank my friends for their encouragement and endless support. Your friendship and presence have been a source of motivation and inspiration, and I am truly fortunate to have you in my life. Special gratitude is extended to one of my closest friends, Damian, for his invaluable assistance in refining the abstract written in Polish.

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LIST OF ABBREVIATIONS AND SYMBOLS

WC: water content

WC_{opt}: optimal water content

CCM: Carbon concentrating mechanism

PPFD: Photosynthetically active photon flux density

$PPFD_{sat}$: saturation point

$PPFD_{comp}$: compensation point

Ψ: Water potential

NE_L: net exchange in light

R_D: dark respiration

GP: the difference between net exchange and dark respiration

ABSTRACT

Throughout the years, significant research has been done on understanding the photosynthetic activity in crustose and foliose lichen species, however, fruticose lichens have received less attention. Fruticose lichens, like *Cladonia stellaris,* play a significant role in the Norwegian landscape, especially in the eastern and central regions. Understanding the photosynthetic dynamics of fruticose lichens could provide valuable insights into their physiology and function in ecosystems. Lichens, like higher plants, are known to exhibit light- and temperature-dependent photosynthetic responses, with water availability being a key factor. They are only active when hydrated, for example, due to fog or dew, but can also become suprasaturated, for example during rainfall events. With lichens facing threats from climate change and shrubification, understanding their contribution to the ecosystem, such as those in the Dovre Mountains in central Norway, is important. In this study, we assessed the photosynthetic activity of six fruticose, mat-forming lichen species, collected from the previously mentioned mountains. Two distinct gas exchange methods were used: open-system gas exchange, focusing on lichen tips, and closed-system gas exchange, involving parts of whole lichen mats. We expected that photosynthesis would increase with decreasing water content, peak at optimal water content, and decline thereafter. However, the results did not fully align with these expectations. Challenges encountered during the experiment, including issues with CO_2 equilibrium, hindered the consistency of the data obtained from both gas exchange methods. While data from the closed-system approach were more promising, they too exhibited inconsistencies, due to factors such as initial CO_2 concentrations, lack of CO_2 control, suprasaturation, and CO_2 recycling. Our observations underscore the complexity of lichen photosynthesis and highlight the need for resilient methods to measure photosynthesis and gas exchange, particularly when working with whole lichen mats.

SAMMENDRAG

Gjennom årene har det blitt gjort betydelig forskning på å forstå fotosyntetisk aktivitet hos skorpe- og bladliknende lavarter, mens buskeformede har fått mindre oppmerksomhet. Buskformede laver, som hvitkrull (*Cladonia stellaris*), spiller en viktig rolle i det norske landskapet, spesielt i de østlige og sentrale regionene. Det å forstå fotosyntese hos buskformede laver kan gi en verdifull innsikt i deres fysiologi og funksjon i økosystemer. Laver, som høyere planter, er kjent for å vise lys- og temperaturavhengige fotosyntetiske responser, med vann tilgjengelighet som en nøkkelfaktor. De er kun aktive når de er våte, for eksempel på grunn av tåke eller dugg, men kan også bli overmettede med vann, for eksempel på grunn av regn. Lav står ovenfor trusler fra klimaendringer og buskvekst (shrubification), og det er viktig å forstå deres bidrag til økosystemet, som de i Dovrefjell i det sentrale Norge. I denne studien vurderte vi fotosyntetisk aktivitet hos seks buskformende, matte-dannende arter, samlet fra de nevnte fjellene. To forskjellige gassutvekslingsmetoder ble brukt: åpen-system, med fokus på lavtupper, og lukket-system, som involverte deler av hele matter. Vi forventet at fotosyntesen ville øke med synkende vanninnhold, nå toppen ved optimalt vanninnhold, og avta deretter. Resultatene samsvarte imidlertid ikke fult med disse forventningene. Utfordringer som oppstod underveis i eksperimentet inkluderte problemer med CO_2-likevekt, og det hindret konsistensen i dataene oppnådd fra begge gassutvekslingsmetodene. Mens dataene fra lukket-system var mer lovende, var også disse mye variasjon, på grunn av faktorer som CO_2 konsentrasjon ved oppstart av målinger, mangel på CO_2-kontroll, overmettelse med vann og CO_2-resirkulering. Våre observasjoner understreker kompleksiteten av lavens fotosyntese og fremhever og fremhever behovet for gode metoder for å måle fotosyntese og gassutveksling, spesielt når man jobber med hele lavmatter.

STRESZCZENIE

Przez lata przeprowadzono znaczną liczbę badań mających na celu zrozumienie aktywności fotosyntetycznej gatunków porostów skorupiastych i liściastych, jednakże krzaczkowate otrzymały już mniejszą uwagę. Odgrywają one istotnę rolę w krajobrazie Norwegii, dobrym przykładem jest chrobotek alpejski (*Cladonia stellaris*), który występuje zwłaszcza na wschodzie i w centralnych regionach tego kraju. Ich dynamika fotosyntezy i jej zrozumienie, mogłoby dostarczyć cennych spostrzeżeń na temat ich fizjologii, oraz funkcji w ekosystemach. Porosty, podobnie jak rośliny wyższe, są znane z wykonywania reakcji fotosyntetycznych, zależnych od światła i temperatury, przy czym dostępność wody jest czynnikiem kluczowym. Są one aktywne tylko w chwili bycia nawilżonymi, na przykład ze względu na mgłę lub rosę, ale mogą też być przesycone, na przykład podczas opadów deszczu. W obliczu zagrożeń zmian klimatycznych i zakrzewienia (shrubification), zrozumienie ich wkładu w ekosystem, takiego jak ten w Górach Dovre w centralnej Norwegii, jest istotne. W niniejszym badaniu oceniono aktywność fotosyntetyczną sześciu gatunków porostów krzaczkowatych tworzących maty, zebranych z Gór wcześniej wymienionych. Zastosowano dwie różne metody wymiany gazowej: otwartą, koncentrującą się na ich końcówkach oraz zamkniętą, obejmującą części całych mat. Oczekiwaliśmy, że fotosynteza wzrośnie wraz ze spadkiem zawartości wody, osiągnie szczyt przy optymalnej ilości, a następnie spadnie, niestety wyniki nie w pełni zgadzały się z tymi oczekiwaniami. Napotkane przeszkody podczas eksperymentu, w tym problemy z równowagą CO_2 w systemie, utrudniły uzyskanie spójnych danych z obu metod wymiany gazowej. Podczas gdy dane uzyskane za pomocą metody zamkniętej były bardziej obiecujące, również one wykazywały niespójności, wynikające z czynników takich jak początkowe stężenie, recykling, brak kontroli nad CO_2 oraz przesycenie próbek. Nasze obserwacje podkreślają złożoność fotosyntezy porostów i wskazują na potrzebę stosowania solidnych metod ich pomiaru oraz wymiany gazowej, zwłaszcza w przypadku pracy z całą ich matą.

1 INTRODUCTION

Global warming has caused the temperature to increase, and this effect has been best seen in recent years. In the mountain environments, the temperature increase has surpassed the global warming rate of 0.2 ± 0.1℃ per decade. Specifically, temperatures in the mountains in Western North America, the European Alps, and Asia have been experiencing a higher rate of warming at 0.3 ± 0.2℃ (Pörtner et al., 2019). No trend has been observed for the changes in annual precipitation, but there has been less snow, according to the IPPC report (Pörtner et al., 2019). The effect of global warming is seen worldwide, in every community, for example in the oceans (Collins et al., 2010). The effect is also seen in the alpine communities, where the species living in the mountains are better adapted to lower temperatures (Pauli et al., 2015). The effects of global warming will change the biodiversity in the alpine communities: the effects can already be seen in the mountains (Pauli et al., 2012). The general expected change in the vegetation caused by global warming is a "warming-driven upward shift of species distribution" (Pauli et al., 2012), as shown by Van de Ven et al. (2007). They predicted that 10 out of 14 species would disappear from the studied area when the temperature increases with 6℃ (Van de Ven et al., 2007). Others, for example, Pauli et al. (2007), found that, at least in the Alps in Austria, global warming seems to be the primary reason for changes in species coverage. It has been shown by several studies, like the ones from Cornelissen et al. (2001) and Moffat et al. (2016), that lichens in the Alpine and Arctic environment decrease in coverage due to an increase in competition with other plants, like dwarf shrubs. Changes in the coverage of the species will thus lead to changes in the mountainous microclimate.

1.1 CULTURAL AND NATURAL HERITAGE

Lichens are an important part of the micro- and macroclimate in the tundra. In some communities, such as Norway, where lichens cover 6% of the land (Bryn et al., 2018), they can have some important roles in affecting the microclimate they occur in. Norwegian lichens are an important part of the landscape (Figure 1) and have a big natural heritage value for many. Another important aspect of the natural and cultural heritage of lichens is the reindeer and reindeer herding Sami in Scandinavia. During the winter reindeer prefer lichens as their food, for example, the *Cladonia* species, like *C. stellaris* and *C. rangiferina* (Inga, 2009). Lichens are one of the factors the herders emphasize in the grazing areas for the reindeer. With climate change, the distribution of the lichens can change, and thus affect the grazing reindeer and the natural and natural heritage in those areas.

Understanding the roles of different components of microclimate, such as lichens, can give a clearer picture of the dynamics in the community, and thus give an insight into how the

community might change during global warming. Since lichens are a big part of the Norwegian mountain landscape, photosynthesis measurements under laboratory conditions can give an insight into how those lichens work and how they contribute to the micro- and macroclimates in the tundra.

Figure 1: A picture from Dovre Mountains (62°14'08.68" N, 9°37'29.22" E). The light-colored areas are lichen communities, mostly *Cladonia stellaris* (picture taken in July 2023, from the personal album)

1.2 Growth Forms and Roles of Lichens

Lichens can be differentiated into three different growth forms: crustose, foliose, and fruticose. Crustose lichens are tightly attached to the surface they grow on, for example, rocks. A crustose lichen (Figure 2A) cannot be removed from the surface without destroying the thallus. Foliose lichens (Figure 2B) are characterized by a "leaf-like structure", they are lobed, and those types of lichens are "only partially attached to the substrate". Fruticose lichens can have distinct characteristics, some of them have a branch-like structure, some have more flat thalli, and others resemble small bushes (Figure 2C). This study involves fruticose, mat-forming species, and those may differ in function compared to crustose and foliose lichens. (Büdel and Scheidegger, 2008)

During the past years, different studies have been conducted to investigate the distinct roles lichen communities have. For example, Nystuen et al. (2019) and van Zuijlen et al. (2020a) found that mat-forming lichens have an insulating effect on the soil, reducing the maximum temperatures and increasing the minimum temperatures. Aartsma et al. (2020) studied the effect of albedo in lichen-dominated and shrub-dominated heaths in Norway and discovered that lichens had higher albedo than shrubs, due to higher reflection of short wave radiation. In her thesis, Orrico (2022) also showed that dry lichens have higher

surface temperatures than vascular plants, due to lack of evaporation and transpiration. As shrubs increase in abundance over the lichen heats, a process called *shrubification* (Mekonnen et al., 2021), the albedo will decrease leading to different consequences for both the micro- and macroclimate in the alpine tundra. Those are just some of the roles that lichens have in the climate they live in. Nevertheless, lichens also perform photosynthesis, but Norwegian lichens and their gas exchange are not much studied.

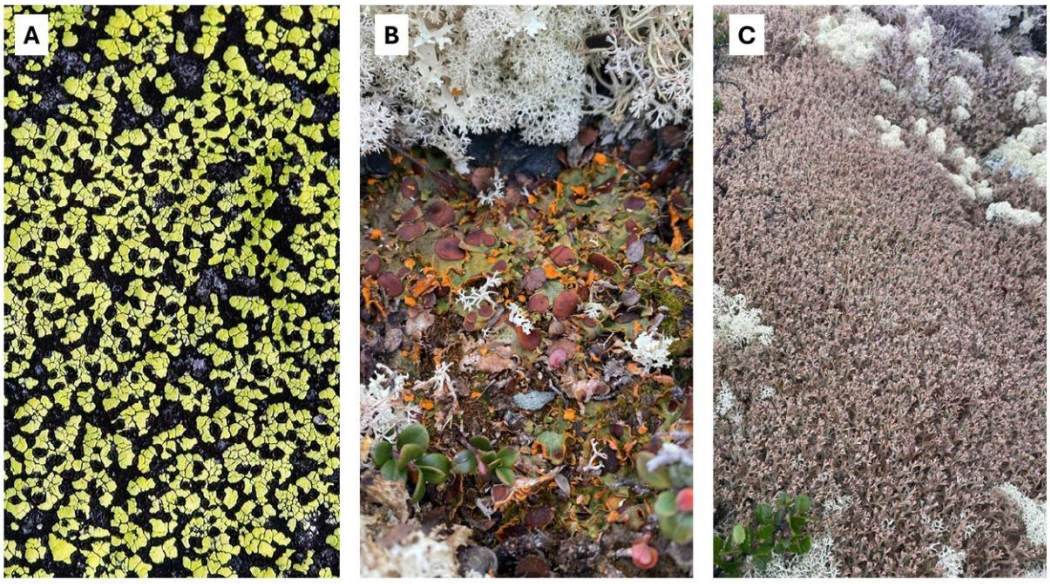

Figure 2: Three growth forms of lichens. A: Crustose lichen *Rhizocarpon geographicum* (Brinker). B: Foliose lichen *Solorina crocea* (lobulated, orange lichen, picture taken in July, from personal album). C: Fruticose lichen *Cladonia rangiferina* (picture taken in July, from the personal album).

1.3 Photosynthesis in Lichens

Seeing as lichens are a symbiosis between usually an ascomycete and an algal or a cyanobacterial photobiont, or both, photosynthesis and primary production are important functions of those organisms (Shukla et al., 2014). As a poikilohydric species, lichen water content (WC) is dependent on the conditions in the environment (Lange et al., 2001), as they have no mechanisms like roots or epidermis/cuticle that enhance the water uptake, and retain the water (Lechowicz, 1982). In higher plants, photosynthesis is dependent on both light and water availability, and lichens display the same dependence. It is worth mentioning that differences in photosynthetic activity may occur not only between the different species but also among the thalli of the same species, and even within the same thallus. Green et al. (2008, Fig. 9.2) discovered that "50% of the dry-weight-related photosynthetic activity occurs in the upper 10% of the podetia", while little to almost no activity was measured in the lower part of the lichen thallus in both *Cladonia stellaris* and *C. rangiferina*.

It is worth noting that when measuring photosynthesis and respiration in leaves, expressing these processes on a mass-based scale can indicate a tighter correlation with growth and provide a more informative perspective compared to area-based measurements (Wright et al., 2004). Wright et al. (2004) mostly concentrated on leaves, however, it is possible that mass-based measurements of photosynthesis and respiration would also be suitable for lichens, as the area of some lichens, especially the fruticose lichens with shrub-like structures, might be hard to determine. Green et al. (2008) also point out that measurements of photosynthesis in lichens are typically given on a dry mass basis "for ease of determination".

It is well established that the symbionts of the lichen exchange water and sugars, the photobiont produces sugars for both the mycobiont and for themselves, while the mycobiont holds water, which is also important for the photobiont (Shukla et al., 2014). Ten Veldhuis et al. (2020) propose to extend the symbiosis. They conducted a study using a foliose lichen *Flavoparmelia caperata* to present the connection between photosynthesis and mycobiont respiration. The study showed that the mycobiont consumes the oxygen produced by the photobiont during photosynthesis and the carbon dioxide produced by the mycobiont during respiration is consumed by the photobiont. They also mention that the CO_2 uptake in this symbiosis involves a Carbon Concentrating Mechanism (CCM), which is enhanced by internal CO_2 production from the mycobiont to boost the photosynthetic O_2 production when the internal CO_2 concentrations are low. Green algal photobiont species have pyrenoids as the carbon concentrating mechanisms. CCMs in lichens are mostly related to the water content of lichens: lichens thalli that are over-saturated with water have a slower diffusion of CO_2 from the environment, and CCMs can increase photosynthesis by concentrating the CO_2 from mycobiont respiration (Koch et al., 2023).

1.3.1 Relationship Between Water Content and Photosynthetic Activity

Rehydrated lichen thalli from the dry state show a linear increase in photosynthesis until optimum WC (WC_{opt}) is reached, as shown by (Green et al., 2011, Fig. 6.6), and the photobiont reaches maximum photosynthetic rate (Barták, 2014). Some lichen thalli can easily become oversaturated with water, for example during heavy rainfall, as their cortex absorbs water continuously, causing the water content to exceed the optimal levels. This phenomenon is called *suprasaturation* (Lange and Green, 1996). High WC can limit the assimilation of CO_2 in the lichens (Lange and Green, 1996, Barták, 2014). Lange et al. (1999) mention in their paper that lichens might be able to reach optimal CO_2 uptake under very narrow hydration levels. Further dehydration of the thallus will lead to a decrease in photosynthesis, while further hydration of the thallus can result in slower diffusion of CO_2

due to increased resistance, also leading to a decrease in photosynthesis (Lange et al., 1999).

Lange et al. (1993) conducted a study with mostly foliose and some fruticose lichen species from a temperate rainforest in New Zealand. They researched CO_2 exchange under different hydration levels, and grouped the lichens into four distinct groups, concerning the degree of photosynthetic depression. They found that most of the studied lichens were affected by the maximum water content, but the depression of CO_2 diffusion and photosynthesis did not occur in all the species. However, most lichens are affected by suprasaturation. In addition, the study showed that suprasaturation can also happen in the field and is not only a laboratory phenomenon.

Lange and Tenhunen (1981) in their study also discovered that water content affects CO_2 diffusion, caused by increased resistance of the diffusion of the gas from the air-filled spaces. They found that the effect of suprasaturation and increased resistance to CO_2 diffusion can be overcome by increasing the ambient CO_2 concentrations. "At 1,600 ppm, the highest CO_2 concentration used, a depression was in most cases fully absent" (Lange and Tenhunen, 1981). The study also confirmed that the CO_2 depression is due to high water content because of the increased resistance to CO_2 diffusion and not because of a decrease in the carboxylation capacity of the lichen.

Del-Prado and Sancho (2000) used two fruticose lichens *Teloschistes lacunosus* and *Ramalina bourgeana* from the Mediterranean semiarid zone Cabo de Gata in Spain, to measure photosynthetic activity under different temperatures and WC. Both species showed a decrease in photosynthetic activity under suprasaturated conditions, although *T. lacunosus* had a more rapid depression than *R. bourgeana*. As the samples dehydrated from high WC, both showed positive photosynthesis, and the maximum photosynthesis for both species was achieved at WC between 95% and 130% (Del-Prado and Sancho, 2000, Fig. 1). Groulx and Lechowicz (1987) studied fruticose and foliose subarctic lichens in which they looked at drying rates of the lichens and photosynthetic recovery in the species after being dormant. *Cladonia sulphurina* and *Nephroma arcticum* reached a compensation point (photosynthesis equals respiration) around 90 minutes after rewetting, indicating that those lichens have a higher water-holding capacity and a slow photosynthetic recovery. *Alectoria ochroleuca*, *Cladonia stellaris,* and *Coleocaulon divergens* had compensation points ranging from 1 to 28 minutes, indicating that those three have a low-water holding capacity and thus faster photosynthetic recovery than *C. sulphurina* and *N. arcticum* (Groulx and Lechowicz, 1987, Fig. 1). They also found that fast-drying species *C. cucullata* and *A. ochroleuca* have minimal rates of resaturation respiration, and slow-drying species *C. stellaris*, *C. sulphurina,* and *N. arcticum* have higher resaturation respiration rates, indicating that "low resaturation can … allow survival and growth of subarctic lichens in an

environment with short or frequent periods of activity" (Groulx and Lechowicz, 1987). This means that fast-drying species can more efficiently transition from a dormant state to a photosynthetically active state, without using a significant amount of energy on respiration in contrast to slow-drying species. Lange et al. (1999) used three crustose lichens *Diploschistes muscorum*, *D. scruposus,* and *Fulgensia fulgens* to check the interaction between external CO_2 and WC. They found that *F. fulgens* had the highest depression of photosynthesis under high WC, and *D. muscorum* had no depression under high WC (Lange et al., 1999, Fig. 1A-B). These observations highlight the diverse water-retention and photosynthesis reactivation capabilities among lichen species, despite their structural similarities in cellular layers. It underscores that each lichen species reacts uniquely to variations in water content and light conditions, even if they live in the same environment. It also highlights that lichens react differently due to morphological structures, like crustose and fruticose lichens.

All the studies mentioned above, agree on a correlation between WC and photosynthesis: a high WC in the thallus can affect the net photosynthesis through hindrance of the CO_2 diffusion path. The studies mentioned used all three lichen growth types: crustose, foliose, and fruticose, and all of them underlined that they can be influenced by high WC and suprasaturation, but the effects of how much photosynthesis is depressed are species-specific, for example as shown by (Lange et al., 1993). It is also clear that lichens have different water-holding capabilities, influencing how fast lichens reactivate their photosynthesis and respiration. It shows that lichens have different strategies to survive and grow, as most of them experience frequent periods of hydration and dehydration throughout the day.

1.3.2 Relationship Between Temperature and Photosynthetic Activity

Photosynthesis in lichens is also temperature dependent, but an increase in the temperature of the thallus will also increase the loss of water (Barták, 2014). In his article, Barták (2014) mentioned that for many of the lichen species, the optimum temperature for photosynthesis lies between 10 and 22℃. Hájek et al. (2001) found out that for *Cetraria islandica*, the critical temperature is around 40℃. Nevertheless, Barták (2014) declares that this is only a theoretical value, since temperatures as high as this, will cause fast dehydration of the thallus. Because lichens are poikilohydric, it seems reasonable that higher temperatures cause loss of water.

Green et al. (2011) and Barták (2014) state that hydration levels as low as 2% thallus WC can be reached when the lichens are heated by the sun, and the temperature can get to 70℃ in the thallus. In a study conducted by Colesie et al. (2018), they looked at three

different lichen species under different temperatures. The study showed that over 6 weeks, none of the species showed any sign of photosynthesis or respiration after 3rd or 4th week, demonstrating that the photosynthetic part of the lichen was dead. Since there was no respiration as well, it could also mean that the mycobiont is dead as well. These results agree with Barták (2014), who stated that the optimum for photosynthesis in lichen lies between 10 and 22℃. The study on Antarctic lichens (Colesie et al., 2018), also showed that *Stereocaulon alpinum*, which is widely distributed on Livingston Island, first grown at 5, and then at 15℃ for 6 weeks, acclimated to warmer temperatures. No or very little acclimation was observed in the two other specialized species. Tegler and Kershaw (1981) focused on *Cladonia rangiferina* and its ability to tolerate heat stress in April, May, and July. They found that in April, *C. rangiferina* had decreased photosynthesis after 7 days under 35℃, but in mid-summer, the same temperature had no harmful effects on photosynthesis. They concluded that this lichen species has an increase of about 10℃ in heat tolerance from April to July. Temperature is an important factor for photosynthesis, and the studies mentioned above, especially the one from Tegler and Kershaw (1981) show that lichens can acclimate to different temperatures. This shows that lichens could acclimate to higher temperatures due to climate warming, but since temperature also influences WC, it is not certain how the lichens will adapt to higher temperatures.

1.3.3 Relationship Between Light and Photosynthetic Activity

When lichens have an optimal WC, they respond to light intensities in the same manner as higher plants do. The photosynthetic response of lichen to light follows the typical curvilinear pattern of higher plants, which reaches a plateau when the light is saturating (Barták, 2014). According to Barták (2014), most of the lichens are saturated in the light range between 100 and 400 μmol m^{-2} s^{-1}. Nonetheless, lichens from shaded habitats have lower saturation ($PPFD_{sat}$ (photosynthetically active photon flux density)) and light compensation ($PPFD_{comp}$) points than lichens from open habitats. Green et al. (1997) measured the photosynthetic response of six foliose and three fruticose lichen species from a temperate rainforest in New Zealand. They found that the studied lichens can be divided into two groups: those with high $PPFD_{sat}$ and $PPFD_{comp}$, and those with low $PPFD_{sat}$ and $PPFD_{comp}$. All fruticose lichens used by Green and his team in this study came from open habitats, and those displayed high $PPFD_{comp}$ (above 50 μmol m^{-2} s^{-1}) and $PPFD_{sat}$ (above 500 μmol m^{-2} s^{-1}). For the rest of the species, the $PPFD_{comp}$ was less than 50 μmol m^{-2} s^{-1}, and $PPFD_{sat}$ was less than 300 μmol m^{-2} s^{-1}. None of the species showed any depression of photosynthesis at higher PPFD values, meaning that no photoinhibition was seen during the measurement, and most of the species, except the fruticose, showed saturated photosynthesis at 500 μmol m^{-2} s^{-1}. Those species came either from the shaded forest or the margins of the forest. It looks like lichens, particularly the ones studied here, might

show the same adaptations as higher plants to different light environments (sun and shade plants), however, since lichens are poikilohydric, the WC and desiccation of the thallus also need to be considered. In the same study, Green et al. (1997) studied the effects of WC on light-saturated photosynthesis for *Pseudocyphellaria coronata*, a foliose lichen. At saturating light, the photosynthesis was depressed when the water content was low, due to low water availability, and when the water content was high, due to suprasaturation.

It is also worth mentioning, that at least what has been measured by Green et al. (1997), the light level outside the forest exceeds 800 µmol m^{-2} s^{-1}. Lichens from the open habitats had lower $PPFD_{sat}$ (549 – 766 µmol m^{-2} s^{-1}) than light in the open, meaning that those lichen achieve maximum photosynthesis at lower light levels, compared to what is found in the open. This could be because of the poikilohydric nature of lichens: they dry out quickly in direct sunlight. As suggested by GREEN et al. (1995) lichens are better adapted to lower light levels when they have an optimal water content, either after a rainfall during the day, or after being moistened during the night.

1.4 Why Is This Important?

As mentioned, the climate in the alpine tundra is changing due to global warming. Several predictions have been made for different regions: according to Miljødirektoratet (2017), the climate in Norway will be wetter and warmer. A lot of studies have been done on crustose and foliose lichens from various parts of the world. Few studies have been found on whole fruticose lichens, most of them focused on tips, and even fewer have been found about the lichens from the Norwegian alpine environment. Hence, this thesis questions how lichens from Dovrefjell, Norway will perform under the changing climate. Lichen-dominated communities are important and conspicuous components of the landscape in Norway, especially in the east. We know little about the productivity of lichens in these communities. It is important to find out what role the lichens play, especially since they may be threatened by climate change and shrubification. Investigating how the photosynthetic activity will change during different hydration levels could help understand why and how lichens are important in ridge-top communities in central Norway in Dovrefjell. This study will also provide background information for future studies investigating changes in alpine lichen ridgetops communities, and, eventually, will also show what we will be losing due to the overtaking of dwarf shrubs, like dwarf birch. The hypothesis assessed in this study is as follows: at a constant temperature, photosynthesis will increase with decreasing WC, reach maximum photosynthesis at optimum WC, and as WC decreases further, photosynthesis will also decrease.

2 METHODS

2.1 SAMPLE COLLECTION

Lichens were collected from the Dovre Mountains in Central Norway (62°14'08.68" N, 9°37'29.22" E, Figure 3). Dovre is a low alpine zone area, around 1100 meters above sea level, and represents a slightly continental zone (Moen, 1999). The continental zone is mainly dominated by mat-forming fruticose lichens, notably by species from *Cladonia spp.,* with dwarf birch–juniper heath also being present (Moen, 1999). The ridgetops are characterized by nutrient-deficient soil, thin snow layers, and frost (Rekdal, 2022). The temperatures in July are around 8-10℃, and annual precipitation varies from 400 mm to 600 mm (Moen, 1999). In 2023, at the closest weather station in Hjerkinn (1012 mamsl), the maximum and minimum temperatures in July and August were 21.4℃, 17.9℃, and 1.5℃, 2.1℃, respectively. The annual precipitation was 667.3 mm, while the precipitation in July and August was 74.9 mm and 206 mm, respectively (Norwegian Centre For Climate Services).

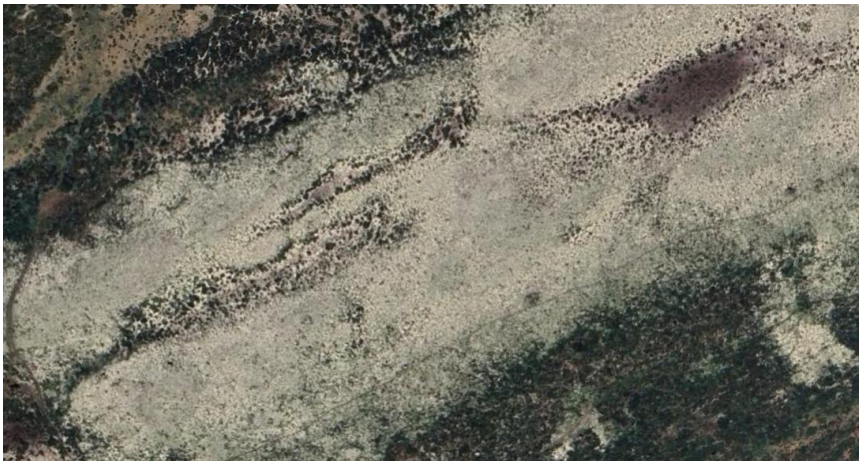

Figure 3: Screenshot from Google Earth presenting the ridgetop where the lichens were collected (62°14'08.68" N, 9°37'29.22" E). The light-shaded areas are dominated by lichens, mainly *Cladonia stellaris*. Other species found on the ridgetop were *C. rangiferina* and *Flavocetraria nivalis*. Moving down from the ridgetop, the areas are dominated by shrubs like dwarf birch (Google, 2023)

Six fruticose lichen species were collected from alpine ridge top communities at the study site in mid-August: *Cladonia stellaris, Cladonia rangiferina, Flavocetraria nivalis, Alectoria ochroleuca, Stereocaulon paschale,* and *Bryocaulon divergens*. They occur on wind-exposed ridges and slopes or other areas where there is thin snow coverage and nutrient-poor conditions. Lichens were dried at room temperature and stored in the dark in paper bags until measurements were taken in the laboratory.

Photosynthetic activity and respiration were assessed for all six species under varying hydration levels at a constant temperature. Two methods were used to dehydrate the lichen samples: vapor pressure equilibration and passive drying.

2.1.1 Vapor Pressure Equilibration and Open System Gas Exchange

To generate the hydration response curves, the lichens were placed in small, weighed beakers, containing only the lichen tips, 2 cm, leaving out the reproductive structures. Only samples of *Cladonia stellaris* were used for these measurements, and lichen tips of all six lichen species are presented in Figure 4. The beakers were then placed in translucent plastic boxes measuring 28 cm x 28 cm, each with a top lid. Those boxes contained solutions with different polyethylene glycol (PEG) concentrations, to establish specific water potentials (ψ) of 0, -1, -2, -4, -8, and -16, all maintained under a constant temperature of 15℃. The required PEG concentrations to achieve the specific water potentials were calculated using an equation from Michel (1983), where PEG concentrations in grams per liter were determined for each ψ value (Table 1).

$$[\mathrm{PEG}] = \frac{4-(5.16\psi\mathrm{T}-560\psi+16)^{0.5}}{2.58\mathrm{T}-280} \qquad (\mathrm{I})$$

Table 1: Calculated PEG concentrations in grams/liter from equation I. These were used to achieve the different water potentials used during this experiment.

PEG in gram for 1L of water	Water potential, ψ
0	0
76	-1
113	-2
166	-4
241	-8
348	-16

After preparation, the boxes containing the samples were stored in a growth chamber, set to cycle through 16 hours of light (ca. 200 µmol m^{-2} s^{-1}), representing day, and 8 hours of darkness, representing night, all at 15℃. The samples were allowed to equilibrate in the growth chamber for 24 hours before the measurements were taken. To ensure comprehensive data collection, samples were rotated after measurements to expose each to various water potentials.

Dry samples were weighed and then placed in the boxes with the PEG solutions, with five samples per box, giving a total of 30 samples for each lichen species. After 24 hours of acclimation, the samples' fresh weight was measured just before assessing the photosynthetic activity. This allowed the calculation of water content later.

Using a modified moss/lichen PLC3 cuvette (25 x 18 mm, 4.5 cm^2) with a cup for the sample attached to a PP Systems CIRAS-3 (Amesbury, USA), the protocol involved initially adjusting the light intensity to 1500 µmol m^{-2} s^{-1} and allowing the sample to acclimate for 2 minutes. Subsequently, three measurements were taken at 10-second intervals. After the third measurement, the light was turned off, and the sample was allowed to acclimate for 1 minute in the dark. After acclimation time (1 minute), three measurements at 10-second intervals were conducted in the dark. After each sample, a Differential Balancing (Diff Bal) calibration procedure was performed, to ensure the accuracy of the reported values. The measurement process for one sample took approximately 7 minutes.

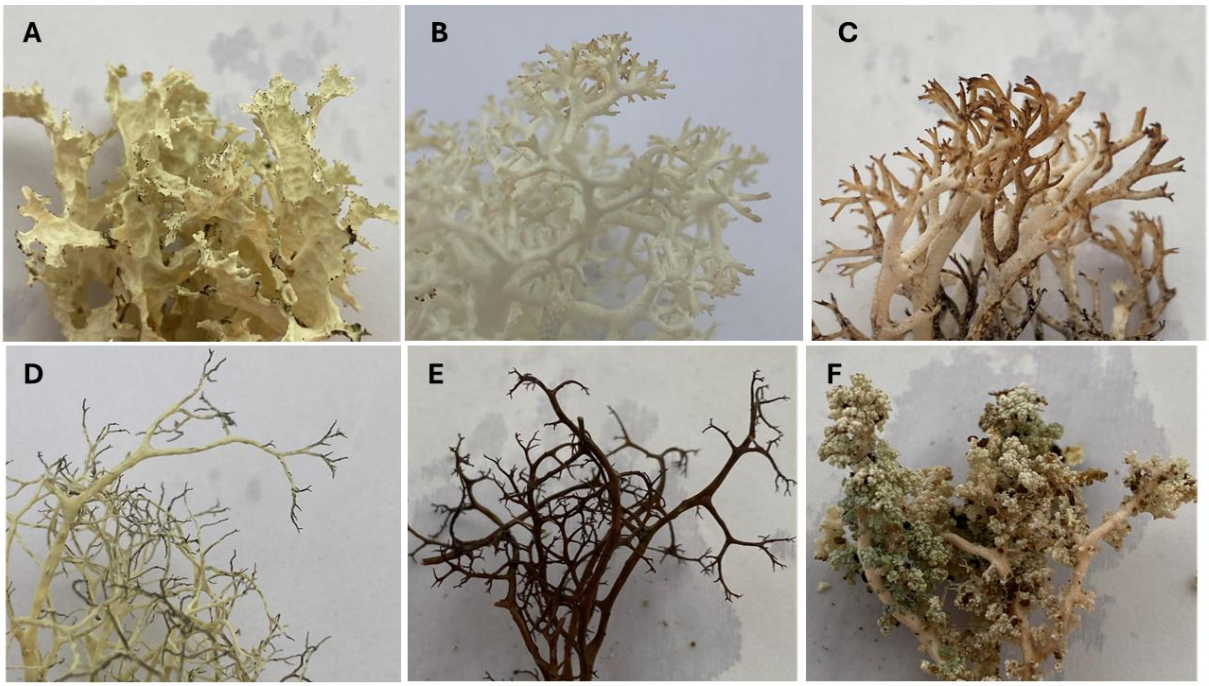

Figure 4: Tips of all six lichen species. A: *Flavocetraria nivalis*, B: *Cladonia stellaris*, C: *C. rangiferina*, D: *Alectoria ochroleuca*, E: *Bryocaulon divergens* and F: *Stereocaulon paschale.* Tips from *C. stellaris* (B) and *C. rangiferina* (C) are similar to each other, but *C. stellaris* (B) is "fluffier." *F. nivalis* (A) has a flat thallus and *S. paschale* (F) has a warty structure, hence defining the tips from those two can be challenging. Tips of both *A. ochroleuca* (D) and *B. divergens* (E) are branch-like.

2.1.2 Passive Drying in Closed System Gas Exchange

Bigger samples were used for this method, not only the tips but also the non-photosynthetic sections of the lichens. The samples had approximately the width of the used Petri dish, 14 cm, or smaller. The samples had also different depths, according to what could be achieved with the collected material. The deepest sample was *C. stellaris* with 11 cm of depth, and the smallest was *S. paschale* with a depth of 3.5 cm. Samples were taken straight from the paper bags and used right away. The measurements were taken using a CPY-4 Canopy Assimilation Chamber (167 cm^2), which operates as a closed system (PP Systems, 2013). For each day, between 3 and 6 samples were measured: one for each lichen. To better explain the method, I will use "S1" and "S2" as examples. Dry weights of S1 and S2 were taken before the measurement. S1 was sprayed with water until it looked wet and was soft to the touch. Excess water was slightly shaken off from S1, and fresh weight was measured. The first measurement was taken right after the sample was wetted. Photosynthetic activity was measured both in light and dark: 530 µmol m^{-2} s^{-1} for light, 2 minutes for both light and dark. After measurement for S1, the sample was stored on a table under constant light of 320 µmol m^{-2} s^{-1}, and the process was repeated with S2. The next step was to water S1 again and record the fresh weight. Photosynthetic activity was measured under both light and dark again. The same step was repeated for S2. For the rest of the laboratory hours, photosynthesis was measured in samples S1 and S2 as they were drying out without further watering, recording the fresh

weight each time before measuring photosynthesis. The system measured the change in CO_2 concentration and other variables every 1.6 seconds.

Each day, fresh samples of the lichens were used, and the samples were reused only when there was no lichen left in the paper bag. The calculations for net photosynthetic activity and respiration were made by the system, but all data were also recorded in case of a recalculation or reevaluation of the data.

2.1.3 Calculations and Statistical Analysis

For the calculations for open system gas exchange, the mean values for light and dark measurements were calculated for each sample, and the mean values were used to further analyze the dataset.

For the data from closed system gas exchange, most of the calculations were made by the PP-System CIRAS 3, but to use the data in this thesis some further calculations were needed. First, the water content of the samples was calculated by comparing the weight of water present in the sample (fresh weight) to the weight of the samples in the dry state (also calculated for the open-system measurements). Further, each sample was inspected in R using a ggplot package, to see what the raw data looked like. Graphs were made both for the dark respiration (R_D) and light measurements (NE_L). Gross photosynthesis (GP) for each sample for each measurement was calculated as the difference between the final estimates of the NE_L measurement and the R_D measurement, which were calculated by the system (A, μmol m^{-2} s^{-1}). As mentioned in the introduction, using mass-based expression for photosynthesis and respiration provides more insight into the plant's growth dynamics, and will also be used in this thesis. To recalculate the gas exchange in R_D and NE_L, the gas exchange measurement was first multiplied by the area of the chamber in m^2 and then divided by the dry weight in grams. The GP in mass was calculated as the difference between NE_L and R_D measurements for each sample for each measurement. Samples with higher CO_2 values than 550 μmol mol^{-1} at the beginning of the NE_L measurements were excluded from further analysis.

To statistically analyze the data, a simple linear model function in R (lm) was used to assess the intercept, the relationship between WC and GP, WC and NE_L, WC and R_D, p-values, and adjusted R^2.

3 RESULTS

3.1 VAPOR PRESSURE EQUILIBRATION AND OPEN-SYSTEM GAS EXCHANGE

Table 2 presents the effect of different water potentials on the water content of lichen *Cladonia stellaris*. Initially, the data appears promising. For instance, CLST34 and CLST9 exhibit a positive gas exchange in the light, and a negative one in the dark, meaning that more photosynthesis is happening in the light. However, there is considerable variation among the samples. For instance, CLST6 demonstrates a negative gas exchange in the light, and a positive one in the dark, meaning that CO_2 is being captured in the dark, and not in the light. Conversely, CLST32 shows a positive gas exchange both in the light and in the dark, while CLST13 exhibits a negative gas exchange both in the light and in the dark.

A graph with gross photosynthesis and water content for the samples was made (Figure S1, supplements). From this graph, photosynthesis decreases with increasing water content, but the results are not statistically significant (p-value > 0.05). The water content (Table 2) also varies among the species and the treatments, and there is no general trend. As the data from the open-system measurements were variable and inconsistent, the procedure was changed to closed-system measurements.

Table 2: Light and dark measurements for *Cladonia stellaris* samples under different treatments (water potentials, ψ). A is the mean gas exchange measurement in μmol m^{-2} s^{-1}, and water content is in grams of water per gram dry weight.

Sample	Treatment	Light or dark	A	Water content
CLST6	0	L	-0.9	
		D	0.033	3.440
CLST32	-1	L	4.833	
		D	0.4	6.298
CLST17	-4	L	1.4	
		D	0,3	6.466
CLST36	-1	L	0.567	
		D	0	5.294
CLST24	-8	L	1.875	
		D	0.167	6.380
CLST15	-4	L	8.667	
		D	0.067	3.632
CLST9	-2	L	9.833	
		D	-0.167	4.739
CLST3	0	L	0	
		D	0	2.895
CLST13	-4	L	0,3	
		D	-0.0333	6.097
CLST23	-8	L	-0.333	
		D	0.5	4.538
CLST29	-16	L	-0.567	
		D	0	5.453
CLST34	-1	L	0.667	
		D	-0.067	6.852

3.2 Passive Drying in Closed-System Gas Exchange

An example of how the raw data looked can be seen in Figure 5. Graph B shows a good example of what the raw data looked like. The line for light measurements (green) has a negative slope, meaning that more photosynthesis is happening, and more CO_2 is consumed than produced. Graph A shows also that some of the raw data were not as expected: the line with the data point for light measurements has a positive slope, which means that more respiration than photosynthesis was happening during the light measurement. More examples of strange measurements are presented in the supplements (Figure S2). It is worth noting that the scale of change in CO_2 in Figure S2 might be different than the scale in Figure 5.

Since the CO_2 conditions in the room were uncontrolled due to human activity, a graph was made to check the relationship between the GP and initial CO_2 for all *C. stellaris* samples (Figure 6). The graph shows a positive relationship between higher CO_2 values and gross photosynthesis. The relationship is also statistically significant (p-value < 0.05), and 16.7% of the variability of gross photosynthesis is explained by the initial CO_2 concentration. This graph is also an example of how uncontrolled the conditions in the room were, and therefore samples with starting CO_2 values higher than 550 µmol mol^{-1} were excluded from further analysis.

To further analyze the data set, the water content of the lichens and gross photosynthesis were calculated. As it is hard to calculate the area of a lichen sample, dry weight was used to get the photosynthesis in µmol g^{-1} s^{-1}.

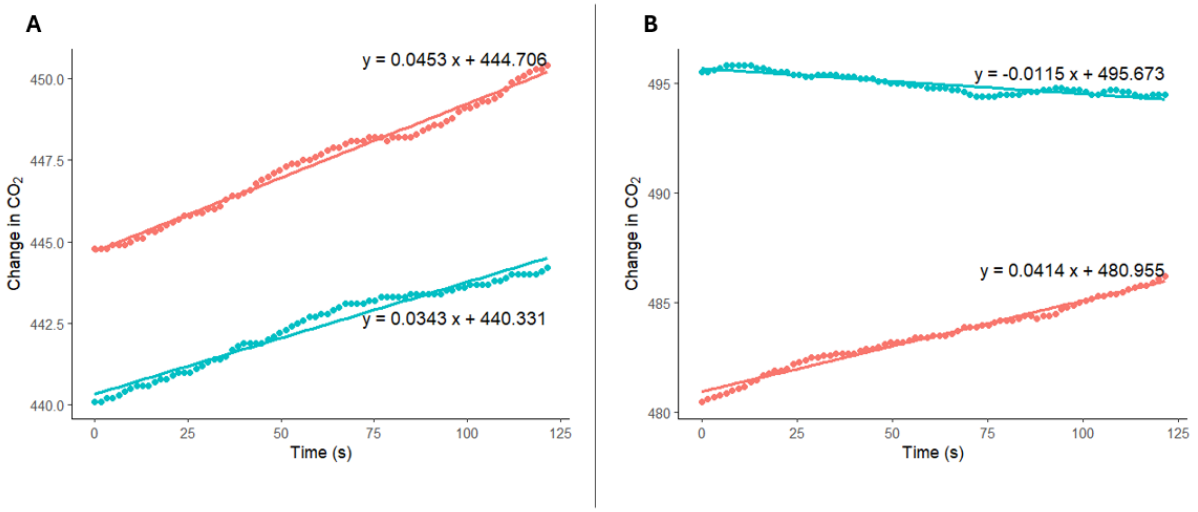

Figure 5: Change in CO_2 concentration in the chamber during the measurement under light (green) and dark (orange) conditions. The CO_2 concentration in graph A under light measurement (green) has a positive slope, while in graph B the CO_2 concentration under light has a negative slope. Both graphs are for the same sample of *C. stellaris*, CLST09. The graphs were made at different WC A: 2.289 and B: 1.873.

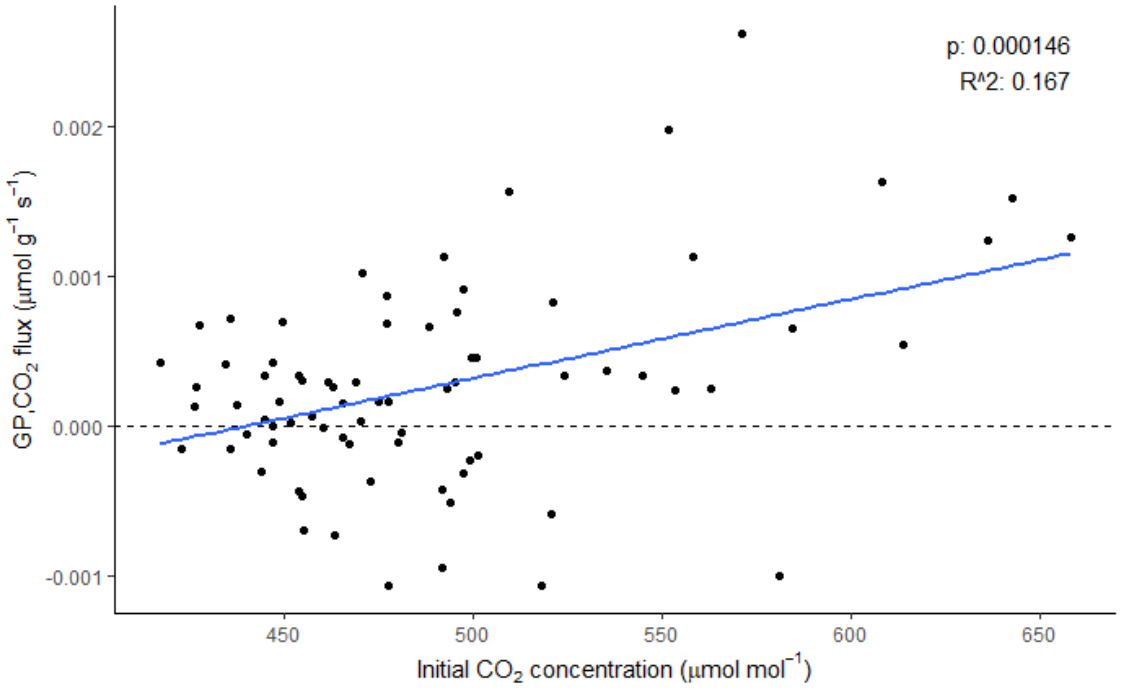

Figure 6: Gross photosynthesis for all *C. stellaris* samples with initial CO_2 levels under light measurements. The p-value and adjusted R^2 are presented.

3.2.1 Individual Samples

Figure 7 is an example of *C. stellaris* samples to show how variable the results are. The graphs for the other species can be found in the supplements (Figure S3). A similar graph with chamber area instead of mass was also made and it can be found in the supplements (Figure S4). From those graphs, it is hard to see for any of the species if there is a general trend for the relationship between water content and GP since the results vary a lot. It is important to mention that initial NE_L measurements were taken right after the samples were moistened with water, so the prediction here was that the samples were not active yet. Some samples, like one of the *C. stellaris* samples, CLST12, had positive photosynthesis from the beginning. It is important to mention, that both in Figure 7, and Figures S3 and S4 in the Supplements, the x-axis goes from low water content to high water content, but the measurements were taken from high water content to low water content.

Linear models were conducted for each sample for all species to check for significance (Table S1). The table was made only for the slopes from graphs with gross photosynthesis in terms of mass. When there were not enough data points (less than 4) to get the slope and/or p-value, the sample was not considered. From this table, twelve *C. stellaris* samples have a negative relationship between water content and gross photosynthesis, and two samples have a positive relationship. Only one *A. ochroleuca* sample, ALOC05, has a

negative relationship between water content and gross photosynthesis, while the other six have a positive relationship. ALOC05 is also the only sample that is statistically significant (p-value < 0.05). Eight *F. nivalis* samples have a negative relationship between water content and gross photosynthesis, and three have a positive relationship. No *F. nivalis* sample has a statistically significant relationship (p-values > 0.05). Three *C. rangiferina* samples have a negative relationship, while five have a positive relationship between water content and gross photosynthesis. None of the samples have a statistically significant relationship (p-values > 0.05). Six *B. divergens* samples have a negative relationship, and only one has a positive relationship, nonetheless, none of the samples have a statistically significant relationship. Only one *S. paschale* sample has a negative relationship, and the other seven have a positive relationship. None of the samples have a statistically significant relationship. As mentioned, from those results it is hard to see any relationship between water content and GP for the six lichen species, as the results are not consistent.

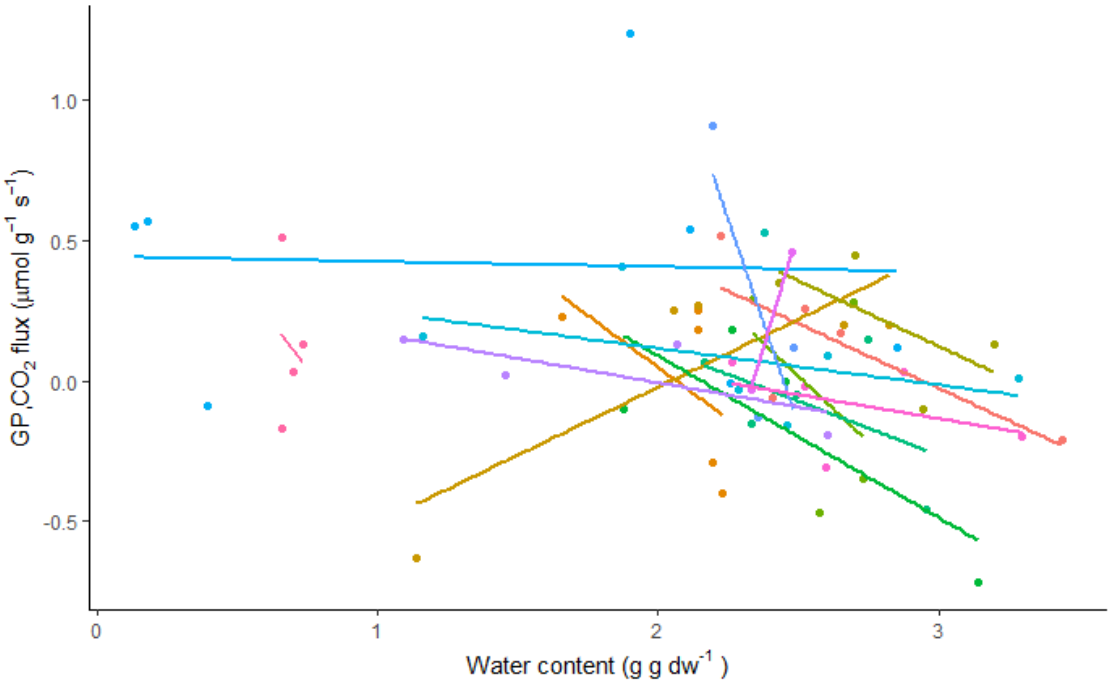

Figure 7: Gross photosynthesis depicted as CO_2 flux per gram of lichen dry mass for *C. stellaris*. The water content represents fresh weight in grams per lichen dry weight. Each data point represents the difference between gas exchange in the light and the dark at the end of the measurement, and each color represents a different sample. The lines represent different samples used during the measurements.

3.2.2 General Trends in Species

To check if there are any general trends from all the samples from each species, one line was fitted across all the samples for each species for NE_L and R_D measurements, Figures 8 and 9, respectively. Both of those show that with higher water content, the gas exchange is more negative, meaning that the samples do more respiration than photosynthesis. For NE_L measurements, only *C. rangiferina* has a statistically significant relationship, and for R_D measurements, only *B. divergens* has a statistically significant relationship (p-values < 0.05). Except for *C. rangiferina* and *B. divergens*, Figures 8 and 9, respectively, all graphs have exceptionally low R^2 values, and some of them are negative. For *C. rangiferina* and *B. divergens*, 11.7% and 26.5% of the variability in gas exchange is explained by water content.

A similar graph was made for all species using the calculated GP (Figure 10). All species except *A. ochroleuca* show a negative relationship between GP and water content, the higher the water content, the lower the photosynthesis. *A. ochroleuca* shows a slightly positive relationship between GP and water content, meaning that GP increases with increasing water content (Table S2). To check if any of the relationships are statistically significant, p-values for each linear model have been calculated (Figure 10). None of the linear models for any of the species have a statistically significant relationship between GP and water content (p-values > 0.05). The adjusted R^2 can show how well water content explains the variability of gross photosynthesis. Values for R^2 are displayed in Figure 10, and all the R^2 values are either exceptionally low or negative. A table with the statistical analysis can also be found in the supplements (Table S2). The same type of graph, but with GP in terms of area can be found in the supplements (Figure S5). From Figure S5, just as in Figure 10, the relationships are negative for all species except for *A. ochroleuca*, which has a positive relationship. One difference in Figure S5 is that the relationship for *C. stellaris* is statistically significant from the displayed p-value in the graph. A linear model was conducted to check the p-value, and it showed 0.0500. When GP is presented as μmol CO_2 m^{-2} s^{-1}, *C. stellaris* is the only species that has a statistically significant linear relationship. Overall, Figures 8, 9, and 10, and Figure S5 in the supplements, show that an increase in water content decreases photosynthesis and increases respiration, but the results are not statistically significant.

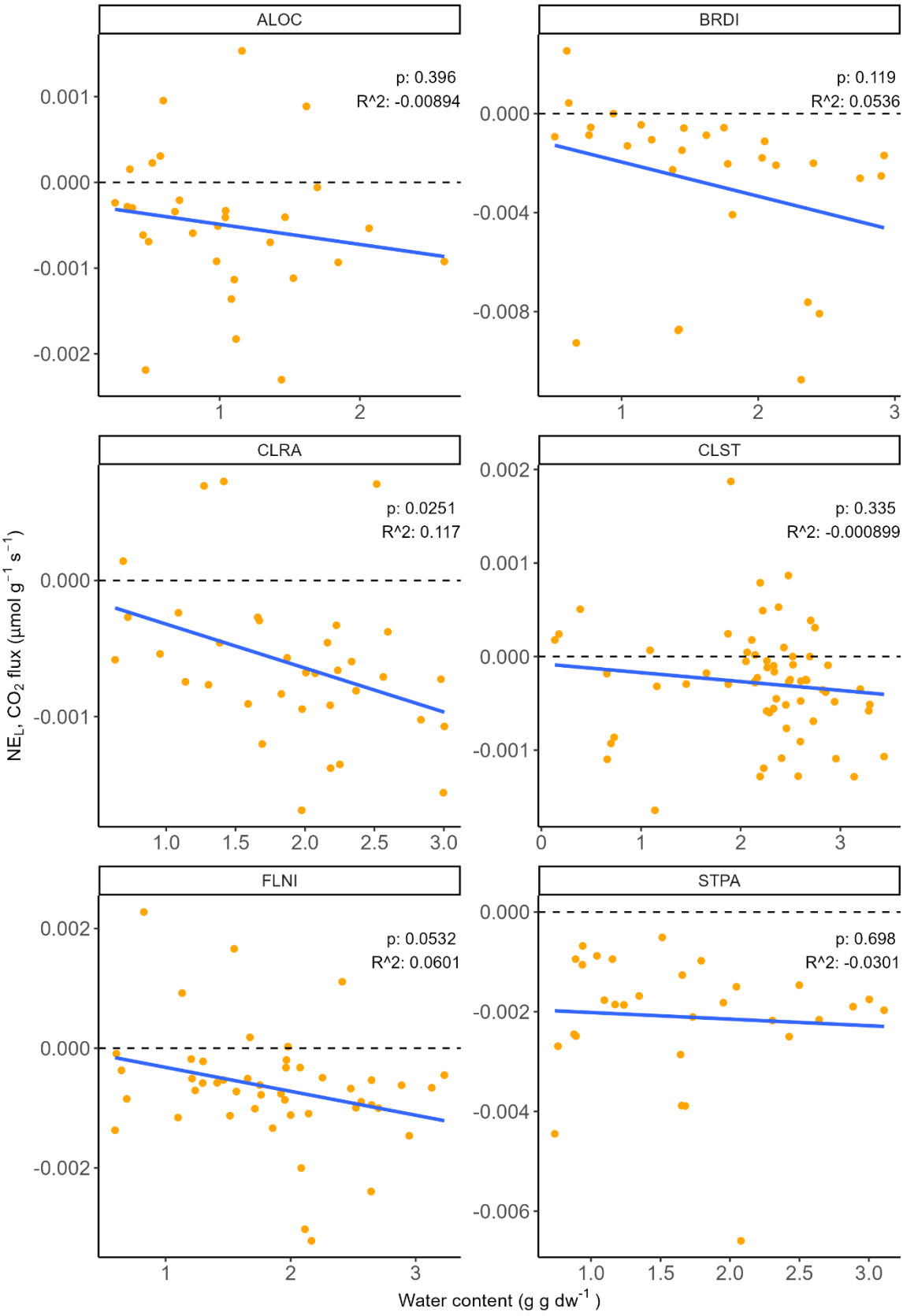

Figure 8: Net exchange in light as CO_2 flux for six lichen species: *A. ochroleuca* (ALOC), *B. divergens* (BRDI), *C. rangiferina* (CLRA), *C. stellaris* (CLST), *F. nivalis* (FLNI) and *S. paschale* (STPA) in the light. Each data point is the difference between the final estimate of light measurement and the final estimate of dark measurement. The graph also displays the p-values and adjusted R^2 for each species.

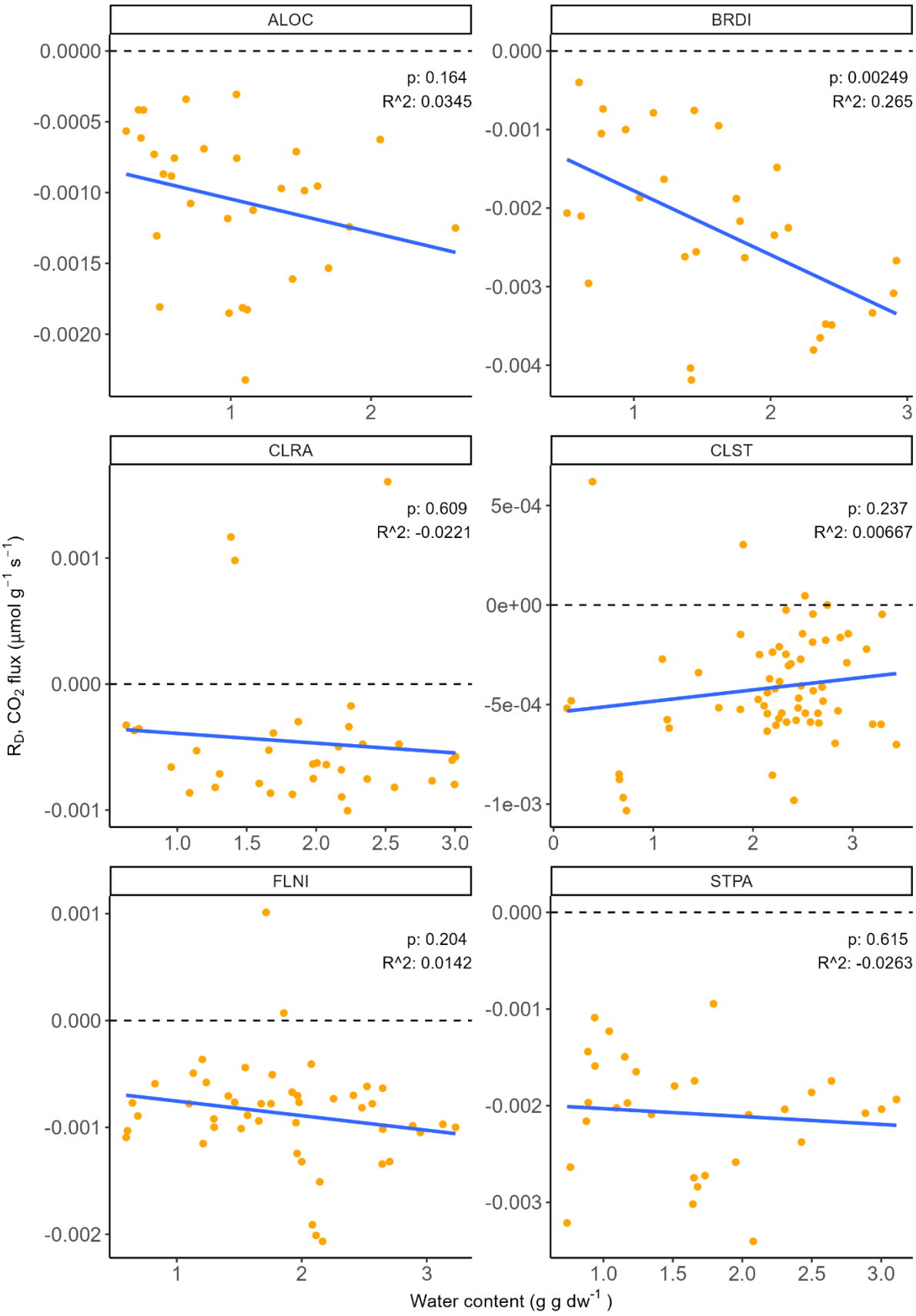

Figure 9: Dark respiration for six lichen species (as in Figure 6). Each data point is the difference between the final estimate of light measurement and the final estimate of dark measurement. The graph also displays the p-values and adjusted R^2.

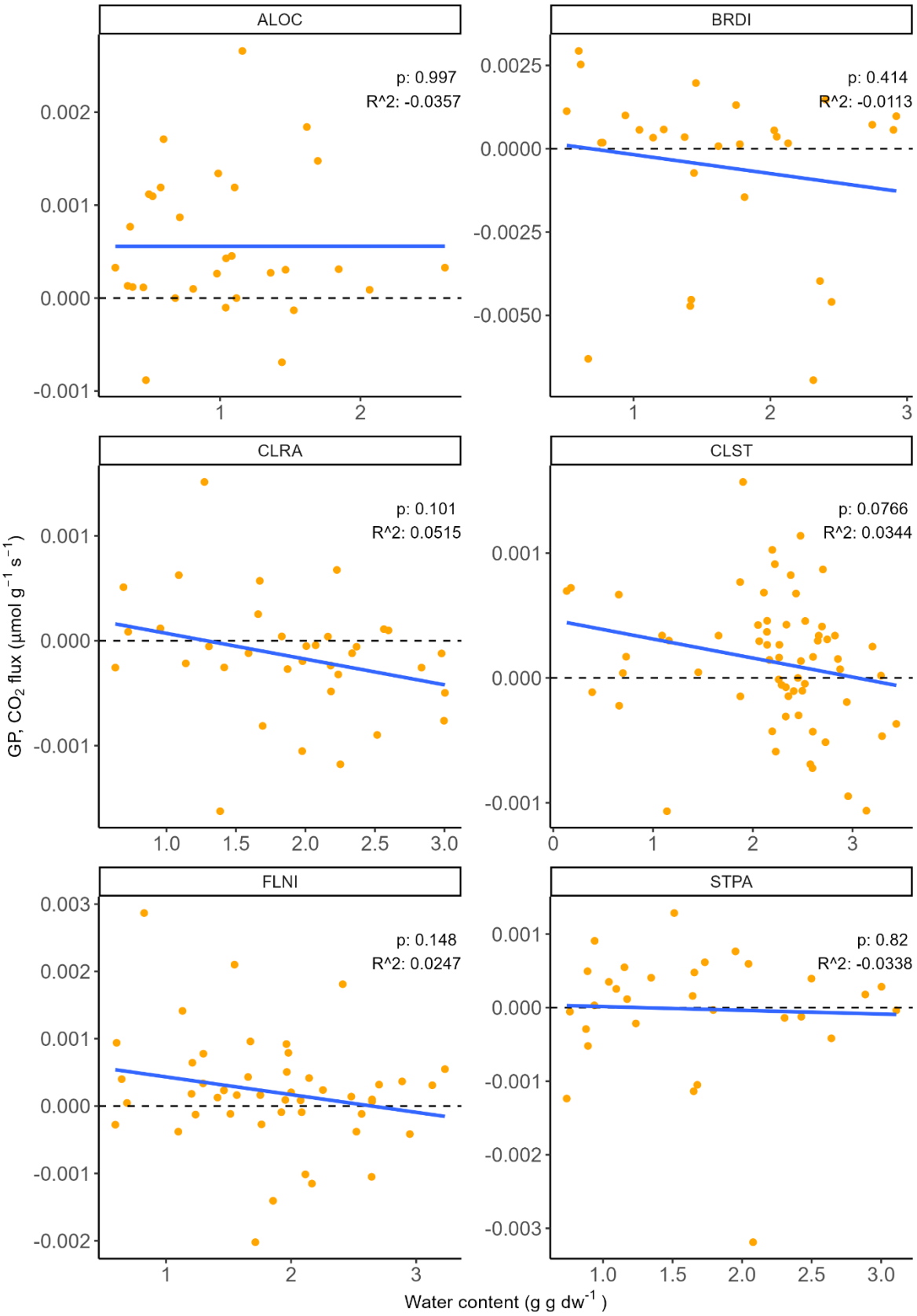

Figure 10: Gross photosynthesis as CO_2 flux for six lichen species (as in Figure 6). A linear regression line was fitted through all the samples to check for any trends in the species. Each data point is the difference between the final estimate of light measurement and the final estimate of dark measurement. The graphs also display the p-values and adjusted R^2 for each species.

4 DISCUSSION

Photosynthesis in lichens, just as in higher plants, depends on many factors, like temperature, light conditions, and water availability. Lichens are adapted to the environment they live in, and the different temperatures and light conditions provided by the environment. For example, Antarctic lichens have shown high net photosynthetic rates at low temperatures (Ino, 1985). Generally, lichens can be photosynthetically active over a wide range of temperatures, with the optimum temperature lying between 10 and 22℃ (Barták, 2014). Different lichen species also have different adaptations to light. Lichens have distinct light saturation and compensation points, corresponding to the environment they live in (Green et al., 1997). Lichens respond to light intensities as higher plants do only when the thalli are at optimum WC. It seems like WC is the most key factor determining the photosynthetic activity of lichens. Lichen thalli are dry most of the time, and they become wet either during the night with the fog or morning dew, or rainfall during the day. They dry out quickly, and they also can become suprasaturated, for example during rainfall. Suprasaturation limits the diffusion rates of CO_2 into the thallus and hence decreases photosynthesis (Lange and Green, 1996). Lichens can be photosynthetically active when the conditions, and especially the hydration levels, are just right (Honegger, 2006). In his paper, Barták (2014) mentions that in a "particular alpine ecosystem, the species were photosynthetically active only 16-25% of the observation time." He also mentions that the most limiting factor is the hydration level of the thallus. Dehydrated lichens cannot do photosynthesis, even if the light and temperature conditions are met.

Measuring photosynthesis through the manipulation of the water content using PEG solutions in the open-system gas exchange presented unforeseen complications, including challenges related to CO_2 recycling, CO_2 equilibrium, and instrument calibration. Treating lichens as leaves in the open-system gas exchange, and as partial communities in the closed-system presented another challenge along the way. However, each difficulty, together with thorough research, provided valuable insights and learning opportunities. The experiment not only provided a framework for refining the measurement methodologies but also expanded the understanding of lichen photosynthesis.

4.1 Instrument Difficulties

One of the problems encountered especially during the open-system measurements, is the CO_2d, the CO_2 Differential in the PP-System CIRAS 3. CO_2d is the difference between the air going into the system and the air going out of the system, and with no sample in the cuvette, the $CO2_d$ should ideally be 0 (PP Systems, 2013). The Operation Manual for CIRAS-3, suggests that for small gas exchange measurements, the errors in the CO_2d could become significant. The problem encountered during the measurements was that the CO_2 would not stay stable around 0, but it varied a lot between different values, sometimes getting to -7 even after waiting for two hours. The problems with the CO_2d made the measurements even more complicated, as the waiting time for equilibration of the CIRAS-3 between each sample was longer.

Several factors may contribute to these fluctuations. If the system had any leakages, it could potentially affect the CO_2 concentrations, resulting in fluctuations. Changes in environmental conditions, like humidity and temperature could also affect the CO_2d, as well as sample handling, like inadequate sealing. Another reason could be that the moisture, both from the samples and the air, affected the CO_2d because the samples were held in beakers with different moisture contents. The system could also have residual CO_2 inside from previous trials with CO_2 control, and this could potentially also affect the CO_2d.

4.2 CO_2 Equilibrium

One of the encountered complications, especially during the open-system gas exchange was the CO_2 concentrations in used lichen samples and the surrounding air. Since the samples were held in beakers with different water potentials, there could be variations in the dissolved CO_2 concentrations in the extracellular water in the thallus and the air. Lower CO_2 concentrations in the thallus water could make CO_2 from air diffuse into the thallus, to reach an equilibrium, and thus make it look as if the samples are photosynthesizing in the dark. Higher CO_2 concentrations in the lichen thallus could make CO_2 diffuse from the thallus water to the air to reach equilibrium and make it look like the samples were respiring in the light. CO_2 dissolves also better in cold water. The temperature in the growth chamber was set to 15℃, and the temperature in the room was 25℃. Different temperatures in the lichen thallus and the ambient air could also influence how the CO_2 diffused either in or from the lichen thallus. Both of those scenarios could lead to misinterpretation of the lichens' activity.

4.3 CO_2 RECYCLING

Numerous vascular plants have different pathways to maximize photosynthesis such as C3, C4, and CAM, collectively known as Carbon Concentrating Mechanisms (CCMs). Similarly, lichens have their CCMs: for instance, green algal lichens feature pyrenoids, and cyanolichens utilize carboxysomes for this purpose (Koch et al., 2023).

However, despite these adaptations, gas exchange measurements in lichens conducted under open-system conditions have yielded inconsistent results (Table 2). Some samples have shown respiration during periods of light exposure and photosynthesis during dark periods. Notably, research by Ten Veldhuis et al. (2020) revealed that exposing lichen samples to high light intensities led to a linear increase in fungal respiration during subsequent dark periods, so the lichen tips were expected to respire more in the dark.

This phenomenon underscores the interplay between the mycobiont and the photobiont components of a lichen. While the photobiont engages in photosynthesis only when light is available, the mycobiont continues to respire all the time when the thallus is hydrated. This mycobiont respiration, coupled with the different CO_2 concentrations within the thallus and the surrounding air, could result in a non-equilibrium state. Due to this non-equilibrium, CO_2 could have diffused from the lichen thallus to the air. Then, the produced oxygen by photosynthesis could be utilized by the mycobiont to produce CO_2. This recycled CO_2 could then become available to the photobiont for further photosynthetic activity, especially when the CO_2 is diffusing out of the lichen thallus due to different conditions.

In these conditions, the respiration may exceed the photosynthesis, to meet the CO_2 requirements of the photobiont, leading to a negative gas exchange during light exposure. There might be ongoing photosynthesis which is "undetected" due to net CO_2 out of the system, which would mean $R_D > NE_L$. These findings highlight the complexity of the symbiosis and the metabolic interaction within the lichen thallus and emphasize the need for further investigation into the factors affecting gas exchange dynamics in the symbiosis.

4.4 OPEN-SYSTEM GAS EXCHANGE

In this part of the experiment, only lichen tips were used to assess the photosynthetic activity of *Cladonia stellaris* under different water potentials (ψ). Twelve different samples were used, which were equilibrated under specific water potentials to achieve different water contents of the samples. The expectation was that with lower water potential, the samples would have lower water content and thus lower photosynthetic activity. The results in Table 2 are not as expected. Most of the samples display different rates of gas exchange than expected, samples ranging from -1 to -8 water potential show photosynthesis under light and respiration in the dark. The rest of the samples showed

only positive, or only negative results under both light and dark conditions. One of the reasons for this could be, because of the different CO_2 concentrations in the lichen's thallus extracellular water and the surrounding air or because of the difficulties with the instrument, as discussed previously. No suprasaturation was expected during this experiment, as the lichen tips had equilibrated with the conditions in the beakers and subsequently the surrounding air. According to Lange and Tenhunen (1981) and Lange et al. (1999), suprasaturation in lichens typically occurs when the samples are exposed to liquid water. In their experiments, they soaked and sprayed the samples with water to achieve suprasaturation. In the open-system measurements, the photobionts obtained water from the air in the beakers and the surrounding air, as green algal lichens are expected to do (Bidussi et al., 2013).

As for water content, the expectation was that with lower water potential, the water content of the lichens would also decrease, as shown by Möller et al. (2022) who studied mosses and lichens. Calculations of the water content have shown that samples with the highest water potential of 0 ψ have the lowest water content, and the highest water content is obtained by a sample held at -1 ψ (Table 2). The lichen sample with the lowest water potential of -16 ψ was expected to have the lowest water content since lower water potentials mean less free water to move. The lichen sample of -16 ψ has an intermediate water content value, compared to other species with lower and higher water contents. The photosynthetic activity did, however, decrease with increasing water content (Figure S1), as was expected, but the results are not statistically significant.

As lichens are poikilohydric, and they have no other structures like roots for taking up water, they become active either in humid air or via contact with liquid water: lichens with green algal photobionts can take up water from the air (Lange et al., 1990, Bidussi et al., 2013). All six lichen species used in this thesis have a green alga as a photobiont, apart from *S. paschale* which also contains a cyanobacterium as a secondary photobiont. This could potentially mean that the lowest used water potential was not low enough to see the effects of decreasing water content. Fundamentally, the photobionts may have retained access to water, ensuring they could uptake as much as required for their metabolic activities.

4.5 Closed-System Gas Exchange

As the first approach with open system measurements and different vapor pressures showed to be insufficient, a decision was made to use closed system measurements with parts of whole lichen mats, not only the tips. The expectation here was also that photosynthesis would increase, then reach maximal photosynthesis with WC_{opt}, and then decrease with decreasing water content, as the samples passively dried out. Figures 7, S3,

S4, and Table S1 show that the photosynthetic activity was more variable than thought. Some of the samples showed decreased photosynthesis with increasing water content, and other samples had higher values with increasing water content.

4.5.1 The Effect of Using Whole Lichen Mats

Green et al. (2008, Fig. 9.2) showed that tips are the most photosynthetically active part of the lichens and that the lower part does not have as much activity. It could be compared with a tree. The leaves of the tree are the photosynthetic part, just as lichen tips, and the roots and the trunk are the non-photosynthetic, just as the mycobiont in the lichen. The photosynthesizing lichen tips, just like leaves, must make enough sugars to sustain both themselves and the mycobiont. The photobionts are, however, not tree leaves. Leaves are supported by the stem and the roots, which give them nutrients and water, and leaves are on the outside of the tree, making the tree trunk a support tissue for the leaves. However, the photobionts are buried within the support tissue, the fungus. Thus, measuring the photosynthetic activity of the lichens is more complicated, especially when using whole lichen mats, compared to tree leaves.

Focusing on the lichen mats instead of the lichen tips can give a picture of how well the photobionts work to sustain not only themselves but also the mycobiont. It could also lead to an underestimation of the overall photosynthetic activity of the photobionts, as the lower part of the lichen respires. For example, it could look like the photobionts did not achieve their maximum activity as the gas exchange was still increasing. Since the CIRAS-3 system measures overall gas exchange, including the respiration of the mycobiont, it might seem like the photobionts did not reach their full potential. However, this could be misleading, as the photobionts might have indeed reached their maximum activity. The ratio of photobiont to mycobionts can influence the interpretation of the results, as the mycobiont "forms the majority of the lichen thallus" (Barták, 2014), and Sundberg et al. (1999) mention that the photobiont can make up as little as 10% of the whole lichen biomass. Even if the photobionts are operating at full capacity, the overall gas exchange may still reflect ongoing metabolic activity, including mycobiont respiration. Thus, it could give the impression that the photobionts are striving to overcome the mycobiont respiration and reach their full activity.

Lichens could also be compared to an ecosystem with producers, consumers, and decomposers. The photobionts in the thallus would be the producers, making sugars for themselves and the fungus. The fungus consumes the sugars made by the photobionts. As lichens grow, the bottom, possibly the dead part of the lichen thallus, is being decomposed, and the decomposability of lichens is affected by many factors, for example, nitrogen concentration (van Zuijlen et al., 2020b). This process may potentially cause a lot of respiration, altering the photosynthetic measurements.

4.5.2 Rewetting and Compensation Point

As for the open system, measurements here were also very variable (Figures 5 and S2). An example of expected data can be seen in Figure 5B, where the *C. stellaris* sample demonstrates a linear relationship where it exhibits photosynthesis under light. The same sample in Figure 5A shows a higher rate of respiration in the light. Measurements from the same samples were used to make graphs, but the time after rewetting for the samples was different. Measurements used in graph A were taken 1.84 hours after rewetting (ca. 110 minutes, WC: 2.289 g gdw^{-1}), and measurements in graph B were taken 2.92 hours after rewetting (ca. 175 minutes, WC: 1.873 g gdw^{-1}). The calculated GP is negative for graph A, meaning that the sample is doing more respiration, and for graph B the GP was positive. According to a study by Groulx and Lechowicz (1987), *C. stellaris* needs 365 minutes to reach 99% of the maximal photosynthesis, but this is an estimate of the full recovery of the photosynthetic activity. They also show that *C. stellaris* needs around 150 minutes to reach the point where the graph begins to plateau when photosynthesis begins to be more stable (Groulx and Lechowicz, 1987, Fig. 1D). This could explain why graph A shows less photosynthesis than graph B, the sample needed more time to overcome the mycobiont respiration. Then again, they found that the compensation time for *C. stellaris* was 15.7 ± 7.1 minutes. According to Groulx and Lechowicz, the sample should have reached the compensation point 110 minutes after rewetting, so the net photosynthesis should be positive. The difference between the samples in this thesis and the samples used by Groulx and Lechowicz lies in the tissue type. They used lichen tips, recognized as the most photosynthetically active tissue (Green et al., 2008), whereas here the entire lichen tissue, including the less active lower parts. The difference in tissue type might clarify why the sample in graph A displayed more respiration than photosynthesis 110 minutes after rewetting, as explained before.

4.5.3 Individual Samples

Looking at the individual samples for each species it is hard to see if the expectations were met, as the results are very variable (Figures 7, S3, and S4). For all species, some of the samples have positive slopes, increasing GP with increasing water content, while others have a negative slope, decreasing GP with increasing water content. It is even possible to see that some samples have positive measurements from the beginning, under the first measurement after rewetting. "Air-dried" lichens are lichens that dry naturally in the air, like the passive drying used in this experiment. According to Green et al. (2008), the air-dried lichens fall into conditions of "activation of the TCA cycle" and have a water layer on the enzyme, and therefore cannot be considered inactive. The samples were stored in bags before the measurements, and before wetting, they were resting on the bench. Given that lichens, particularly those with green algae, are capable of absorbing the water from the

atmosphere (Lange et al., 1990, Bidussi et al., 2013), they could still be active, either having retained their activity in the bags or become reactivated while resting on the bench before being rewetted.

Honegger (2006) discovered that photobionts are coated with metabolites derived from the mycobiont which "prevent waterlogging at high levels of thalline hydration". This hydrophobic layer maintains the algal layer air-filled during wetting periods and facilitates the movement of solutes from the surface (Honegger, 2006, Büdel and Scheidegger, 2008). Typically, these hydrophobic layers are found atop the hydrophilic layers (Honegger, 2006), and together they may enhance water retention, ensuring sufficient moisture around the photobionts, even at low water content (WC << 1). Therefore, it might be crucial to dehydrate the samples to WC << 1 to maximize the effects on the photosynthetic activity. As noted, green algal lichens can absorb water from humid air and conduct photosynthesis. The combined action of the hydrophilic and hydrophobic layers can possibly retain adequate moisture around the photobiont to photosynthesize, even though the samples might feel dry to the touch.

4.6 CO_2 Levels, CO_2 Control and Suprasaturation

Due to human activity in the room, it was hard to control the CO_2 levels. This influenced the photosynthetic activity of the lichens, as mentioned before, some of the samples had positive levels from the first measurements after first rewetting. Photosynthesis also increases with increasing CO_2 concentration (Figure 6).

Since the lichens were just sprayed with water until they felt wet with no control of the actual levels of water content and suprasaturation, it could have been that some of the samples were suprasaturated. As mentioned above, some of those samples could have been active because of the air-dried state, and together with the suprasaturated state after rewetting and high CO_2 levels, the samples could then have positive NE_L measurements from the beginning after the first rewetting. A study conducted by (Lange and Tenhunen, 1981) showed that suprasaturated samples exposed to high CO_2 levels can overcome the suprasaturated sate and the decreased CO_2 diffusion into the thallus. This could be the reason some of the samples showed photosynthesis during the first measurement, hence the decision to exclude the measurements where the initial CO_2 concentration was higher than 550 μmol mol^{-1}.

4.7 General Trends in the Species

The general expectation was that GP would increase with decreasing WC, reach a maximal value of GP with WC_{opt}, and then decrease again with decreasing WC. This expectation was only partially met.

While the WC decreased, the NE_L increased for all the species, yet none showed positive NE_L values (Figure 8). Notably, a significant portion of the NE_L measurements is attributed to the mycobiont, which, as mentioned earlier, respires when the thallus is wet. The mycobiont is also a bigger part of the lichen symbiosis compared to the photobionts, and it is essential to consider its impact on the overall net exchange.

The reasons why none of the species showed positive NE_L measurements could lie in the used samples. As the mycobionts respire, the photobionts must overcome the respiration to reach positive net photosynthesis. From Figure 8, it looks like none of the lichens' photobionts were able to overcome the mycobiont respiration. Some species have a steeper increase in NE_L than others, for example, *C. rangiferina* has a much steeper increase than *S. paschale* (Figure 8). This variability suggests species-specific adaptations and functionalities within the fruticose, mat-forming lichens.

Examining R_D, all samples, except *C. stellaris*, show a decrease in respiration with decreasing WC, while *C. stellaris* shows an increase in respiration (Figure 9). Moreover, the rate of decrease in R_D varies among species, with some experiencing a more pronounced decline compared to others. For instance, *B. divergens* shows a sharper decline in respiration than *F. nivalis*.

As previously discussed, lichens are only active when hydrated, this applies both to photosynthesis and respiration. As the lichens dry out, not only will photosynthesis decrease, but also the respiration until the lichen reaches a state of metabolic dormancy due to desiccation. Sundberg et al. (1999) measured respiration in five foliose lichen species after rehydration. The study showed that for all species respiration was highest in the beginning after rehydration and decreased with time after rehydration. This supports the results for five of the fruticose species in this experiment, where *A. ochroleuca*, *B. divergens*, *C. rangiferina*, *F. nivalis,* and *S. paschale* show higher respiration with higher WC and a decrease in respiration with decreasing WC over time.

As for *C. stellaris*, the results for R_D are not as expected since this species shows an increase in respiration with decreasing water content. For all species, the measurements have varied a lot, making it hard to measure the activity under light and dark measurements and calculate the GP (Figures 5 and S2). These variabilities could be the reason *C. stellaris* shows a different result in R_D than the other species.

Looking at GP, the difference between NE_L and R_D provides insights into the photosynthetic activity of lichens. Notably, all samples, except for *A. ochroleuca*, exhibit an increase in GP as WC decreases (Figure 10). Examining Figure 10 alone shows that *A. ochroleuca* has no increase in GP, but according to conducted linear models (Table S2), *A. ochroleuca* displays a subtle increase in GP. All samples, except for *A. ochroleuca*, have also reached the compensation point (y = 0) at different WC, but none of the samples have reached a maximal GP (Figure 10). This clearly shows that the photobionts of the species are photosynthesizing at different rates.

Several factors contribute to the absence of maximal photosynthetic rates observed in this experiment. Firstly, the duration of measurements may have influenced the results. Groulx and Lechowicz (1987) showed in their study on subarctic lichens that distinct species need substantial time to reach the maximal photosynthetic activity. For instance, *B. divergens*, *A. ochroleuca*, and *C. stellaris* needed 143, 257, and 365 minutes, respectively, to reach 99% of their maximal photosynthetic activity. However, these durations were not consistently reached in this experiment, potentially explaining the lack of observed maximal GP.

Furthermore, the choice of tissue used for measurements may have impacted the results. In this study, whole lichen mats were used, whereas Groulx and Lechowicz (1987) focused on lichen tips. The differences in tissue type could have influenced the photosynthetic activity, as previously discussed.

Moreover, the presence of CCMS in some lichen species introduces another layer of complexity, complicating the interpretation of GP recalculated from NE_L and R_D. The PP CIRAS-3 System has no way of distinguishing photosynthesis in lichens that comes from the CO_2 from the ambient air and that coming from CCMs. In leaves, it is possible to calculate C_i ("sub-stomatal CO_2 concentration", (PP Systems, 2013)), which if very low while achieving net photosynthesis, would indicate a C4-type CCM. CO_2 recycling by photobionts in response to mycobiont respiration may have influenced GP results, especially if samples were suprasaturated during the initial measurements.

Given the variability in the data, distinguishing genuine trends within NE_L, R_D, and GP becomes a formidable task (Figures 8-10). Few samples exhibit statistical significance, with notable exceptions such as *C. rangiferina* in NE_L measurements and *B. divergens* in R_D measurements. Furthermore, the adjusted R^2 values remain modest across all species and graphs.

These observations underscore the complex nature of lichen photosynthesis. The dynamic interplay of the photobionts and mycobionts, influenced by several factors, presented a challenge when measuring photosynthesis.

4.8 Recommendations For Gas Exchange Measurements In Mat-Forming Lichens

Measuring photosynthesis using the open-system method posed unforeseen challenges, including instrument difficulties, CO_2 equilibrium, and moisture content. Among these, achieving CO_2 equilibrium within the system emerged as the most formidable obstacle. To address this challenge, several potential solutions present themselves. One approach could involve ensuring uniform air conditions by employing the same air for the samples both in the beakers and during the measurement. This could be achieved using a bubbler: air can be bubbled through the same PEG solutions as used for the equilibration, which would ensure that the air in the system has the same vapor pressure and relative humidity as the air in the beakers, and hopefully the same CO2 concentrations. This idea comes from Groulx and Lechowicz (1987), who bubbled air through distilled water to avoid drying of the samples thalli. Alternatively, integrating the passive drying of samples, while utilizing the open-system approach could offer a promising opportunity for enhancing measurement accuracy. However, managing sample water content, and when to measure it, would require careful consideration, given the rapid drying observed in cuvette-based setups.

The closed-system method had challenges like the lack of CO_2 control, resulting in varying initial CO_2 concentrations among samples. Implementing CO_2 control measures to standardize initial concentrations could mitigate this issue, potentially leading to more consistent and reliable results. Additionally, extending the drying times to reach maximum photosynthetic activity and identify optimal water content levels could further enhance the depth of data collection.

Reflecting on these challenges and exploring potential solutions allows us to gain valuable insights into the complexities of measuring lichen photosynthesis and gives us the possibility to better our methodologies to ensure consistent and reliable measurement

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SUPPLEMENTS FOR RESULTS

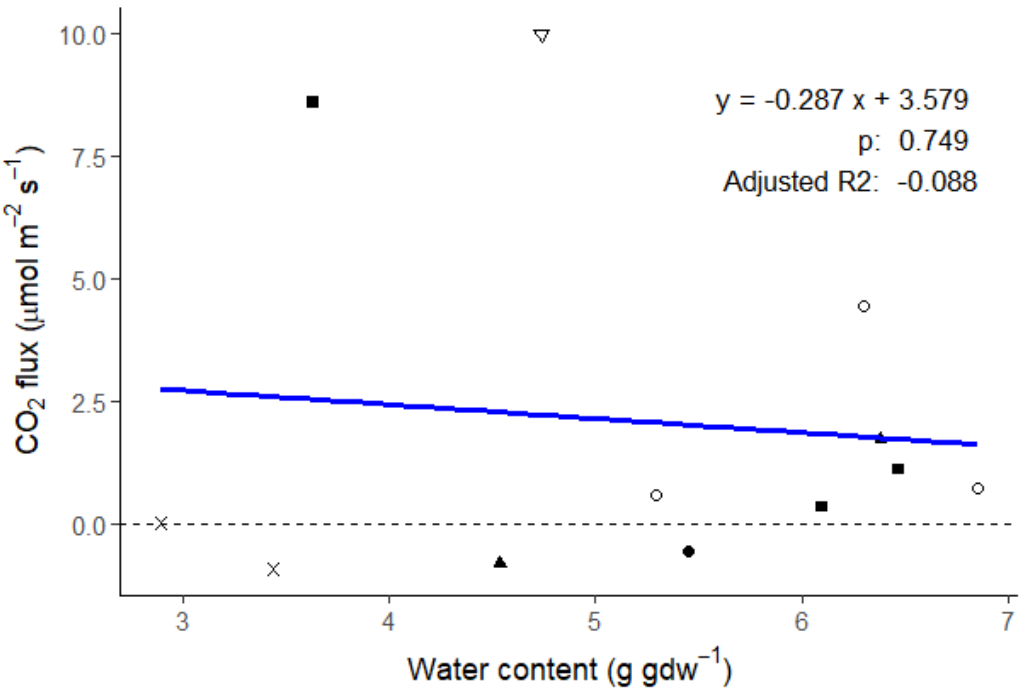

Figure S1: Gross photosynthesis calculated as the difference between the mean of light measurements and the mean of dark measurements for twelve samples of *C. stellaris* under different treatments (circles: -16 ψ, triangles: -8 ψ, squares: -4 ψ, open triangles: -2 ψ, open circles: -1 ψ, and x: 0 ψ).

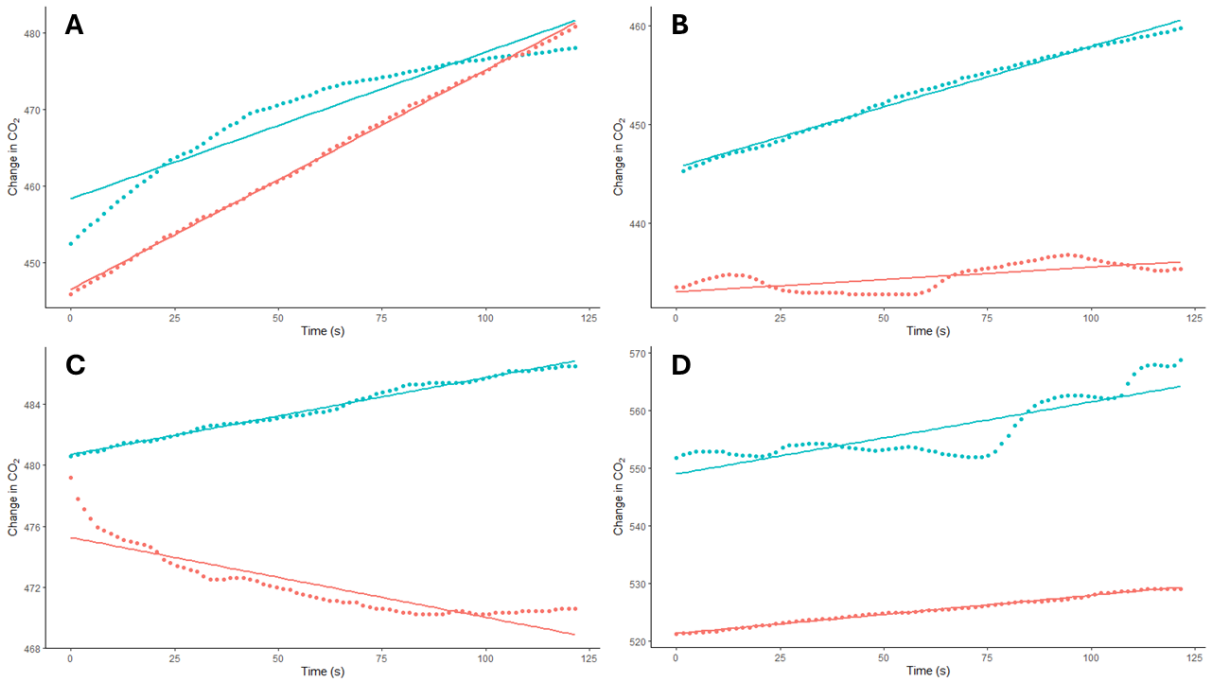

Figure S2: Examples of strange results during the measurements in the closed-system gas exchange. Green are light measurements and orange are dark measurements. A: *S. paschale*, B: *F. nivalis*, C: *C. rangiferina* and D: *C. stellaris*.

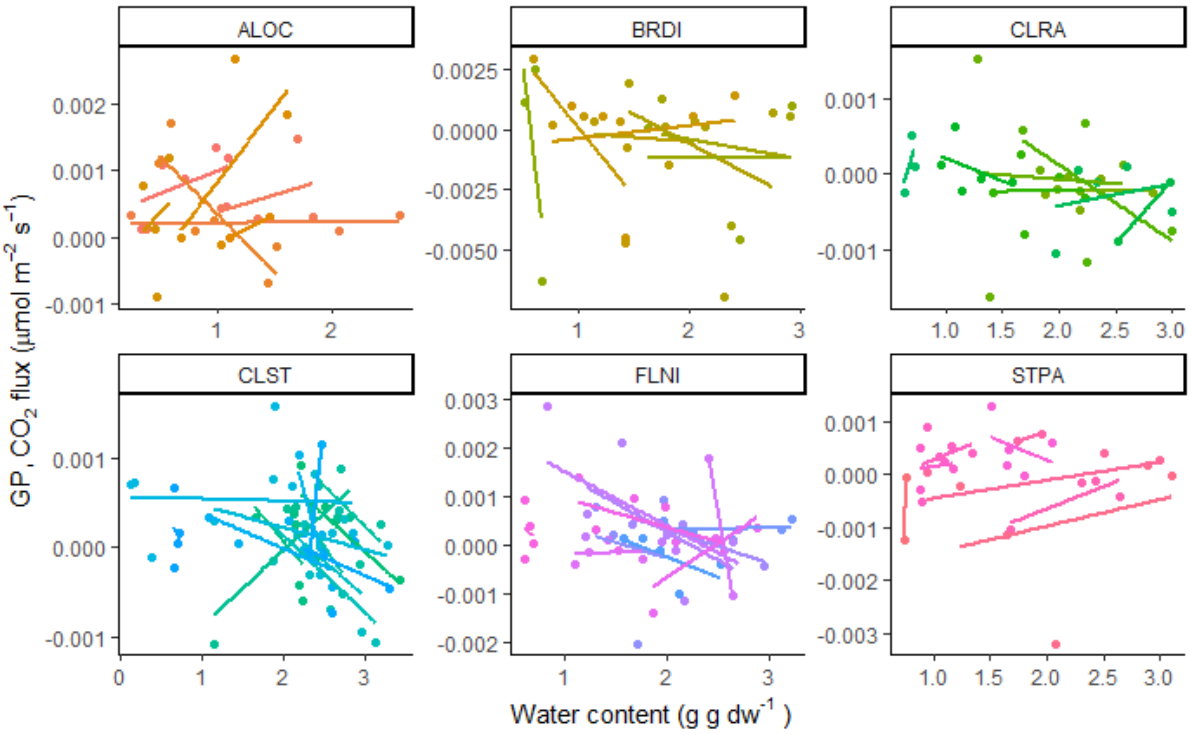

Figure S3: Gross photosynthesis depicted as CO_2 flux per gram of lichen dry mass for the six lichen species: *Alectoria ochroleuca* (ALOC), *Bryocaulon divergens* (BRDI), *Cladonia rangiferina* (CLRA), *Cladonia stellaris* (CLST), *Flavocetraria nivalis* (FLNI) and *Stereocaulon paschale* (STPA). The water content represents gram of lichen per lichen dry weight. Each data point represents the difference between the final estimates of gas exchange in the light and the dark, and each color represents different samples. The lines represent different samples used for each species during the measurements.

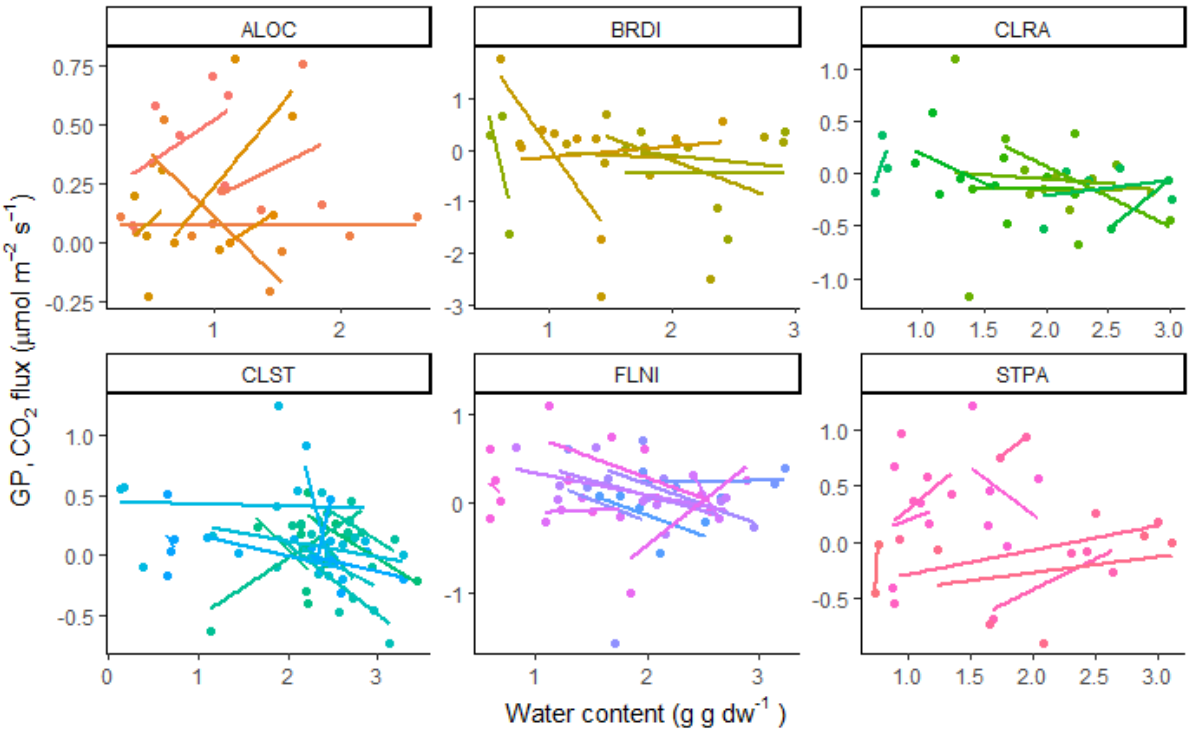

Figure S4: Gross photosynthesis as CO_2 flux per m^2 area of the sample (in this case used chamber) for six lichen species: *A. ochroleuca* (ALOC), *B. divergens* (BRDI), *C. rangiferina* (CLRA), *C. stellaris* (CLST), *F. nivalis* (FLNI) and *S. paschale* (STPA). Water content is presented as gram per gram of dry weight. Each data point is the difference between gas exchange under light and dark measurements, and each color represents different measurements. The final estimates of light measurements and dark measurements were used to calculate the difference giving the gross photosynthesis for each species. The lines represent different samples used for each species.

Table S1: Each row corresponds to a different sample for six lichens species: *C. stellaris* (CLST), *A. ochroleuca* (ALOC), *F. nivalis* (FLNI), *C. rangiferina* (CLRA), *B. divergens* (BRDI) and *S. paschale* (STPA), identified by the sample name. The slope values represent the estimated rate of change in gross photosynthesis for each sample in relationship with water content. The p-values were calculated for each slope and indicated the statistical significance. Significance codes: "***" 0, "**" 0.001, "*" 0.05, "." 0.1, " " 1.

Sample	Slope	p-value
CLST01	-0.0008095	0.134
CLST02	-0.0010850	0.331
CLST03	0.0008190	0.0837 .
CLST04	-0.0009012	0.375
CLST05	-0.0013775	0.590
CLST06	-0.0008504	0.222
CLST07	-0.0007880	0.380
CLST08	NA	NA
CLST09	-0.0002441	0.336
CLST10	-0.0000231	0.923
CLST11	-0.0032978	0.435
CLST12	-0.0003922	0.258
CLST13	0.0082657	NaN
CLST14	-0.0003943	0.493
CLST15	-0.0016807	0.843
ALOC01	0.0006782	0.358
ALOC02	0.0005140	0.679
ALOC04	0.0000050	0.954
ALOC05	-0.0017260	0.0330 *
ALOC06	0.0008713	NaN
ALOC07	0.0022476	0.366
ALOC08	0.0018538	0.817
FLNI01	-0.0006869	0.220
FLNI02	0.0000278	0.897
FLNI03	-0.0006412	0.863
FLNI04	-0.0006933	0.234
FLNI13	-0.0007218	0.186
FLNI05	-0.0011691	0.332
FLNI06	-0.0011166	0.214
FLNI07	-0.0123985	NaN
FLNI12	0.0013920	0.218
FLNI11	0.0000951	0.875
FLNI14	-0.0015810	0.871
CLRA01	0.0000063	0.984
CLRA02	-0.0001161	0.916
CLRA03	-0.0010047	0.416
CLRA04	0.0000029	0.997
CLRA06	-0.0006024	0.458
CLRA07	0.0016775	NaN

CLRA08	0.0002906	0.693
CLRA09	0.0047631	0.608
BRDI01	0.0005469	0.766
BRDI02	-0.0057290	0.152
BRDI04	-0.0003475	0.843
BRDI05	-0.0024406	0.572
BRDI06	-0.0008348	0.892
BRDI07	-0.0000247	0.997
BRDI08	-0.0394916	0.538
STPA01	-0.0008770	0.563
STPA02	0.0003033	0.863
STPA03	0.0008684	0.624
STPA04	0.0008358	0.201
STPA05	0.0006725	NaN
STPA06	0.0003410	0.557
STPA07	0.0005053	0.730
STPA08	0.0511481	NaN

Table S2: A linear model for the relationship between gross photosynthesis and water content for the lichens: *A. ochroleuca* (ALOC), *B. divergens* (BRDI), *C. rangiferina* (CLRA), *C. stellaris* (CLST), *F. nivalis* (FLNI) *and S. paschale* (STPA). The table shows values for the intercept and water content, with standard error, p-values, and adjusted R^2. Significance codes: "***" 0, "**" 0.001, "*" 0.05, "." 0.1, " " 1.

	Estimate	**Standard error**	**p-value**	**Adjusted R^2**
		ALOC		
Intercept	5.552e-04	2.952e-04	0.0704 .	-0.03571
Water content	1.038e-06	2.521e-04	0.997	
		BRDI		
Intercept	0.0003930	0.0012066	0.747	-0.01131
Water content	-0.0005687	0.0006862	0.414	
		CLRA		
Intercept	0.0003162	0.0002918	0.286	0.05146
Water content	-0.0002454	0.0001455	0.101	
		CLST		
Intercept	4.639e-04	1.953e-04	0.0206 *	0.03439
Water content	-1.523e-04	8.455e-05	0.07766 .	
		FLNI		
Intercept	0.0006964	0.0003532	0.0548 .	0.02467
Water content	-0.0002632	0.0001789	0.1483	
		STPA		
Intercept	6.519e-05	3.978e-04	0.871	-0.03377
Water content	-5.078e-05	2.214e-04	0.820	

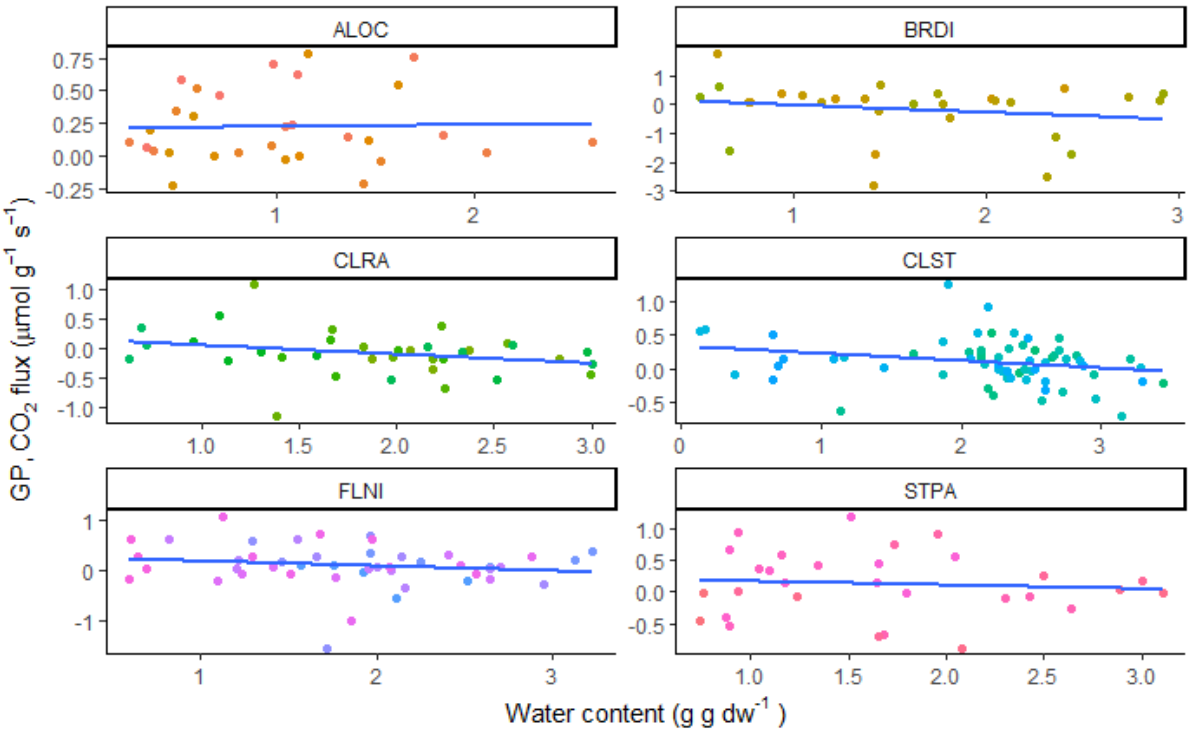

Figure S5: Gross photosynthesis as CO_2 flux per m^2 of the chamber area for six lichen species: *A. ochroleuca* (ALOC), *B. divergens* (BRDI), *C. rangiferina* (CLRA), *C. stellaris* (CLST), *F. nivalis* (FLNI) and *S. paschale* (STPA). The linear regression line was fitted through all the samples for each species. Each data point is a difference between the final estimate of measurement in the light and the final estimate of measurement in the dark, and each color represents different samples.

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