UNIVERSITÀ DI PARMA

UNIVERSITÀ DEGLI STUDI DI PARMA

DOTTORATO DI RICERCA IN

"Biologia Evoluzionistica ed Ecologia"

CICLIO XXXVI

Effects of microclimatic variations on lichen diversity and ecophysiology

Coordinatore:
Chiar.mo Prof. Pierluigi Viaroli

Tutore:
Prof. Alessio Papini

Co-tutori:
Prof. Renato Benesperi

Prof. Paolo Giordani

Prof. Juri Nascimbene

Dottorando: Luca Di Nuzzo

Anni accademici 2020/2021 - 2023/2024

Contents

Riassunto

Il cambiamento climatico è una delle principali minacce per la biodiversità a scala globale. Il principale motivo è che il clima è uno dei fattori principali che influenzano la distribuzione degli organismi. Le condizioni climatiche che gli organismi sperimentano sono spesso diverse dal clima generale della zona in cui vivono a causa dell'interazione di quest'ultimo con fattori come vegetazione e topografia. Queste condizioni, definite microclima, sono ecologicamente rilevanti per gli organismi di piccole e medie dimensioni. In alcune situazioni, condizioni microclimatiche favorevoli possono permanere anche dopo cambiamenti del macroclima. Questi microrifugi possono giocare un ruolo importante in ambito conservazionistico, permettendo alle specie di persistere e eventualmente di ricolonizzare le aree circostanti in futuro. Questo è particolarmente significativo per organismi la cui fisiologia è strettamente legata alle condizioni ambientali, come nel caso dei licheni, noti per essere molto sensibili alle variazioni climatiche. L'influenza del clima su questi organismi li rende da una parte molto sensibili al cambiamento climatico ma anche degli ottimi biomonitor di quest'ultimo. Attraverso quattro capitoli l'obiettivo di questa tesi di dottorato è quella di studiare gli effetti delle variazioni microclimatiche sull'ecofisiologia e la diversità dei licheni, considerando diverse componenti del microclima e con un focus particolare sul cambiamento climatico.

Nel **Capitolo I**, è stata analizzata l'influenza delle condizioni microclimatiche sulle forme di crescita dei licheni epifiti in ambiente mediterraneo e la possibilità che gli alberi con più capacità "microrifugio" possano mantenere una diversità lichenica epifita in diversi scenari di cambiamento climatico futuro. Con questo obiettivo sono stati campionati 70 punti nella Sardegna occidentale nei quali abbiamo rilevato la frequenza delle specie licheniche epifite. Per ogni albero campionato è stata stimata la capacità di modificare le condizioni sotto chioma rispetto al macroclima e in base a questo è stata classificata la sua capacità di funzionare da

microrifugio. Dopo aver testato l'influenza delle variabili microclimatiche su questo aspetto abbiamo utilizzato i risultati per studiare la frequenza futura dei licheni epifiti in diversi scenari di cambiamento climatico. Le forme di crescita dei licheni risultano significativamente influenzate dalle variabili microclimatiche. Inoltre, i risultati mostrano una significativa riduzione della presenza di tutte le forme di crescita in ognuno degli scenari considerati. Anche gli alberi con la maggior capacità di microrifugio non saranno in grado di supportare una diversità lichenica comparabile a quella odierna.

Nel **Capitolo II** abbiamo indagato l'effetto di un incremento della temperatura di circa 2°C sui licheni alpini e sulla possibilità che alcuni di essi possano modificare la loro distribuzione altitudinale per seguire le condizioni climatiche in cui si trovano attualmente. Sono state selezionate tre specie con un diverso range altitudinale: *Cetraria islandica* che ha un ampio range altitudinale, *Solorina crocea* che si trova solo ad altitudini elevate e *Peltigera malacea* che invece non raggiunge le stesse quote delle precedenti. I talli di tutte e tre le specie sono stati raccolti e trapiantati a 2700m, con quelli delle prime due specie prelevati vicino all'area di trapianto e i talli di *P. malacea* raccolti a un'altitudine inferiore. Tramite l'utilizzo di Open Top Chambers è stata incrementata la temperatura su metà dei talli trapiantati e per due estati sono state misurate sia l'efficienza fotosintetica che le condizioni microclimatiche ogni due settimane. I risultati mostrano che le condizioni microclimatiche precedenti i due giorni le misure, in particolar modo la temperatura media del suolo, hanno una influenza significativa sull'efficienza fotosintetica. La termofila *P. malacea* a basse temperature del suolo nei giorni precedenti la misura risulta avere una efficienza fotosintetica maggiore nei plot riscaldati, mentre tale differenza si riduce a temperature del suolo più alte. *S. crocea* mostra delle risposte comparabili ma ad alte temperature del suolo tende ad avere valori di efficienza fotosintetica più bassi indipendentemente dal trattamento. Al contrario, *C. islandica* mostra valori simili a basse temperature del suolo ma la sua efficienza fotosintetica è inferiore all'interno dei plot riscaldati che all'esterno quando le temperature del suolo precedenti la misura erano più alte. Questi risultati mostrano come, in un contesto di riscaldamento globale di circa 2°C specie più termofile come *P. malacea* siano in grado di colonizzare altitudini dove adesso trovano condizioni non favorevoli. Mentre le specie già presenti ad altitudini maggiori avranno risposte diverse a seconda delle loro caratteristiche specifiche.

Nel **Capitolo III** è stata studiata l'influenza delle variazioni microclimatiche in seguito al taglio del bosco sulla crescita del lichene *Lobaria pulmonaria.* Con questo obiettivo sono stati raccolti e trapiantati talli di *L. pulmonaria* in un'area precedentemente soggetta a taglio del bosco e in una confinante preservata dal taglio. I talli sono poi sono stati trapiantati sugli alberi selezionati a due diverse esposizioni (N e S) e a due diverse altezze dal suolo (50 e 100cm). Dopo due anni, è stata misurata la crescita dei talli nelle diverse condizioni. I risultati ottenuti hanno mostrato come i campioni esposti a sud nell'area tagliata avessero una bassa probabilità di crescita, diversamente da quelli nell'area non tagliata dove la probabilità era maggiore. Inoltre, i coefficienti di crescita annuale più elevati sono stati registrati nei campioni esposti a sud a 50 cm da terra nell'area non tagliata. Questo risultato mostra come anche a scala di albero le condizioni microclimatiche possano determinare forti variazioni della crescita nei licheni.

Infine, nel **Capitolo IV** tramite l'utilizzo del modello process-based LiBry abbiamo studiato la probabilità di sopravvivenza e l'attività metabolica di diversi stadi di crescita di *L. pulmonaria* lungo un gradiente climatico. Abbiamo campionato 50 plot lungo la penisola italiana e misurato diverse caratteristiche di *L. pulmonaria* come il suo *water holding capacity*, *specific thallus mass* e lo spessore del tallo in talli adulti. Inoltre, in un subset di aree abbiamo misurato gli stessi parametri anche per talli giovani. Questi dati sono stati accorpati in nove regioni e utilizzati per settare il modello LiBry. I risultati delle simulazioni mostrano come i talli giovani hanno in generale una minor probabilità di sopravvivenza rispetto agli adulti. Probabilità che tende decrescere a temperature più elevate in entrambi gli stadi di crescita. Infine, i talli adulti sono risultati essere attivi fotosinteticamente/metabolicamente per un tempo più lungo rispetto ai giovani. Sulla base di questi risultati si ipotizza che il riscaldamento dovuto al cambiamento climatico in futuro potrà ridurre sia la sopravvivenza che la crescita soprattutto dei talli giovani.

Abstract

Among the factors that threaten biodiversity at the global scale, climate change is becoming one the most important. The principal reason lies in the fact that organisms' distribution is often highly dependent on climate. Nevertheless, the climatic conditions that organisms experience are often different from the macroclimatic conditions that studies on climate change normally consider. The presence of these conditions, which could vary over short distances, is due to the interaction between local factors, such as topography or vegetation, with macroclimate. These conditions, called microclimate, are ecologically relevant, especially for small organisms. As macroclimate changes, one could expect that these microclimatic conditions change proportionally. This is not always the case, as they may persist in specific areas even after general climatic conditions are changed. These areas are very important from a conservation point of view, as they may act as microrefugia for some species. This is particularly relevant for organisms whose physiology is closely linked to environmental conditions, as in the case of lichens. The significant influence of climate on these organisms makes them on the one hand very sensitive to climate change but also excellent biomonitors of this latter. The aim of this PhD thesis is to study the effects of microclimatic variations on the ecophysiology and diversity of lichens, considering different components of the microclimate and with a particular focus on climate change. Firstly, I analysed the influence of microclimatic variations on the frequency of growth forms, growth and photosynthetic efficiency of epiphytic and terricolous lichens in different habitats. Subsequently, the data obtained were used to study the effects of climate change on lichens. In this context, the capacity of certain trees to act as microrefugia for epiphytic lichens under different climate change scenarios in the Mediterranean environment was also evaluated. The results of this thesis show that microclimatic conditions have a strong influence on lichens and that even small microclimatic variations can lead to significant alterations in the performance of

these organisms. The effects of climate change differ depending on the organism, its life stage and the scenario considered. Furthermore, the microrefuge capacity was found to be insufficient to preserve the diversity of epiphytic lichens in all future scenarios considered.

Introduction

Global changes and biodiversity

In the last millennia, due to the development of human civilization and related activities, ecosystems have undergone profound changes. Nevertheless, humans rely on ecosystems' functioning for their sustainment and well-being (Díaz et al. 2015; Isbell et al. 2017). As biodiversity is the foundation of ecosystem functioning and of all the ecosystem services we take advantage of, conserving and protecting it is fundamental, if not ethically, surely for the survival of our civilisation and future generations (Tilman et al. 2014; van der Plas 2019). There are many ways anthropogenic activities impact biodiversity, but among them could be mentioned habitat degradation and fragmentation, pollution, invasive species, and climate change (Sage, 2020). In addition, these negative impacts can occur simultaneously, leading to more severe consequences for ecosystems (Côté et al., 2016).

Climate change has drawn significant attention from the academic community in the last decades due to its negative impacts on most of Earth's ecosystems. A recent study has tentatively estimated the climate-related extinction of species in a range of 14% - 32% of macroscopic species before 2100, even in intermediate greenhouse gas emission scenarios (Wiens & Zelinka, 2024). The recent IPCC sixth assessment report (IPCC, 2022) highlighted an increase of global surface temperature of 1.09°C in the period 2011 - 2020 compared to the pre-industrial period (1850-1900) with a high likelihood of exceeding 1.5°C warming in the near term, even in a scenario with low greenhouse gas emission (IPCC, 2022). Before the end of the century in the intermediate and high greenhouse gas emission scenarios, the increase in temperature is expected to be 2.7°C and 4.4°C, respectively, with different effects worldwide. For example, in the Mediterranean region, mean temperatures, heat extremes, heavy precipitation and drought are expected to increase. At the same time, cold extremes and summer precipitation are forecasted to decrease (IPCC, 2022). As climate is an essential driver of species distribution, such changes in the climatic regime had and will have (Parmesan et al., 2022) a wide range of impacts on biodiversity, spanning from organisms to biomes (Bellard et al., 2012). The climatic niches of species determine their response to climate change. For example, species with specialized climatic niches are particularly vulnerable to changes in climate, while species with broader climatic niches, capable of tolerating a wider range of environmental conditions, may exhibit greater adaptability (Thuiller et al., 2005, Yu et al., 2017, Hällfors et al., 2023). Nevertheless, not all species are predicted to be negatively affected; for example species adapted to arid climates are expected to expand their range extension (Dew et al., 2019). Moreover, the responses to environmental changes can vary among different populations within a single species. For example, range-edge populations are normally adapted to different environmental conditions from those in the core area (Bennett et al., 2019). In addition, organisms with complex life histories might have different climatic niches or be tolerant to wider or lower extremes in environmental conditions at different life stages (Kingsolver et al., 2011; Radchuk et al., 2013). Often, studies only consider one life stage, thus underestimating the effects of climate change. For example, when considering embryonic performances in lizards, the predicted loss of fitness is tenfold (Levy et al., 2015).

Species have various ways to respond to climate changes (Bellard et al., 2012; O' Connor et al., 2012). For example, organisms could shift their spatial distribution following their climatic niche to follow suitable climatic conditions, with expected poleward and upward migrations (Parmesan, 2006). Nevertheless, this is probably an oversimplification as species ranges are often not only limited by climate (Thomas, 2010). Moreover, many expected range changes are still not observed due to acclimatization (Luo et al. 2001; Enriquez-Urzelai, et al., 2020) and the buffering effect on climatic variations exerted by local conditions (see paragraph on microclimate). Alteration of distributional ranges could determine the invasion of species in areas previously not colonized with adverse effects on the native biota. Organisms may also acclimatize to the new climatic conditions through plasticity in their phenology, physiology, and behaviours (O'Connor et al., 2012). For example, plants may acclimate to higher temperatures by regulating photosynthesis and respiration (Smith & Duke, 2013). Similarly, many species have been observed to shift in time their phenology to track favourable conditions, as in the case of earlier flowering (Menzel & Doze, 2005). Nevertheless, this has already driven a mismatch in the interactions between organisms, for instance, plants and their pollinators, with negative effects on all organisms involved (Bellard et al., 2012). Such mismatches could also be induced by differential speeds in range shifting, with one of the interacting species moving faster than the other (Lurgi et al., 2012). Finally, species may adapt to newer climatic conditions over generations. Nevertheless, the pace and magnitude of climate change often exceed generation time hindering the possibility to adapt (O'Connor et al., 2012). Thus, to understand and predict the impacts of climate change on biodiversity, there is an urgent need to study the different effects of predicted changes on organsisms, from the genetic to wider levels. Moreover, comparing different aspects of organisms' life cycles, like growth or different life stages, could give a better capacity to predict negative effects and plan effective management measures to buffer or eliminate such threats.

Microclimate

In climate studies, attention is frequently given to large-scale patterns. Neverhteless, these conditions often deviate from those effectively experienced by organisms, i.e. the microclimate. This latter refers to those climatic conditions present in a limited area of variable size, generally from a few millimetres to some meters, and fine temporal resolution, distinct, and often significantly different, from the broad climatic conditions found in the region (Zellweger et al., 2019). Microclimate is generally considered for spatial resolutions lower than 100m and with time frequencies of an hour or higher (Bramer et al., 2018). Like macro- or mesoclimate, microclimate is characterized for example, by temperature, humidity, solar radiation, precipitation, and wind. These different conditions are determined by the interaction of local climate with, for example, topography, altitude, vegetation, aspect, water bodies and soil type (Rotach & Calanca, 2003). On a smaller scale, even aerodynamic characteristics, radiative and thermal properties of objects or organisms could influence microclimatic conditions (Rotach & Calanca, 2003). The conditions that arise from the interplay between the aforementioned local factors and free-air conditions can lead to either buffered conditions, such as the case of reduced temperature extremes in understories (De Frenne et al., 2021; Gril et al., 2023), or amplified conditions (Miller et al., 2021). Microclimatic conditions are commonly characterised by high variability in terms of space and time. For example, in the mountains, the complex topography coupled with strong elevation gradients determines variation in mean annual temperature up to 2°C in a few meters (Scherrer & Korner 2011; Graae et al., 2018). In forests, microclimate could vary horizontally, as close to forest gaps (Stoutjesdijk & Barkman, 2015), or even vertically along tree trunks (Eliáš et al., 1989). In the forest understory, the temperature is, on average, 2.1°C colder in the summer and 2°C warmer in winter compared with free air temperature (Haesen et al., 2023). Regarding temperature variation within a single organisms there can be a significant difference of up to 10°C between the temperature of the ovaries and that of the petals in plants (Dietrich & Körner, 2014). Such a broad range of variations translates into a wide variety of microhabitats at a very small scale that is difficult to model or include in ecological studies.

In ecology, what to consider microclimate depends on the organisms and the question studied (Mislan & Helmuth, 2008). A small-sized organism could be influenced by a millimetre or centimetre scale variation (Pincebourde & Woods, 2020). For example, slight differences in the microclimate at 2mm around plant leaves can determine significant variations in the egg metabolic rate of some insects (Potter et al., 2009). On the other hand, the same microclimate is not able to influence the metabolism of larger organisms and, therefore, has to be considered on a larger scale (Mislan & Helmuth, 2008; Pincebourde et al., 2021), for example, temperatures in the tree canopies are often strongly correlated with macroclimatic conditions. Due to instrumental and budget limitations, in the past, climatic factors used in ecological studies were those collected by weather stations or calculated by models constructed on this latter with a spatial resolution of a maximum of $\sim$1 km^2

(Karger et al., 2017; Fick et al., 2017). The position at which these weather stations record meteorological measurements is normally standardised, as they are positioned 1.5-2 meters above short grass. Thus, the weather conditions recorded often fail to intercept the microclimatic conditions experienced by organisms even a few meters away (Suggit et al., 2011; Lembrechts et al., 2019). In fact, as seen before, with complex topography and vegetation, small spaces could encompass a wide range of microclimatic conditions. Nevertheless, these latter are those effectively experienced by organisms and are thus more ecologically relevant than coarser free-air conditions (Varner & Dearing, 2014; Bramer et al., 2018). Thus, microclimate is fundamental to a proper understanding of the climatic niche of specific organisms. Often, studies observe that even if a species is found in a wide range of climatic conditions, the true climatic niche in which it can survive is rather narrower than what could be expected by macroclimate (Potter et al., 2013). This results, for example, in the inability of species distribution models (SDMs) based on macroclimatic data to adequately encompass the factors that constrain species distribution (Lembrechts et al., 2019; Haesen et al., 2023; Maclean & Early, 2023). Consequently, this limitation could lead to inaccurate predictions of global change drivers' effects on biodiversity (Storlie et al., 2014; Suggit et al., 2018). The unexpected differences between the predicted and observed change in species distribution due to global warming can often be ascribed to the role of microclimates in either mitigating or even amplifying the impact of general climatic conditions (Maclean et al., 2015; Carnicer et al., 2021).

Accurate predictions of species distribution become increasingly crucial for species conservation planning. Understanding the climatic preferences of species is fundamental to identifying areas with favourable conditions in future climate change scenarios. The areas where a suitable microclimate is maintained, even if surrounding climatic conditions have changed, are called microrefugia (Ashcroft, 2010; Dobrowski, 2011). Here, small populations can survive in future climate change scenarios, serving as reservoirs of genetic diversity (Tonin et al., 2023) and stepping stones from which species may later recover (Patsiou et al., 2014).

Lichens as model organisms to study environmental changes

Lichens are generally described as symbiotic organisms composed of a mycobiont, normally a fungus belonging to the phylum Asocomycota, or, in fewer cases, to Basidiomycota, and a photobiont, commonly a green alga or a cyanobacterium. Yet, the growing body of evidence that other organisms, such as yeast (Morrillas et al., 2022), bacteria (Grimm et al., 2021) or other fungi (Morrillas et al., 2022) are integrated and have functions inside the symbiosis has recently led to a re-definition of lichens (Hawksworth & Grube, 2020; Lücking et al., 2021). Here, drawing from the holobiont theory (Simon et al., 2019), lichens are defined as composed of a stable association, able to self-sustain in which one fungal species is the main mycobiont and the host, the algae or cyanobacteria are primary photosynthetic partner and other microscopic microorganisms, mainly bacteria and other fungi, are obligated associated elements (Lücking et al., 2021).

Various environmental factors play a significant role in shaping the ecophysiology of lichens, including temperature, water source availability and type, and light. A crucial aspect that particularly defines their ecophysiological characteristics is their poikilohydric nature. In fact, they cannot control water loss and their water content is in equilibrium with the surrounding environment. Such poikilohydry is coupled with the capacity to withstand periods of desiccation in which all their metabolic activity is suspended, allowing species to survive periods with little or no water availability. This desiccation tolerance is present in almost all lichen species but with a wide range of tolerance in terms of time and degree of desiccation (Green and Lange, 1991; Kranneret al., 2008, Osyczka et al., 2023). Water sources are key factors in lichen physiology, in fact, to be photosynthetically active, lichens need to be hydrated. Under sufficient light conditions, net photosynthesis arises until an optimal water content is reached, after which any increase in thallus water content decreases due to supersaturation. This condition reduces gas exchanges due to excess water in the extracellular spaces, lowering the amount of CO_2 that can reach the algal layer (Stanton et al., 2023). Being so dependent on water availability, lichens can use different sources of hydration, such as air humidity, dew and rain. The first main difference in water source use comes from the photobiont type: lichens with green algal photobiont can rehydrate with rain and relative humidity. In contrast, species with cyanobacteria as the main photobiont need liquid water to reactivate (Lange et al., 1993; Gauslaa, 2014). Growth form is another major determinant of what kind of water source is used in lichens. For example, fruticose filamentous lichens are adapted to take advantage of air humidity (Phinney et al., 2021). At the same time, lichens with thicker thallus, such as foliose growth form, needs more water to reach a saturation level sufficient for starting photosynthesis and may need higher air humidity or even dew formation or rain. On the other hand, temperature plays a crucial role in controlling processes associated with the growth, physiology, and overall performance of lichens (Green et al., 2008). Increasing temperatures may lead to a higher drying rate, which in turn results in reducing the overall length of activity. Despite lichens need cycles of dessication, and are negatively affected by long periods of continuous hydration (Solhaug & Gauslaa, 2004; Gauslaa, 2023), longer periods of dehydration can reduce the time for physiological activity and have detrimental effects (Osyczka et al., 2023). At the same, increasing temperatures during hydration periods can lead to reduced net photosynthesis (Green et al., 2008).

Due to the features mentioned above, lichens are deeply influenced by the surrounding abiotic and biotic conditions and are thus very sensitive to environmental changes. Lichens have been widely used as a biomonitor of air pollution (Nimis et al., 2002), but have also been proposed to be an effective biomonitor of climate change (Nimis et al., 2002; Sancho et al., 2019) and of global change in general (Matos et al., 2017; Morillas, 2024). Moreover, despite their small size lichen are key components of many ecosystems, and have important roles in, for example, water and nutrient cycles (Asplund & Wardle 2017; Porada et al., 2018; Concostrina-Zubiri et al., 2022). Thus, developing a wider comprehension of lichen ecophysiology and ecology, with a focus on the effects of microclimatic conditions in climate change scenarios could aid not only in developing conservation targeting these organisms but also provide a tool to monitor global changes.

Objectives and structure of the thesis

The aim of this thesis is to provide a comprehensive study of the effects of climatic variations on lichen, with a focus on microclimate in the context of global changes. Through four chapters we investigated how variations in key microclimatic parameters affect lichen diversity and ecophysiology. In Chapter I, the effects of microclimatic variation, and other small-scale biotic and abiotic factors, on the abundance of epiphytic lichen growth forms in a Mediterranean climate were evaluated. In addition, based on the observed relationship we analyzed the capacity of microrefuges to mitigate the effects of climate change. In Chapter II we studied the effects of temperature warming on photosynthetic efficiency in three terricolous alpine lichens. In addition, we compared the relative importance of microclimatic conditions over different time spans before measurements. In Chapter III we used an epiphytic lichen species, *Lobaria pulmonaria* Hoffm., as a model species to understand the effects of microclimatic variations on its growth and survival probability. Such study case took into account also the interaction between microclimate and anthropological impacts like forest management and logging. Finally, in Chapter IV, by broadening the picture, we aimed at evaluate the growth capacity and survival of *L. pulmonaria* along a climatic gradient in a climate change framework.

Bibilography

Aragón, G., Martínez, I., Hurtado, P., Benítez, Á., Rodríguez, C., & Prieto, M. (2019). Using growth forms to predict epiphytic lichen abundance in a wide variety of forest types. Diversity, 11(4), 51.

Ashcroft, M. B. (2010). Identifying refugia from climate change. *Journal of Biogeography*, 37(8), 1407–1413.

Asplund, J., & Wardle, D. A. (2017). How lichens impact on terrestrial community and ecosystem properties. *Biological reviews*, 92(3), 1720-1738.

Bellard, C., Bertelsmeier, C., Leadley, P., Thuiller, W., & Courchamp, F. (2012). Impacts of climate change on the future of biodiversity. *Ecology letters*, 15(4), 365-377.

Bennett, S., Duarte, C. M., Marbà, N., & Wernberg, T. (2019). Integrating within-species variation in thermal physiology into climate change ecology. *Philosophical Transactions of the Royal Society B*, 374(1778), 20180550.

Bramer, I., Anderson, B. J., Bennie, J., Bladon, A. J., De Frenne, P., Hemming, D., ... & Gillingham, P. K. (2018). Advances in monitoring and modelling climate at ecologically relevant scales. In *Advances in ecological research* (Vol. 58, pp. 101-161). Academic Press.

Carnicer, J., Vives-Ingla, M., Blanquer, L., Méndez-Camps, X., Rosell, C., Sabaté, S., ... & Barbeta, A. (2021). Forest resilience to global warming is strongly modulated by local-scale topographic, microclimatic and biotic conditions. *Journal of Ecology*, 109(9), 3322-3339.

Concostrina-Zubiri, L., Valencia, E., Ochoa, V., Gozalo, B., Mendoza, B. J., & Maestre, F. T. (2022). Biocrust-forming lichens increase soil available phosphorus under simulated climate change. *European Journal of Soil Science*, 73(4), e13284.

Côté, I. M., Darling, E. S., & Brown, C. J. (2016). Interactions among ecosystem stressors and their importance in conservation. *Proceedings of the Royal Society B: Biological Sciences*, 283(1824), 20152592. De Frenne, P., Lenoir, J., Luoto, M., Scheffers, B. R., Zellweger, F., Aalto, J., ... & Hylander, K. (2021). Forest microclimates and climate change: Importance, drivers and future research agenda. *Global Change Biology*, 27(11), 2279-2297.

Dew, R. M., Silva, D. P., & Rehan, S. M. (2019). Range expansion of an already widespread bee under climate change. *Global Ecology and Conservation*, 17, e00584.

Díaz, S., Demissew, S., Carabias, J., Joly, C., Lonsdale, M., Ash, N., ... & Zlatanova, D. (2015). The IPBES Conceptual Framework—connecting nature and people. *Current opinion in environmental sustainability*, 14, 1-16.

Dietrich, L., & Körner, C. (2014). Thermal imaging reveals massive heat accumulation in flowers across a broad spectrum of alpine taxa. *Alpine Botany*, 124, 27-35.

Dobrowski, S. Z. (2011). A climatic basis for microrefugia: the influence of terrain on climate. *Global Change Biology*, 17(2), 1022-1035.

Eliáš, P., et al. "Stand microclimate and physiological activity of tree leaves in an oak-hornbeam forest: I. Stand microclimate." *Trees*, 3 (1989): 227-233.

Enriquez-Urzelai, U., Tingley, R., Kearney, M. R., Sacco, M., Palacio, A. S., Tejedo, M., & Nicieza, A. G. (2020). The roles of acclimation and behaviour in buffering climate change impacts along elevational gradients. *Journal of Animal Ecology*, 89(7), 1722-1734.

Fick, S. E., & Hijmans, R. J. (2017). WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. *International journal of climatology*, 37(12), 4302-4315.

Gauslaa, Y. (2014). Rain, dew, and humid air as drivers of morphology, function and spatial distribution in epiphytic lichens. *The Lichenologist*, 46(1), 1–16. doi: 10.1017/S0024282913000753

Gauslaa, Y. (2023). Recovery kinetics of epiphytic lichen diversity after dieback during a continuously wet season. *Fungal Ecology*, 66, 101299.

Graae, B. J., Vandvik, V., Armbruster, W. S., Eiserhardt, W. L., Svenning, J. C., Hylander, K., ... & Lenoir, J. (2018). Stay or go–how topographic complexity influences alpine plant population and community responses to climate change. *Perspectives in Plant Ecology*, Evolution and Systematics, 30, 41-50.

Green, T. G. A., & Lange, O. L. (1991). Ecophysiological adaptations of the lichen genera Pseudocyphellaria and Sticta to south temperate rainforests. *The Lichenologist*, 23(3), 267-282.

Green, T. G. A., Nash T. H. III, and Lange, O. L. (2008). “Physiological ecology of carbon dioxide exchange,” in *Lichen Biology*, ed. T. H. Nash III (Cambridge: University Press), 152–181.

Gril, E., Spicher, F., Greiser, C., Ashcroft, M. B., Pincebourde, S., Durrieu, S., ... & Lenoir, J. (2023). Slope and equilibrium: A parsimonious and flexible approach to model microclimate. *Methods in Ecology and Evolution*, 14(3), 885-897.

Grimm, M., Grube, M., Schiefelbein, U., Zühlke, D., Bernhardt, J., & Riedel, K. (2021). The lichens’ microbiota, still a mystery?. *Frontiers in Microbiology*, 12, 714.

Haesen, S., Lenoir, J., Gril, E., De Frenne, P., Lembrechts, J. J., Kopecký, M., ... & Van Meerbeek, K. (2023). Microclimate reveals the true thermal niche of forest plant species. *Ecology Letters*, 26(12), 2043-2055.

Hällfors, M., Heikkinen, R., Kuussaari, M., Lehikoinen, A., Luoto, M., Pöyry, J., ... & Kujala, H. (2023). Recent range shifts of moths, butterflies, and birds are driven by the breadth of their climatic niche. *Evolution Letters*, qrad004.

Hawksworth, D. L., & Grube, M. (2020). Lichens redefined as complex ecosystems. *The New Phytologist*, 227(5), 1281.

IPCC, 2022: Climate Change 2022: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [H.-O. Pörtner, D.C. Roberts, M. Tignor, E.S. Poloczanska, K. Mintenbeck, A. Alegría, M. Craig, S. Langsdorf, S. Löschke, V. Möller, A. Okem, B. Rama (eds.)]. Cambridge University Press.

Cambridge University Press, Cambridge, UK and New York, NY, USA, 3056 pp., doi:10.1017/9781009325844.

Isbell, F., Gonzalez, A., Loreau, M., Cowles, J., Díaz, S., Hector, A., ... & Larigauderie, A. (2017). Linking the influence and dependence of people on biodiversity across scales. *Nature*, 546(7656), 65-72.

Karger, D. N., Conrad, O., Böhner, J., Kawohl, T., Kreft, H., Soria-Auza, R. W., ... & Kessler, M. (2017). Climatologies at high resolution for the earth's land surface areas. *Scientific data*, 4(1), 1-20.

Kearney, M. R., Isaac, A. P., & Porter, W. P. (2014). microclim: Global estimates of hourly microclimate based on long-term monthly climate averages. *Scientific dat*a, 1(1), 1-9.

Kingsolver, J. G., Arthur Woods, H., Buckley, L. B., Potter, K. A., MacLean, H. J., & Higgins, J. K. (2011). Complex Life Cycles and the Responses of Insects to Climate Change. *Integrative & Comparative Biology*, 51(5).

Kranner, I., Beckett, R., Hochman, A., & Nash III, T. H. (2008). Desiccation-tolerance in lichens: a review. *The Bryologist*, 111(4), 576-593.

Lange, O. L., Büdel, B., Meyer, A. & Kilian, E. (1993) Further evidence that activation of net photosynthesis by dry cyanobacterial lichens requires liquid water. *The Lichenologist* 25: 175–189.

Lembrechts, J. J., Nijs, I., & Lenoir, J. (2019). Incorporating microclimate into species distribution models. *Ecography*, 42(7), 1267-1279.

Levy, O., Buckley, L. B., Keitt, T. H., Smith, C. D., Boateng, K. O., Kumar, D. S., & Angilletta Jr, M. J. (2015). Resolving the life cycle alters expected impacts of climate change. Proceedings of the Royal Society B: *Biological Sciences*, 282(1813), 20150837.

Lücking, R., Aime, M. C., Robbertse, B., Miller, A. N., Aoki, T., Ariyawansa, H. A., ... & Schoch, C. L. (2021). Fungal taxonomy and sequence-based nomenclature. *Nature* microbiology, 6(5), 540-548.

Luo, Y., Wan, S., Hui, D., & Wallace, L. L. (2001). Acclimatization of soil respiration to warming in a tall grass prairie. *Nature*, 413(6856), 622-625.

Lurgi M, Lopez BC, Montoya JM. Novel communities from climate change. Philos Trans R Soc Lond B Biol Sci. 2012;367: 2913–2922. pmid:23007079

Maclean, I. M., & Early, R. (2023). Macroclimate data overestimate range shifts of plants in response to climate change. *Nature Climate Change*, 13(5), 484–490.

Maclean, I. M., & Klinges, D. H. (2021). Microclimc: A mechanistic model of above, below and within-canopy microclimate. *Ecological Modelling*, 451, 109567.

Maclean, I. M., Hopkins, J. J., Bennie, J., Lawson, C. R., & Wilson, R. J. (2015). Microclimates buffer the responses of plant communities to climate change. *Global Ecology and Biogeography*, 24(11), 1340-1350.

Maclean, I. M., Mosedale, J. R., & Bennie, J. J. (2019). Microclima: An r package for modelling meso-and microclimate. *Methods in Ecology and Evolution*, 10(2), 280-290.

Matos, P., Geiser, L., Hardman, A., Glavich, D., Pinho, P., Nunes, A., ... & Branquinho, C. (2017). Tracking global change using lichen diversity: towards a global-scale ecological indicator. *Methods in Ecology and Evolution*, 8(7), 788-798.

Menzel, A., & Dose, V. (2005). Analysis of long-term time series of the beginning of flowering by Bayesian function estimation. *Meteorologische Zeitschrift* (Berlin), 14.

Miller, B. D., Carter, K. R., Reed, S. C., Wood, T. E., & Cavaleri, M. A. (2021). Only sun-lit leaves of the uppermost canopy exceed both air temperature and photosynthetic thermal optima in a wet tropical forest. *Agricultural and Forest Meteorology*, 301, 108347.

Mislan, K.; Helmuth, B. Microclimate. In Encyclopedia of Ecology; Elsevier BV: Amsterdam, The Netherlands, 2008; pp. 472–475.

Morillas, L. (2024). Lichens as Bioindicators of Global Change Drivers. *Journal of Fungi*, 10(1), 46.

Morillas, L., Roales, J., Cruz, C., & Munzi, S. (2022). Lichen as multipartner symbiotic relationships. *Encyclopedia*, 2(3), 1421-1431.

Nimis, P. L., C. Scheidegger, and P. A. Wolseley. 2002. Monitoring with lichens–monitoring lichens. An introduction, pp. 1–4. In : Nimis, P. L., C. Scheidegger, and P. A. Wolseley, eds. Monitoring with Lichens–Monitoring Lichens. Springer Netherlands, Dordrecht, Netherlands.

O'Connor, M. I., Selig, E. R., Pinsky, M. L., & Altermatt, F. (2012). Toward a conceptual synthesis for climate change responses. *Global Ecology and Biogeography*, 21(7), 693-703.

Osyczka, P., Kościelniak, R., & Stanek, M. (2023). Old-growth forest versus generalist lichens: Sensitivity to prolonged desiccation stress and photosynthesis reactivation rate upon rehydration. *Mycologia*, 0(0), 1–13. doi: 10.1080/00275514.2023.2275460

Parmesan, C. (2006). Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology, Evolution, and Systematics*, 37, 637-669.

Parmesan, C., M.D. Morecroft, Y. Trisurat, R. Adrian, G.Z. Anshari, A. Arneth, Q. Gao, P. Gonzalez, R. Harris, J. Price, N. Stevens, and G.H. Talukdarr, 2022: Terrestrial and Freshwater Ecosystems and Their Services. In: Climate Change 2022: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [H.-O. Pörtner, D.C. Roberts, M. Tignor, E.S. Poloczanska, K. Mintenbeck, A. Alegría, M. Craig, S. Langsdorf, S. Löschke, V. Möller, A. Okem, B. Rama (eds.)]. Cambridge University Press, Cambridge, UK and New York, NY, USA, pp. 197–377, doi:10.1017/9781009325844.004

Patsiou, T., Conti, C. E., Zimmerman, N. E., Theodoridis, S., & Randin, C. F. (2014). Topo-climatic microrefugia explain the persistence of a rare endemic plant in the Alps during the last 21 millennia. *Global Change Biology*, 20(7), 2286–2300. https://doi.org/10.1111/gcb.12515

Phinney, N. H., Gauslaa, Y., Palmqvist, K., & Esseen, P. A. (2021). Macroclimate drives growth of hair lichens in boreal forest canopies. *Journal of Ecology*, 109(1), 478-490.

Pincebourde, S., & Woods, H. A. (2020). There is plenty of room at the bottom: microclimates drive insect vulnerability to climate change. *Current Opinion in Insect Science*, 41, 63-70.

Pincebourde, S., Dillon, M. E., & Woods, H. A. (2021). Body size determines the thermal coupling between insects and plant surfaces. *Functional Ecology*, 35(7), 1424-1436.

Porada, P., Van Stan, J. T., & Kleidon, A. (2018). Significant contribution of non-vascular vegetation to global rainfall interception. *Nature Geoscience*, 11(8), 563-567.

Potter, K. A., Arthur Woods, H., & Pincebourde, S. (2013). Microclimatic challenges in global change biology. *Global Change Biology*, 19(10), 2932-2939.

Potter, K., Davidowitz, G., & Woods, H. A. (2009). Insect eggs protected from high temperatures by limited homeothermy of plant leaves. *Journal of Experimental Biology*, 212(21), 3448-3454.

Radchuk, V., Turlure, C., & Schtickzelle, N. (2013). Each life stage matters: the importance of assessing the response to climate change over the complete life cycle in butterflies. *Journal of Animal Ecology*, 82(1), 275-285.

Rotach M.W., Calanca P. (2003). *Microclimate, Encyclopedia of Atmospheric Sciences*, Academic Press, Editor(s): James R. Holton, 2003, Pages 1301-1307, ISBN 9780122270901.

Sage, R. F. (2020). Global change biology: a primer. *Global Change Biology*, 26(1), 3-30.

Sancho, L. G., Pintado, A., & Green, T. A. (2019). Antarctic studies show lichens to be excellent biomonitors of climate change. *Diversity*, 11(3), 42.

Scherrer, D. and Körner, C. (2010) Infra-red thermometry of alpine landscapes challenges climatic warming projections. *Global Change Biology* 16, 2602–2613

Scherrer, D., & Körner, C. (2011). Topographically controlled thermal-habitat differentiation buffers alpine plant diversity against climate warming. *Journal of Biogeography*, 38(2), 406-416.

Simon JC, Marchesi JR, Mougel C, Selosse MA. 2019. Host–microbiota interactions: from holobiont theory to analysis. *Microbiome* 7: 5

Smith, N. G., & Dukes, J. S. (2013). Plant respiration and photosynthesis in global-scale models: incorporating acclimation to temperature and CO 2. *Global Change Biology*, 19(1), 45-63.

Solhaug, K. A., & Gauslaa, Y. (2004). Photosynthates stimulate the UV-B induced fungal anthraquinone synthesis in the foliose lichen Xanthoria parietina. *Plant, Cell & Environment*, 27(2), 167-176.

Stanton, D. E., Ormond, A., Koch, N. M., & Colesie, C. (2023). Lichen ecophysiology in a changing climate. *American Journal Of Botany*, 110(2), e16131.

Storlie, C., Merino-Viteri, A., Phillips, B., VanDerWal, J., Welbergen, J., & Williams, S. (2014). Stepping inside the niche: microclimate data are critical for accurate assessment of species' vulnerability to climate change. *Biology Letters*, 10(9), 20140576.

Stoutjesdijk, P. H., & Barkman, J. J. (2015). Microclimate, vegetation & fauna. Brill.

Suggitt, A. J., Wilson, R. J., Isaac, N. J., Beale, C. M., Auffret, A. G., August, T., ... & Maclean, I. M. (2018). Extinction risk from climate change is reduced by microclimatic buffering. *Nature Climate Change*, 8(8), 713-717.

Thomas, C. D. (2010). Climate, climate change and range boundaries. *Diversity and Distributions*, 16(3), 488-495.

Thuiller, W., Lavorel, S., & Araújo, M. B. (2005). Niche properties and geographical extent as predictors of species sensitivity to climate change. *Global Ecology and Biogeography*, 14(4), 347-357.

Tilman, D., Isbell, F., & Cowles, J. M. (2014). Biodiversity and ecosystem functioning. *Annual Review of Ecology, Evolution, and Systematics*, 45, 471-493.

Tonin, R., Wilhelmi, S., Gültas, M., Gerdol, R., Paun, O., Trucchi, E., ... & Wellstein, C. (2023). Ice holes microrefugia harbor genetically and functionally distinct populations of Vaccinium vitis-idaea (Ericaceae). *Scientific Reports*, 13(1), 13055.

van der Plas, F. (2019). Biodiversity and ecosystem functioning in naturally assembled communities. *Biological Reviews*, 94(4), 1220-1245.

Varner, J., & Dearing, M. D. (2014). The importance of biologically relevant microclimates in habitat suitability assessments. *PLoS One*, 9(8), e104648.

Wiens, J. J., & Zelinka, J. (2024). How many species will Earth lose to climate change?. *Global Change Biology*, 30(1), e17125.

Yu, F., Groen, T. A., Wang, T., Skidmore, A. K., Huang, J., & Ma, K. (2017). Climatic niche breadth can explain variation in geographical range size of alpine and subalpine plants. *International Journal of Geographical Information Science*, 31(1), 190-212.

Zellweger, F., De Frenne, P., Lenoir, J., Rocchini, D., & Coomes, D. (2019). Advances in microclimate ecology arising from remote sensing. *Trends in Ecology & Evolution*, 34(4), 327-341.

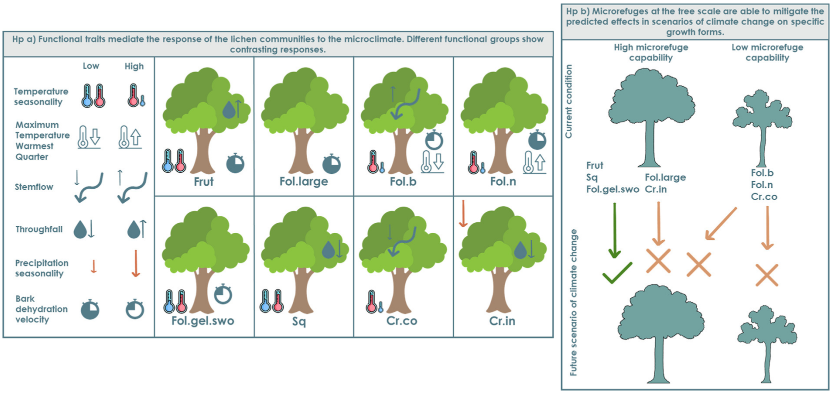
Chapters

Chapter 1

Little time left. Microrefuges may fail in mitigating the effects of climate change on epiphytic lichens

Luca Di Nuzzo, Renato Benesperi, Juri Nascimbene, Alessio Papini, Paola Malaspina, Guido Incerti, Paolo Giordani

Published in *Science of The Total Environment*, June 2022, volume 825, DOI: 10.1016/j.scitotenv.2022.153943.



Abstract

Climate change is already causing considerable reductions in biodiversity in all terrestrial ecosystems. These consequences are expected to be exacerbated in biomes that are particularly exposed to change, such as those in the Mediterranean, and in certain groups of more sensitive organisms, such as epiphytic lichens. These poikylohydric organisms find suitable light and water conditions on trunks under the tree canopy. Despite their small size, epiphytic communities contribute significantly to the functionality of forest ecosystems.

In this work, we surveyed epiphytic lichen communities in a Mediterranean area (Sardinia, Italy) and hypothesized that 1) the effect of microclimate on lichens at tree scale is mediated by the functional traits of these organisms and that 2) micro-refuge trees with certain morphological characteristics can mitigate the negative effects of future climate change.

Results confirm the first hypothesis, while the second is only partially supported, suggesting that the capability of specific trees to host specific conditions may not be sufficient to maintain the diversity and ecosystem functionality of lichen communities in the Mediterranean.

1.1 Introduction

Climate change is causing increasing impacts on biodiversity, and future projections agree on predicting negative impacts on a wide range of biota and ecosystems (Thomas et al., 2004; Pacifici et al., 2015). Still, there are some groups of organisms which, due to their biological characteristics, are more prone to change. Several multitaxon studies have led to robust arguments supporting the existence of a response gap between organisms with different sensitiveness to climate change (Maclean & Wilson, 2011; Ovaskainen et al., 2020). For example, non-vascular cryptogams, and in particularly lichens (Ellis, 2019), could be more affected than vascular plants and even bryophytes (Di Nuzzo et al., 2021; Nascimbene and Spitale, 2017). Lichen biological features make them extremely sensitive to climate change. They are poikilohydric symbiotic organisms that maintain a complex internal micro-ecosystem based on the interaction between non-lichenized fungi and bacteria, in addition to the two primary symbionts represented by ascomycetes and green algae or cyanobacteria (Hawksworth & Grube, 2020; Spribille et al., 2016, Spribille et al., 2020). Lichens lack protective tissues and therefore depend on the surrounding atmosphere for gas exchange, light and water supply (Kranner et al., 2008).

Various studies have shown how climate change can impact lichens at different levels, including a decrease in population size (Rubio-Salcedo et al., 2015), loss of alpha diversity, variations in beta diversity (Di Nuzzo et al., 2021), alterations in functional composition (Giordani et al., 2019), shifts or reductions in climatic suitability and ecological niche (Nascimbene et al., 2016, Nascimbene et al., 2020; Hurtado et al., 2020; Rubio-Salcedo et al., 2015; Vallese et al., 2021). Although the small size of these organisms may suggest that they are a secondary element of ecosystems, several studies have demonstrated the importance of their ecological functionality, which could be seriously altered as a result of climate change (Asplund & Wardle, 2017; Porada et al., 2013, Porada et al., 2018; Ellis et al., 2021). Some of these impacts derive from direct effects that hamper lichen dehydration/hydration cycles with negative consequences on their vitality (Phinney et al., 2018; Proctor & Tuba, 2002). In other cases, indirect effects may occur that alter the biotic interactions between lichens and other organisms. For example, fire regimes alteration, induced by increasing temperatures, can negatively affect for long time lichen communities by altering local microclimatic conditions (Miller et al., 2018, Miller et al., 2021). At the same time, Nascimbene et al. (2020) showed the consequences of the increased suitability for invasive tree species that are less suitable to lichen colonization. However, most of these models inform on climate change projections at landscape scales which describe the macroclimatic conditions likely occurring over large areas (Rubio-Salcedo et al., 2015). If, on one hand, it is evident that there is a strict connection between macroclimate and the microclimate occurring at a more detailed scale, on the other hand, it is likely that these relationships are not constant either along spatial gradients or on a temporal scale (Haesen et al., 2021).

In recent years, numerous studies debated the relevance of microclimate in determining the probability of species occurrence in climate change scenarios (De

Frenne et al., 2019; Maclean et al., 2015; Zellweger et al., 2019; Schall & Heinrichs, 2020; Miller et al., 2017). Most of these studies indicate that forests, and trees in general, play a fundamental role in shaping the microclimate and in establishing potential climatic microrefugia (De Frenne et al., 2021). Ultimately, microrefugia can serve to buffer climate variability and thus slow down the process of extinction caused by it (Morelli et al., 2020; Keppel & Wardell-Johnson, 2015; Hannah et al., 2014). The interaction between topographic concavity of the terrain and canopy structure delineates the capability of a site to act as a climate microrefuge (Lenoir et al., 2017). This effect is potentially observable at any scale and, indeed, the scale plays a key role. In fact, to better understand what the effects of climate change might be, it is essential to circumscribe the microclimate to which a given target organism is actually subject (De Frenne et al., 2019). For example, in the case of epiphytic lichens, the microrefuge effect could be already observable at the tree scale. In fact, canopy increases shading and distributes precipitations in terms of throughfall, stemflow and water intercepted by the trunk (Porada & Giordani, 2021; Porada et al., 2018; Van Stan et al., 2020). Tree crown also causes a considerable decrease in sub-canopy vs free-air temperatures (Lenoir et al., 2017) lowering the maximum temperature down to -3 °C and potentially counteracting the expected temperature increase in future scenarios of up to 1 °C. As temperature rise, the capacity of a forest to maintain different temperature could be a consequence of different dynamics. On the one hand, the temperature under the canopy could increase proportionally with the free-air temperature. This has been described as a “perfect coupling” (sensu De Frenne et al., 2021) to which hereafter will be referred as “mitigation”. On the other hand, the canopy could influence temperature by maintaining a steadier temperature, i.e., the increase of temperature under the canopy is not perfectly related to the increase in free-air temperature. Hereafter we will refer to this dynamic as “buffer” (De Frenne et al., 2021).

Proportionally, the gap between macro- and microclimate may be less relevant for populations of large species (e.g. tree species), compared to those of small organisms (De Frenne et al., 2019). Microclimate mitigation is merely decisive for obligate epiphytes whose relationships with tree crown and trunk determine each step of their life cycle (Giordani et al., 2020; Ellis et al., 2014; Ellis & Eaton, 2021). For example, for hygrophilous lichens, microclimatic refugia have a significant effect in maintaining a growth rate on vital levels (Ellis, 2020), or in determining the probability of survival and development of recruits (Benesperi et al., 2018). The relevance of microrefuges is considerably higher the harsher the climatic conditions, for example in semi-arid Mediterranean environments where models predict the most drastic changes in terms of temperature increase and precipitation decrease (Giorgi & Lionello, 2008). In fact, similarly to what has been predicted for semi-arid forests in North America (Smith et al., 2020), lichen species are more susceptible to climate-induced changes in these environments, determining the importance of microclimatic refugia.

However, the effect of optimal microclimatic conditions on lichen communities is not apparent, nor unique, since functional traits mediate the response of each species to environmental variations (Violle et al., 2007). Traits come into play individually or interactively, in a more or less marked way and determine the possibility of species occurrence and survival (Ellis et al., 2021). As for lichens, several works have highlighted how some functional traits are decisive in response to climatic factors (Giordani et al., 2012, Giordani et al., 2019; Matos et al., 2015; Hurtado et al., 2020, Hurtado et al., 2019; Ellis et al., 2021). For example, the photobiont type determines the type of water source preferred, as cyanolichens require liquid water to activate photosynthesis (Gauslaa, 2014; Lange et al., 1986). Among others, thallus growth form seems to be one of the most responsive traits, being relevant in establishing a trade-off between photosynthetic capacity and photorespiration (Gauslaa, 2014; Merinero et al., 2014).

For the first time, in this work we explicitly take into consideration the relevance of growth form in the response of epiphytic lichen communities to microclimatic factors, highlighting the differences, that exist and that we could expect in the future, in sites with greater or lesser capacity to act as climatic microrefuges.

We formulated two consequential hypotheses:

a) functional traits mediated the response of lichen communities to microclimate in the Mediterranean environment, and this response is detectable against the confounding effect of other microenvironmental variables. Moreover, different functional groups show contrasting responses to microclimatic drivers, and.

b) based on the relationships between functional traits and microclimate, microrefuges at the tree scale, characterized by particular conditions of water and temperature, can mitigate the predicted effects of climate change on growth forms that require more humid and colder conditions. By contrast, the mitigation on growth forms linked to dryer and warmer conditions it is possible, though it could be hindered by the absence of other environmental factors.

1.2 Materials & Methods

1.2.1 Study area

We carried out the study in a 1260-km^2 area of western Sardinia, Italy, where human population density is very low (40 persons/km^2), and local sources of air pollution are negligible. Along an altitudinal gradient that ranged from sea level to 1200 m, the main vegetation types were Mediterranean maquis, Mediterranean garigue, and evergreen holm oak forest. This latter was mixed with deciduous oaks, which demonstrated a progressive compositional shift from xero-thermophilic to mesophilic communities up to the highest altitude. Stone pine plantations, cork oak stands, arable fields, and pastures for sheep breeding locally replaced natural plant communities along the same altitudinal gradient. Mean annual rainfall and temperature ranged from 600 mm and 15 °C, respectively, along the coast to 1100 mm and 13 °C, respectively, at the highest elevations.

1.2.2 Sampling

Based on a stratified random sampling design, we selected coordinates pairs to obtain 70 sampling points, which were allocated into nine strata obtained by aggregation of CORINE land cover classes, proportionally to the surface occupied by each stratum within the survey area. In the field, each sampling point was positioned using a GPS and used as the SW corner of an N-oriented 20 × 20-m plot. Within each plot, proportionally to the tree cover, we randomly selected and sampled 1 to 6 trees. Following Asta et al. (2002), we recorded the occurrence of corticolous lichen species in each 10 × 10-cm squares of a sampling grid, which consisted of a 10 × 50 cm ladder that was divided into five quadrants and systematically placed on the N, E, S, and W sides of each tree bole, with the top edge 1.5 m above ground level.

1.2.3 Growth form

All lichen species were categorized by their growth form. We used a modified version of the categorization proposed by Aragón et al., 2016 and Aragón et al., 2019 (Figure 1.1, Appendix A.7). To better differentiate crustose lichens, species were split into conspicuous (e.g. *Pertusaria*) and inconspicuous (e.g. *Catillaria*, *Arthonia*) on the basis of the capability of the species to develop a well-defined thallus or not, respectively. Squamulose species were considered all those species with squamulose thallus (e.g. *Fuscopannaria*, *Normandina*), without considering further sub-divisions of this category. Regarding foliose species, we differentiated between foliose narrowed-lobed (lobes narrower than 0.5 mm: e.g. *Physcia*) and foliose broad-lobed (lobes wider >0.5 mm:). In addition, we used a foliose large category that comprehended larger foliose species (e.g. *Lobaria*, *Peltigera*). Moreover, we also categorized those species with foliose gelatinous swollen thallus (e.g. *Collema*). For fruticose species we did not consider sub-categories (e.g filamentous as in *Usnea* or composite thallus as in *Cladonia*).

1.2.4 Tree-level measurements of environmental variables

A set of environmental variables were also recorded on each tree. Some of these variables have been used to quantify the sub-canopy microclimate (see paragraphs 1.2.6 and 1.2.7). In contrast, others have been directly used as predictors in the fourth corner analysis (see paragraph 1.2.8) to estimate the effect of non-climatic confounding factors on the composition of lichen communities. Variables included chemical and physical characteristics of the bark and some aspects related to the habitat in which the trees were located. We report brief descriptions of the variables

Figure 1.1: Examples of lichen species and growth forms considered in this study: a) fruticose (Frut), *Ramalina farinacea*; b) Large foliose (Fol.large), *Lobaria pulmonaria*; c) broad-lobed *Parmelia*-like foliose (Fol.b), *Parmotrema perlatum*; d) narrow-lobed *Physcia*-like foliose (Fol.n), *Physconia distorta*; e) gelatinous foliose (Fol.gel.swo), *Collema furfuraceum*; f) squamulose (Sq), *Normandina pulchella*; g) conspicuous crustose (Cr.co), *Lepra albescens*; h) inconspicuous crustose (Cr.in), *Chrysothryx candelaris*. A detailed list of all detected species and their corresponding growth forms can be found in the Appendix A.

along with recording procedures, calculations and range values in Table 1.1. More details on the protocols are given in Appendix A.

1.2.5 Statistical downscaling of bioclimatic variables

Bioclimatic variables with 1 km resolution were obtained from CHELSA database (Karger et al., 2017). To minimize model overfitting, we performed a pairwise Pearson correlation between bioclimatic predictors. We retained four predictors that were not highly correlated ($r < |0.70|$). We selected temperature seasonality (BIO4), maximum temperature of the warmest month (BIO5), annual precipitation (BIO12) and seasonality of precipitation (BIO15). Moreover, we downloaded the same variables also for four climate change scenarios: RCP 2.6 and RCP 8.5 for two time periods 2041–2060 and 2061–2080. RCPs were selected from the CESM1-CAM5 model. We downscaled each bioclimatic variable, both current and future, following the procedure used by Lenoir et al. (2017). In particular, we used a Geographic Weighted Regression (GWR) model (Fotheringham et al., 2002). As predictor variables we used Northness, Eastness, altitude, slope, land use, insolation, and distance from the sea. These variables are frequently used in similar studies to model the topoclimate and, as in our case, microclimate (Lenoir et al., 2017). Topographic predictors were calculated using the open-source software QGIS 3.10.12 using a 10 m DTM. Finally, the GWR was run using R 3.6 (R Core Team) through the *gwr* function in the spgwr package (Bivand et al., 2020). Bandwidth was calculated through the *gwr.sel* function.

1.2.6 Partitioning precipitations into stemflow and throughfall at tree level

The overall precipitation was partitioned into stemflow and throughfall at tree level. These two facets of precipitation are strictly related to canopy and bark characteristics. Throughfall represents the precipitation that passes through the canopy due to presence of gaps or branch drips. Conversely, stemflow is the water that flows on the bark drained from the canopy (Sadeghi et al., 2020). In general, comparing the same amount of rainfall, bark thickness and branch angles are important factors in determining the amount of stemflow and throughfall. Though, for stemflow, the ratio between canopy height and width seems to play a more important role (Sadeghi et al., 2020). To model stemflow and throughfall for each tree we used the Gash Analytical Model as reported in Valente et al. (1997). Tree features were measured both in the field and in laboratory, while species-specific traits were retrieved from the available literature. A detailed description of the whole process is presented in the Appendix (paragraph A.1). Stemflow and throughfall are two facets of the overall precipitation in forests as they are an important source of water, nutrients and other chemical compounds for lichens attached to the trunk. Stemflow could be a relevant source of liquid water. For example, high amount of stemflow could led to suprasaturation in certain species, hindering photosynthesis (Lakatos et al., 2006). At the same time, the throughfall could act as a source of vapor water, as the evaporation following a rain event enhance the air relative humidity or, more rarely, of liquid water, when rain falls directly on the thallus. Thus, different regimes of stemflow and throughfall could select different species based on their functional traits, e.g. growth forms, photobionts.

1.2.7 Modelling sub-canopy temperature

Following Lenoir et al. (2017), we assessed the impact of the climatic mitigation effect on sub-canopy temperature by setting a maximum of 3 °C reduction in T max of the warmest month (BIO5) due to the combined effect of topographic concavity (−1 °C) and canopy structure (−2 °C). With a similar procedure, we have described the potential mitigation of T seasonality (BIO4) by setting a maximum of −1.5 °C of reduction (−1 °C due to the effect canopy, −0.5 °C to the concavity effect). These values were supported by periodic direct measurements at sites within the study area where above- and below-canopy temperature data were available.

To quantify the canopy effect, we used a PCA to explore the patterns of variables related to the structure of the sampled trees. In particular, we included tree height, canopy height, canopy area, Leaf Area Index (LAI), and tree cover of the plot. Then, we used the loadings of each tree on the dimensions associated with increasing canopy size and coverage to calculate a canopy effect for each tree. Similarly, the percentage value of topographic concavity in the area surrounding each tree was used to estimate the contribution of the concavity effect to temperature mitigation. The concavity was obtained from the digital terrain model (DTM) of the study area at 10 m resolution, using the SAGA processing module 'terrain surface texture', integrated into QGIS 3.10. Finally, the sub-canopy temperature mitigation of each tree to above-canopy conditions was calculated as follows:

$$\Delta Tmax\ (BIO05) = 2°C\ x\ CaE + 1°C\ x\ CoE$$

$$\Delta Tseasonality\ (BIO04) = 1°C\ x\ CaE + 0.5°C\ x\ CoE$$

where CaE is Canopy Effect and CoE is Concavity Effect.

1.2.8 Fourth corner analysis

To explore the presence and strength of possible associations between functional traits and environmental variables we performed a fourth corner analysis. This method combines three matrices: (i) a sample units × species abundance, (ii) sample units × environmental variables and (iii) a species × traits matrix. Different type of solution of the 'fourth corner problem' have been proposed (Dray & Legendre, 2008; Dray et al., 2014; Brown et al., 2014). We used the model-based approach proposed by Brown et al. (2014) as it allows to test the strength of the interaction between environmental variables and functional traits. The method proceeds by fitting a model with all species abundances at the same time as a function of environmental variables, species traits and their interaction. We used a negative binomial error distribution in the generalized linear model using the traitglm function in the mvabund R package (Wang et al., 2020). For model selection, a least absolute shrinkage and selection operator (LASSO penalty) was used, which is used to simplify interpretation as it switches any terms that do not explain any variation to zero. The model was used to predict abundances in the four different climate change scenarios (RPC 2.6 and 8.5, 2040–2061 and 2061–2080). All predictors based on tree measurements were kept the same for prediction, while those which comprehend also temperature or precipitation (e.g. throughfall) were parameterized based on the ratios between current and future conditions. Predicted abundances were relativized to the maximum frequency in each square to be more comparable. These ratios were modelled using habitat, type of future climatic model (RPC2.5, etc.), and microrefuge capacity and the interaction between these two latter. Models were performed through glmmTMB function from glmmTMB package (Brooks et al., 2017), using *beta_family* as family error distribution. To obtain more robust confidence intervals and p-values all models were boostrapped with 1000 iterations using the *parameters* package (Lüdecke et al., 2020).

1.2.9 Identification of climatic microrefuge capacity of trees

We assessed the climatic microrefuge capacity of each sampled trees using a species-neutral approach. This method does not take into consideration the different microclimatic requirements of individual species or functional groups but assesses the microrefuge capacity based solely on the relationship between the morphological characteristics of the site and the buffering effect that it can exert on macroclimate.

In particular, we used the mitigation effects calculated as described in paragraph 1.2.7 to define the ability of each tree to act as a climatic microrefuge for epiphytic lichens. We quantified the microrefuge capacity in terms of percentile distribution of the mitigation effect of the temperature on the trees.

1.3 Results

1.3.1 Quantifying the microrefuge capacity of trees

Based on the combination of the canopy and the concavity effects, we have defined the ability of each tree to act as a climatic microrefuge for epiphytic lichens.

The first 3 components of the PCA on the structural characteristics of trees accounted for 92.9% of the overall variance (Figure 1.2a). The first component (Dim1

Predictor	Description	Units	Source	Mean	Std. dev.	Min	Max
Nitrogen	Potential NH3 emission	Kg ha^{-1}	Calculated	2602.376	4165.895	0.000	18,668.660
Light	Direct solar radiation	MJm^{-2}d^{-1}	Measured	0.506	0.653	0.035	4.670
Buffer pH	Bark buffer pH	pH unit	Measured	3.96E-05	5.62E-06	0.000	0.000
pH	Bark pH	pH unit	Measured	6.411	0.513	4.070	7.220
Ivy	Ivy cover	Proportion	Estimated	0.019	0.089	0.000	0.613
Moss	Bryophyte cover	Proportion	Estimated	0.059	0.168	0.000	0.925
Bark Micro	Bark microstructure	No unit	PCA on collected data	0.426	0.188	0.000	1.000
T50	Bark loss water halftime	min	Measured	114.190	76.265	13.000	341.000
Maximum temperature of wettest quarter subcanopy	Maximum temperature of wettest quarter	°C*10	Modelled on CHELSA BIO5	246.242	17.671	211.300	280.500
Temperature Seasonality subcanopy	Temperature variation over the year (Standard deviation of monthly mean temperature)	NA	Modelled on CHELSA BIO4	4741.476	225.095	4116.649	5191.110
Precipitation Seasonality subcanopy	Variation in monthly precipitation over the year (Coefficient of variation of monthly precipitation)	NA	Modelled on CHELSA BIO15	54.318	1.708	51.227	57.869
Stemflow	Stemflow	mm y^{-1}	Modelled on CHELSA BIO12	59.815	59.540	0	448.2
Trunk interception	Water intercepted and retained by the tree bark	mm y^{-1}	Modelled on CHELSA BIO12	40.239	29.432	1.300	231.055
Throughfall	Throughfall precipitation	mm y^{-1}	Modelled on CHELSA BIO12	434.064	101.657	80.000	706.700

Table 1.1: Descriptive statistics of the predictors used to determine the relationships between epiphytic lichen communities and tree microenvironment in the study area.

= 49.6%) was associated with increasing tree height, canopy height and canopy area. Consistently with a distinction between trees located in open vs forested areas, the second component (Dim2 = 25.3%) described contrasting gradients of LAI vs tree cover. However, both latter variables were positively associated with the third dimension (Dim3 = 16.6%). As positive values of Dim1 and Dim3 were associated with increasing canopy coverage, we used the loadings of trees on Dim1 and Dim3 to calculate the canopy effect on the microclimatic mitigation of each tree. When taking into account also the effect of topographic concavity, we estimated that on average the sampled trees would be able to lower BIO5 by -1.3 °C (min = -0.3 °C, max = -2.7 °C) and BIO4 by -0.4 °C (min = -0.1 °C, max = -1 °C) (Figure 1.2b).

1.3.2 Hypothesis a) The response of the lichen communities to microclimate is mediated by functional traits. Different functional groups show contrasting responses to the microclimate

The Fourth Corner analysis returns interactions between microenvironmental variables and the abundance of epiphytic lichens that are mediated by their growth form (Figure 1.3).

The growth form was involved in mediating the response to both microclimatic variables, and other microenvironmental factors related to other characteristics of the tree bark. For example, among others, bryophyte coverage had strong positive effects on the abundance of foliose large, fruticose and squamulose species. This latter group was also positively influenced by bark pH and buffer. The capability of the bark of buffering pH was also relevant for foliose gelatinous swollen and crustose conspicuous species.

Considering microclimate descriptors, foliose narrow-lobed species were positively influenced by long dehydration times of the bark (T50) and by high Tmax of the warmest quarter (BIO5), and by temperature seasonality (BIO4). The same variables strongly limited the occurrence of foliose gelatinous swollen species. The

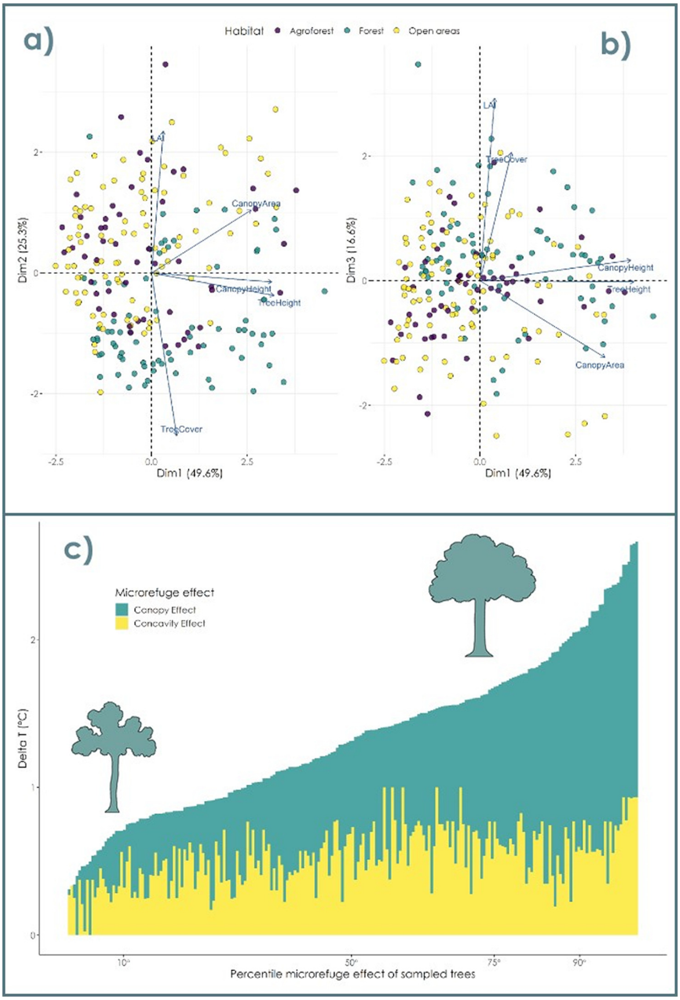


Figure 1.2: Determination of the microrefuge capacity of the trees surveyed in the study area. Figures (a) and (b) show Principal Component Analysis (PCA) of tree morphological characteristics used to calculate weights to be assigned to the maximum canopy capacity for temperature buffering suggested by Lenoir et al. (2017) as 2 °C: PC1 vs PC2 (a) and PC1 vs PC3 (b). Figure (c) shows the percentile distribution of the overall micro-refuge capacity of the trees, determined by the sum of the canopy effect and the concavity effect and expressed as the difference between the temperature outside the canopy and the temperature below the canopy.

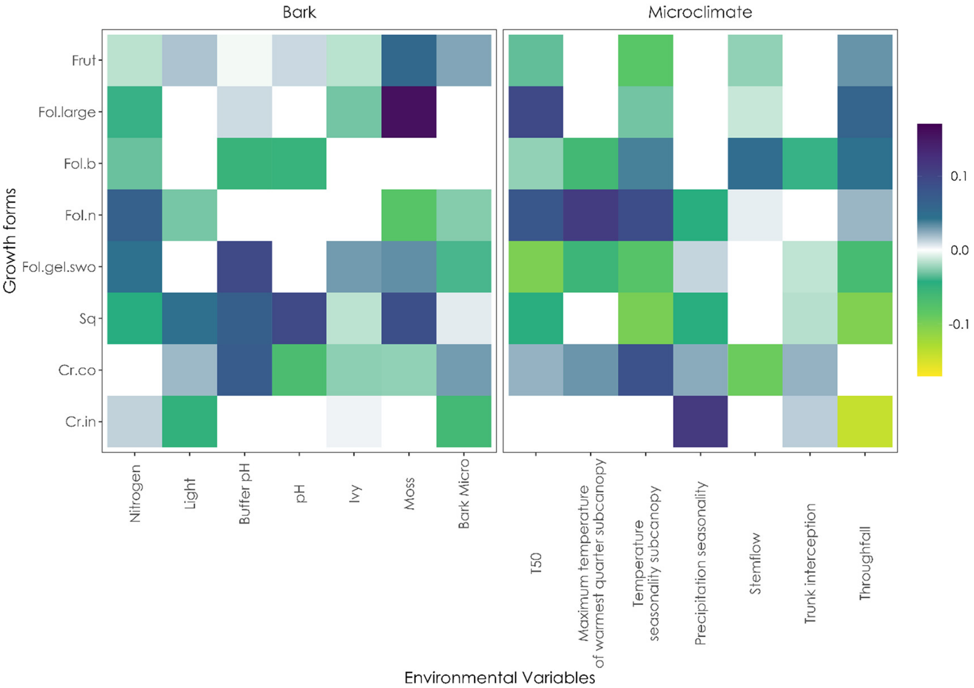


Figure 1.3: Results of the fourth corner analysis relating the functional trait "growth form" of lichen species to the related microenvironmental variables found on trees colonized by epiphytic communities. The micro-environmental variables are distinguished between a set of descriptors of the physical and chemical characteristics of the bark of the trees and a set of descriptors of the microclimatic characteristics found at the trunk under the canopy. Boxes are coloured according to traits fourth-corner coefficients: blue and green indicate positive and negative significant trait-variable association respectively. Details on the measurements and/or calculation of the predictors are given in Table 1.1. Abbreviations of lichen growth forms are illustrated in Figure 1.1

seasonality of precipitations (BIO15) determined contrasting responses between crustose inconspicuous and foliose narrow-lobed species. Among the components of sub-canopy precipitation, throughfall inhibited the presence of crustose inconspicuous and squamulose, while enhancing fruticose species. Water intercepted by the trunk inhibited the presence of broad-lobed foliose species, which, in turn, were enhanced by a high amount of stemflow. Foliose large species were enhanced by a longer dehydration time of the bark and partially by a high throughfall.

	Estimate	**Bootstrap 2.5%**	**Bootstrap 97.5%**	***p value***
Frut				
(Intercept)	-2.55979	-2.96205	-2.13833	<0.001
Microref. cap.	1.321963	0.962605	1.645346	<0.001
26_y40	-0.77399	-0.98687	-0.54807	<0.001
26_y60	-0.91185	-1.13522	-0.6958	<0.001
85_y40	-1.08517	-1.32776	-0.84858	<0.001
85_y60	-2.60806	-2.9045	-2.31093	<0.001
Agroforest vs. Forest	-0.25453	-0.80262	0.31558	0.332
Open areas vs. Forest	-0.20703	-0.64812	0.249092	0.432
Microref. cap.:26_y40	-0.78797	-1.16892	-0.43679	<0.001
Microref. cap.:26_y60	-0.34804	-0.68681	0.013868	0.058
Microref. cap.:85_y40	-0.70881	-1.08343	-0.3443	<0.001
Microref. cap.:85_y60	-0.85842	-1.33889	-0.37569	<0.001
Fol.large				
(Intercept)	-4.39015	-4.90619	-3.9515	<0.001
Microref. cap.	0.893105	0.349385	1.508417	<0.001
26_y40	-0.08356	-0.41228	0.259263	0.658
26_y60	-0.18622	-0.55671	0.152556	0.28
85_y40	-0.14095	-0.49545	0.224067	0.462
85_y60	-0.65808	-1.03568	-0.29468	<0.001
Agroforest vs. Forest	-0.39221	-0.90514	0.082726	0.104
Open areas vs. Forest	-0.4019	-0.80635	0.00119	0.052
Microref. cap.:26_y40	-0.40316	-1.01798	0.147889	0.16
Microref. cap.:26_y60	0.087804	-0.52534	0.670662	0.774
Microref. cap.:85_y40	-0.43735	-1.03118	0.185285	0.164
Microref. cap.:85_y60	-1.0006	-1.61876	-0.36759	0.004
Fol.b				
(Intercept)	-1.51582	-1.96892	-1.04916	<0.001
Microref. cap.	-0.7708	-1.10393	-0.42382	<0.001
26_y40	-1.48988	-1.69194	-1.28663	<0.001
26_y60	-1.58445	-1.78125	-1.38803	<0.001
85_y40	-1.47457	-1.68798	-1.29236	<0.001
85_y60	-3.52721	-3.80121	-3.26963	<0.001
Agroforest vs. Forest	-0.22464	-0.81144	0.368837	0.468
Open areas vs. Forest	-0.16726	-0.6624	0.377866	0.566
Microref. cap.:2.6_y40	0.231567	-0.15864	0.583362	0.248
Microref. cap.:26_y60	0.671496	0.32135	1.047184	<0.001
Microref. cap.:85_y40	0.430144	0.088599	0.793104	0.012
Microref. cap.:85_y60	1.040393	0.58037	1.511842	<0.001
Fol.n				
(Intercept)	-0.48309	-0.87232	-0.12586	0.006
Microref. cap.	-1.09123	-1.39784	-0.77934	<0.001
26_y40	-1.05582	-1.23457	-0.86559	<0.001
26_y60	-1.44367	-1.63084	-1.25708	<0.001
85_y40	-0.3255	-0.49959	-0.15423	<0.001
85_y60	-3.35856	-3.6242	-3.11683	<0.001
Agroforest vs. Forest	0.133386	-0.32255	0.592381	0.558
Open areas vs. Forest	0.305482	-0.08917	0.691862	0.126
Microref. cap.:26_y40	0.119892	-0.22714	0.48357	0.506
Microref. cap.:26_y60	0.606761	0.261905	0.949966	<0.001
Microref. cap.:85_y40	0.052707	-0.25243	0.366688	0.722
Microref. cap.:85_y60	1.264895	0.811891	1.708384	<0.001

	Estimate	**Bootstrap 2.5%**	**Bootstrap 97.5%**	***p value***
Fol.gel.swo				
(Intercept)	-4.40195	-4.82627	-3.98591	<0.001
Microref. cap.	2.299685	1.823958	2.722609	<0.001
26__y40	-0.43129	-0.76523	-0.09253	0.012
26__y60	-0.5296	-0.86068	-0.21096	0.002
85__y40	-0.65003	-1.01289	-0.31682	<0.001
85__y60	-1.30284	-1.65399	-0.97319	<0.001
Agroforest vs. Forest	-0.45237	-0.86981	-0.01574	0.04
Open areas vs. Forest	-0.00232	-0.37064	0.40275	0.996
Microref. cap.:26__y40	-1.66445	-2.20199	-1.12987	<0.001
Microref. cap.:26__y60	-1.62125	-2.14259	-1.1188	<0.001
Microref. cap.:85__y40	-1.75612	-2.31957	-1.18184	<0.001
Microref. cap.:85__y60	-2.06046	-2.62015	-1.47204	<0.001
Sq				
(Intercept)	-5.46386	-5.87602	-5.0409	<0.001
Microref. cap.	2.24863	1.726404	2.723809	<0.001
26__y40	-0.01546	-0.35752	0.360566	0.924
26__y60	-0.23763	-0.61836	0.125461	0.202
85__y40	-0.1408	-0.52514	0.193805	0.44
85__y60	-0.73508	-1.11096	-0.37383	<0.001
Agroforest vs. Forest	-0.16484	-0.49812	0.197715	0.346
Open areas vs. Forest	-0.04942	-0.32949	0.24881	0.716
Microref. cap.:26__y40	-1.42207	-2.04448	-0.84031	<0.001
Microref. cap.:26__y60	-1.54103	-2.12869	-0.9025	<0.001
Microref. cap.:85__y40	-1.56831	-2.14699	-0.94007	<0.001
Microref. cap.:85__y60	-1.84875	-2.46533	-1.22613	<0.001
Cr.co				
(Intercept)	-0.68742	-1.03964	-0.35016	<0.001
Microref. cap.	-1.09178	-1.46479	-0.7499	<0.001
26__y40	-1.22292	-1.42543	-1.00083	<0.001
26__y60	-1.28549	-1.50463	-1.06184	<0.001
85__y40	-0.7545	-0.96389	-0.5535	<0.001
85__y60	-2.80736	-3.09346	-2.53896	<0.001
Agroforest vs. Forest	-0.17057	-0.54689	0.206239	0.394
Open areas vs. Forest	0.044111	-0.28483	0.378201	0.812
Microref. cap.:26__y40	0.181531	-0.22703	0.60397	0.376
Microref. cap.:26__y60	0.563198	0.170539	0.953536	0.006
Microref. cap.:85__y40	0.253043	-0.12244	0.616367	0.192
Microref. cap.:85__y60	1.085712	0.614793	1.549444	<0.001
Cr.in				
(Intercept)	-1.97729	-2.29193	-1.68265	<0.001
Microref. cap.	0.538341	0.214804	0.858051	0.002
26__y40	-1.3592	-1.5791	-1.1387	<0.001
26-y60	-1.3756	-1.59011	-1.17053	<0.001
85__y40	-1.33423	-1.55018	-1.12003	<0.001
85__y60	-2.73486	-3.01492	-2.45481	<0.001
Agroforest vs. Forest	-0.03794	-0.39978	0.306838	0.804
Open areas vs. Forest	0.175993	-0.12055	0.455345	0.246
Microref. cap.:26__y40	-0.38913	-0.75826	-0.03239	0.026
Microref. cap.:26__y60	-0.05552	-0.41727	0.316675	0.786
Microref. cap.:85__y40	-0.23859	-0.64042	0.147611	0.22
Microref. cap.:85__y60	0.098796	-0.36649	0.579981	0.718

Table 1.2: Results of the GLMM models. Confidence intervals and p-values were obtained using bootstrap with 1000 iterations. Abbreviations of lichen growth forms are illustrated in Fig. 1. Other abbreviations: Microref. cap.: Microrefuge capacity, 26__y40: RCP 2.6 year 2040, 26__y60: RCP 2.6 year 2060, 85__y40: RCP 8.5 year 2040, 85__y60: RCP 8.5 year 2060.

1.3.3 Hypothesis b) Microrefuges at the tree scale can mitigate the predicted effects on hosted lichen communities in scenarios of climate change

Using GLMM models, we analyzed the relationship between the abundance of each growth form as a function of the microrefuge capacity of trees in the different climate change scenarios (Table 1.2, Figure 1.4). Under the current conditions, a strong microrefuge effect has been observed for fruticose and foliose gelatinous swollen, foliose large, squamulose and crustose inconspicuous species whose abundance increases linearly or even exponentially with microrefuge capacity of the trees. Although in a context of progressive reduction of abundance, among these growth forms, fruticose, squamulose and foliose gelatinous swollen species are expected to maintain a significant relationship with the microrefuge capacity in all future scenarios, while for foliose large and crustose inconspicuous species in 2040 and 2060, both in the optimistic scenario RCP 2.6 and in the pessimistic scenario RCP 8.5, the models predicted a drastic reduction in abundance, regardless of the microrefuge capacity of the host trees.

On the other hand, broad-lobed foliose, foliose narrow-lobed and crustose conspicuous species under the current conditions were more abundant on trees with lower microrefuge capacity, showing a negative trend according to this variable. According to the model, these growth forms are expected to undergo a progressive decrease in abundance which may be more marked on trees with less microrefuge capacity.

1.4 Discussion

Exploring the relationships between microclimate and biodiversity is a key issue to better understand the direct and indirect impacts of global change on the biota (De Frenne et al., 2021). In particular, unravelling species-climate relationships at the local scale will likely provide a more comprehensive, precise, and detailed picture of the interactions between abiotic factors and organisms and, consequently, enable more accurate predictions on potential community changes (Bramer et al., 2018; De Frenne et al., 2019; Zellweger et al., 2019). Following this research line, as an innovative contribution of this work, we have been able to delineate the interactions between microclimatic variables in Mediterranean epiphytic lichen communities, providing a detailed picture of the expected changes in the near future. Our results partially support our two consequential hypotheses about the response of epiphytic lichen communities to microclimate and to global changes, which are hereafter discussed.

1.4.1 Hypothesis a) Growth form mediates the response of epiphytic lichen communities to microclimate

Our results reveal significant relationships between lichen functional traits and different environmental variables related to microclimate. Thallus growth form primarily characterizes the response to microclimatic variables, with contrasting responses between different growth form-based functional groups (Figure 1.5). In particular, community compositional shifts correspond to different growth forms prevailing under different conditions of sub-canopy temperatures and precipitation components, consistent with the effects of the amount, duration, and physical state of water availability for epiphytic communities (Gauslaa, 2014; Giordani and Incerti, 2008; Ås Hovind et al., 2020; Phinney et al., 2019; Gauslaa and Solhaug, 1998). Along the microclimatic variation, we found a gradient of growth form turnover connected with specific water requirements. In conditions of throughfall precipitation prevalence, high light availability and low temperature seasonality, fruticose lichens are favored. Under larger canopies with reduced maximum temperatures and high rainfall interception and stemflow along the trunk, broad-lobed foliose lichens thrive as their thalline structure is more suitable for intercepting running water. Interestingly, when stemflow decreases, as in both cases of lower annual rainfall and higher bark water retention capacity, community composition shifts from broad-lobed foliose lichens to crustose growth forms. As such, the water retention capacity of the bark seems to play a fundamental role in defining the duration of the activity periods of lichen communities. In sub-arid Mediterranean environment where water is a limiting factor, the uptake of bark water extends the period of activity with positive net photosynthesis by up to 21% (Porada and Giordani, 2021). Irrespectively of the total precipitation amount, narrow-lobed foliose lichens respond to temperature conditions, being fostered by high maximum values and large seasonal differences. Their prevalence under the harshest temperature conditions could be simply related to the minimal competitive pressure by species with different growth form in such conditions, more than to specific advantage provided by lobe narrowness per se.

1.4.2 Hypothesis b) Micro-refuge trees locally mitigate climate change effects on epiphytic lichen community

Our models provide a complex picture that is only partially consistent with the hypothesis of a positive effect of micro-refuge trees on the abundance of epiphytic lichen functional groups. In fact, the current distribution pattern of many lichen growth forms is strongly associated to the microclimatic mitigation capacity exerted by the host trees by means of specific morpho-physical-chemical canopy and bark traits. However, these combinations of host and epiphyte traits may not still hold with the same balance in the future. In particular, we have outlined three distinct situations which are summarized schematically in Figure 1.6:

1.4.2.1 Micro-refuge trees will continue to preserve some lichen functional groups in the future

We estimate that the climatic microrefuge capacity of some trees could prove decisive in enabling the survival of certain functional groups that are already present in these environmental conditions. These groups are rather morphologically heterogeneous, including fruticose, squamulose and foliose gelatinous growth forms. Fruticose survival could be due to the mitigation potential of the trees on which they live combined with their intrinsic resilience. On the other hand, the trees colonized by squamulose and foliose gelatinous lichens are located in areas less impacted by macroclimatic scenarios, so their mitigation potential seems sufficient to neutralise macro-scale exacerbation.

1.4.2.2 Micro-refuge trees will not be enough to save species that have already paid their part of extinction debt

A second situation can be depicted for the growth forms preferentially found under mild conditions, on trees with high mitigation potential. According to our results, two of these groups, large foliose and crustose inconspicuous lichens, shall not resist to the future water shortage and warming, with even the trees with highest mitigation potential apparently unable to ensure micro-refuge conditions for these lichens, hence destined to an irreparable decline. Most likely, these groups in the Mediterranean have already paid a large part of their extinction debt (Ellis et al., 2017; Ellis and Coppins, 2017). Presumably, these lichens have already been relegated for a long time to climatic refugia, where they are maintaining residual populations. On the other hand, for large foliose lichens, which include well-known species of the genus Lobaria, several works have already predicted a drastic decrease in the climatic suitability for these species and their host trees (Nascimbene et al., 2020).

1.4.2.3 Xerophilous species will decline and will not be able to exploit the micro-refuges

Conspicuous crustose, narrow- and broad-lobed lichens, which include some of the most common taxa, are currently more abundant on trees with low micro-refuge capacity. As shown by the fourth corner analysis results, this situation can certainly be traced back to their ecological demands already outlined in the previous section. In fact, these growth forms are favored by harsh microclimatic environment that can hardly be found on the trunk of trees with high capacity of climate mitigation. Consistent with this preference for more extreme context, even in future climate change scenarios, these growth forms shall not increase their abundance in micro-refuge trees. However, contrary to what might be expected, our results for both the optimistic and pessimistic scenarios indicate that these lichens shall undergo a drastic abundance decrease on trees more suited to their ecological requirements. Therefore, even for more xerophilous and thermophilic species, the future water shortage and temperature regimes shall exceed the limit of their potential ecological niche under the canopy of trees.

1.4.3 Consequences for Mediterranean forest ecosystem

What would happen if micro-refuge trees were no longer able to provide a suitable microclimate for epiphytic lichens? In addition to conservation issues related to the reduction and/or loss of lichen diversity, the scenarios outlined by our models also raise some considerations at the scale of forest ecosystems in Mediterranean regions. It is clear that in these environments lichens are a minor component in terms of biomass, but, especially with reference to epiphytic communities, they constitute, together with bryophytes, a unique microhabitat for several groups of organisms (Asplund et al., 2018; Asplund & Wardle, 2017). Small arthropods and terrestrial mollusks are primarily or even exclusively linked to lichens (Asplund & Wardle, 2017). For these organisms, epiphytic communities represent sources of water and food, refuge, hunting and nesting areas. The effects of a local decrease in epiphytic communities can also translate into considerable consequences at regional or continental scales on basic ecosystem functions such as those related to the water cycle. For example, Porada et al. (2018) have shown that in terrestrial ecosystems the total evaporation of free water from the forest canopy and soil surface increases by 61% when non-vascular vegetation is included.

1.4.4 Limitations and perspectives

Although our work has provided a detailed picture of the environmental relationships controlling the composition of epiphytic lichen communities, there are certainly some limitations that need to be considered and which could be the starting point for further studies.

First, it is well established that obligate epiphytes have a close relationship with their tree substrate. Recent studies have shown that the decoupling of these relationships could be an additional indirect effect of climate change (Nascimbene et al., 2020). Our models did not take into account the potential changes of host tree species. In other words, in our model, results of the future scenarios refer to

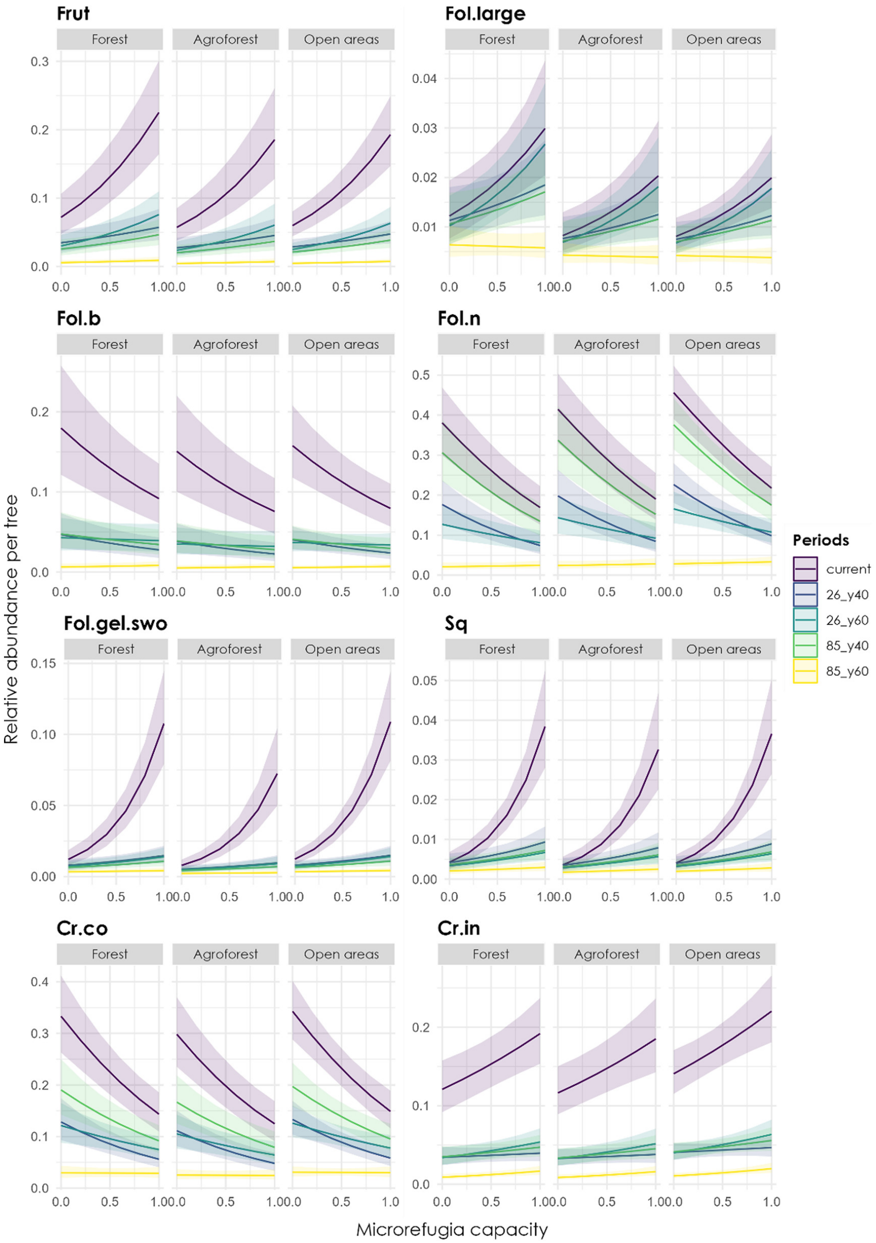


Figure 1.4: Expected differences in terms of relative abundance for different microrefugia capacity comparing current conditions with different climate change scenarios (RCP2.6 and RCP8.5), different years (2040 and 2060) and different habitat (Forest, Agroforest and Open areas) for each growth form. Abbreviations of lichen growth forms are illustrated in Figure 1.1

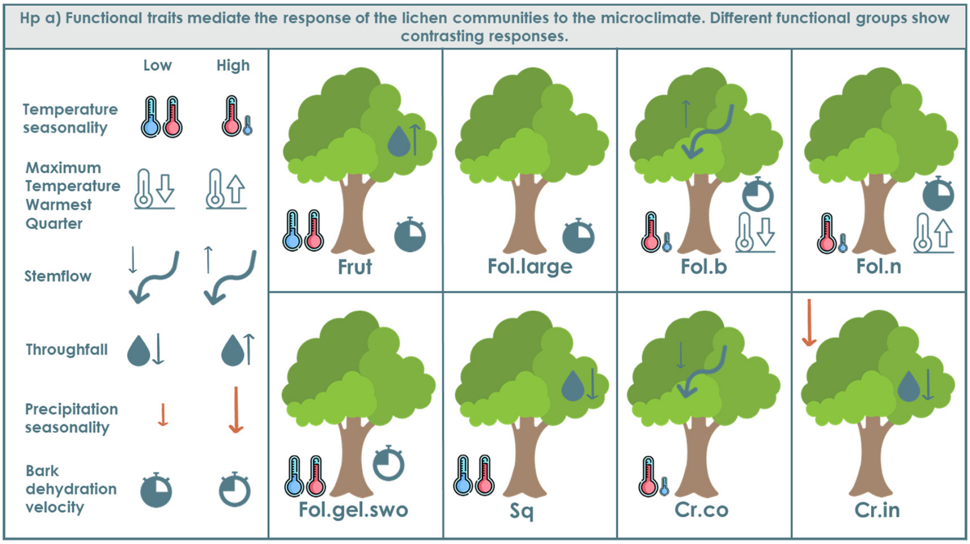


Figure 1.5: Summary diagram of the main responses of epiphytic lichens to the microclimate mediated by the growth form tested with hypothesis a) and according to the results obtained from the fourth corner analysis shown in Figure 1.3. Abbreviations of lichen growth forms are illustrated in Figure 1.1.

trees in the study area that have equivalent micro-refuge capacity to those actually observed. This may be an oversimplification, especially when considering our results for predictive purposes. However, under a pure research perspective, it allows us to focus on the microclimatic effect net of other confounding factors, including, as non-exhaustive examples, effects of warming and water shortage on morpho-physical-chemical tree properties. Ideally, integrating the study of the functional ecology of epiphytic communities with the development of models capable of simulating the growth of their tree substrates (Trotsiuk et al., 2020) under different environmental conditions could lead to a more refined prediction of epiphyte dynamics. Another possible limitation of this work is that we take into account mitigation and not buffering. Maintaining a more stable temperature could lead to less dramatic changes in terms of temperature in respect to those predicted considering mitigation, leading to a less pronounced impact on lichen species. Moreover, we have modelled the lichen abundances by taking a static approach that is unable to weigh any differences that the various species may show throughout their life cycle (Benesperi et al., 2018),

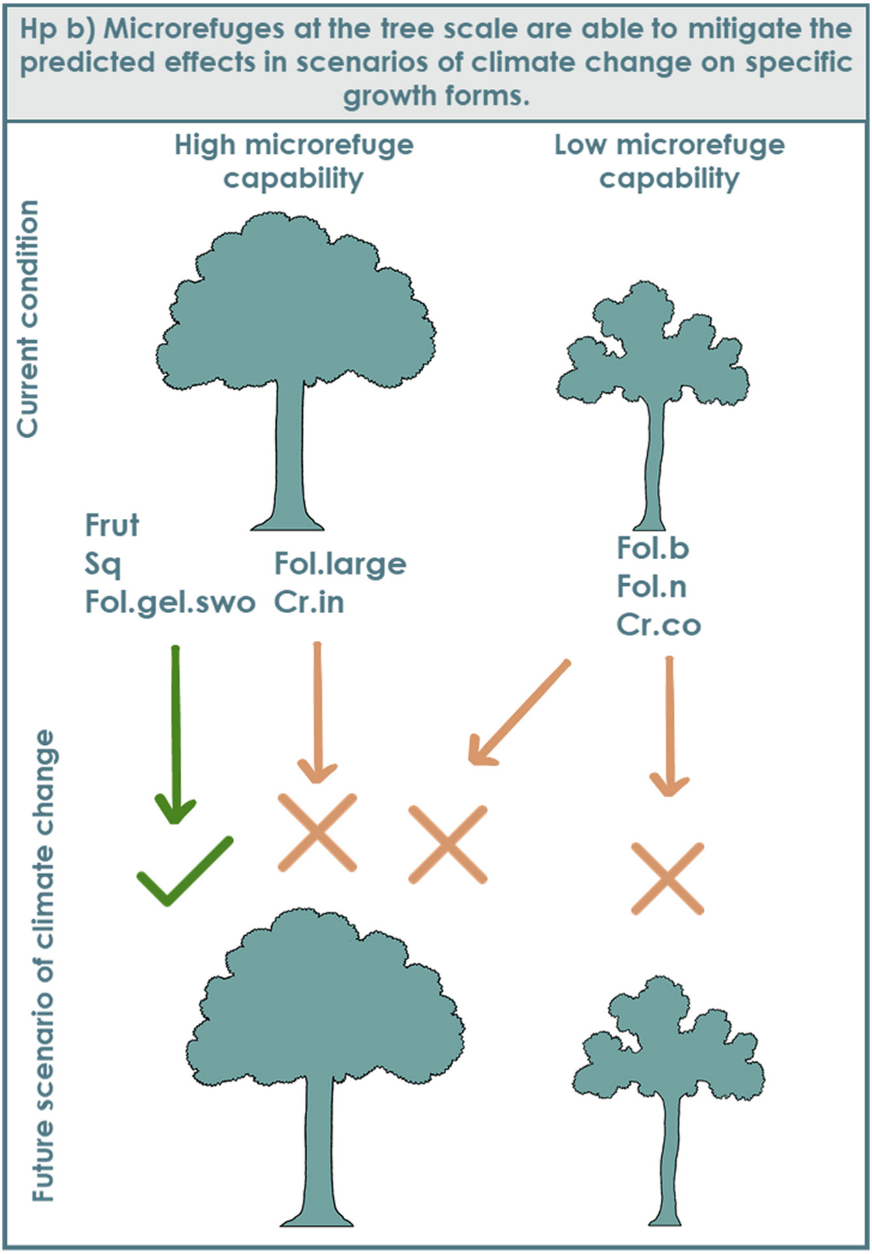


Figure 1.6: Traits-mediated future variations of lichen communities on trees with high vs low microrefuge capacity according to hypothesis b). Abbreviations of lichen growth forms are illustrated in Figure 1.1.

including the establishment and development phases of new thalli that can be very critical for determining the continuity of the colonization.

A further limitation is that our models consider functional groups separately and exclude community interactions, which obviously occur in the real system and can shape community composition. These interactions include both competitive and facilitative processes that may contribute to slowing, accelerating or modifying the effects of abiotic factors on communities (Saiz et al., 2021). The relationship between community interactions and the severity of environmental conditions is a hot topic of interest in plant ecology research (Brooker et al., 2008; Le Bagousse-Pinguet et al., 2014; Bonanomi et al., 2016). In the case of epiphytes, and lichens in particular, much less is known and it is certainly a field of research worthy of investigation in the near future.

Our models predict drastic changes and reduction of epiphytic lichen communities in the worst climate change scenarios. Nevertheless, lichens can colonize much more extreme habitats, such as deserts, where they face high temperatures and low water availability. Lichens, like many other organisms, are predicted to migrate to their track suitable climate space (Ellis, 2019). Consequently, it could be hypothesized that, in the future, species adapted to dry and warm conditions could find here their suitable conditions, replacing the native flora.

Acknowledgements. We are thankful to Dr. Gabriele Gheza for help with the figures. The authors would like to thank Prof. John T. Van Stan (Cleveland State University) and two anonymous reviewers for their constructive suggestions to the manuscript.

1.5 Bibliography

Aragón, G., Belinchón, R., Martínez, I., & Prieto, M. (2016). A survey method for assessing the richness of epiphytic lichens using growth forms. *Ecological Indicators*, 62, 101–105. https://doi.org/10.1016/j.ecolind.2015.11.034.

Aragón, G., Martínez, I., Hurtado, P., Benítez, Á., Rodríguez, C., & Prieto, M. (2019). Using growth forms to predict epiphytic lichen abundance in a wide variety of forest types. *Diversity*, 11(4), 51. https://doi.org/10.3390/d11040051.

Ås Hovind, A. B., Phinney, N. H., & Gauslaa, Y. (2020). Functional trade-off of hydration strategies in old forest epiphytic cephalolichens. *Fungal Biology*, 124(10), 903–913. https://doi.org/10.1016/j.funbio.2020.07.008.

Asplund, J., & Wardle, D. A. (2017). How lichens impact on terrestrial community and ecosystem properties. *Biological Reviews*, 92(3), 1720–1738. https://doi.org/10.1111/brv.12305.

Asplund, J., Strandin, O. V., & Gauslaa, Y. (2018). Gastropod grazing of epiphytic lichen-dominated communities depends on tree species. *Basic and Applied Ecology*, 32, 96–102. https://doi.org/10.1016/j.baae.2018.07.007.

Asta, J., Erhardt, W., Ferretti, M., Fornasier, F., Kirschbaum, U., Nimis, P. L., ..., & Wirth, V. (2002). *Mapping lichen diversity as an indicator of environmental quality.* In P. L. Nimis, C. Scheidegger, & P. A. Wolseley (Eds.), Monitoring With Lichens—Monitoring Lichens (pp. 273–279). Springer. https://doi.org/10.1007/978-94-010-0423-7_19.

Benesperi, R., Nascimbene, J., Lazzaro, L., Bianchi, E., Tepsich, A., Longinotti, S., ... & Giordani, P. (2018). Successful conservation of the endangered forest lichen Lobaria pulmonaria requires knowledge of fine-scale population structure. *Fungal Ecology*, 33, 65–71. https://doi.org/10.1016/j.funeco.2018.01.006.

Bivand, R., Yu, D., Tomoki, N., & Garcia-Lopez, M.-A. (2020). SPGWR: Geographically Weighted Regression (Version 0.6-34) [R]. CRAN.

Bonanomi, G., Stinca, A., Chirico, G. B., Ciaschetti, G., Saracino, A., & Incerti, G. (2016). Cushion plant morphology controls biogenic capability and facilitation effects of Silene acaulis along an elevation gradient. *Functional Ecology*, 30(7), 1216–1226. https://doi.org/10.1111/1365-2435.12596.

Bramer, I., Anderson, B. J., Bennie, J., Bladon, A. J., De Frenne, P., Hemming, D., ... & Gillingham, P. K. (2018). Advances in monitoring and modelling climate at ecologically relevant scales. In D. A. Bohan, A. J. Dumbrell, G. Woodward, & M. Jackson (Eds.), *Advances in Ecological Research* (Vol. 58, pp. 101–161). Academic Press. https://doi.org/10.1016/bs.aecr.2017.12.005.

Brooker, R. W., Maestre, F. T., Callaway, R. M., Lortie, C. L., Cavieres, L. A., Kunstler, G., ... & Anthelme, F. (2008). Facilitation in plant communities: the past, the present, and the future. *Journal of Ecology*, 96(1), 18–34.

Brooks, M.E., Kristensen, K., van Benthem, K.J., Magnusson, A., Berg, C.W., Nielsen, A., ... & Bolker, B.M. (2017). GlmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *R Journal*, 9(2), 378–400.

Brown, A.M., Warton, D.I., Andrew, N.R., Binns, M., Cassis, G., & Gibb, H. (2014). The fourth-corner solution—using predictive models to understand how species traits interact with the environment. *Methods in Ecology and Evolution*, 5(4), 344–352. https://doi.org/10.1111/2041-210X.12163

De Frenne, P., Zellweger, F., Rodríguez-Sánchez, F., Scheffers, B.R., Hylander, K., Luoto, M., ... & Lenoir, J. (2019). Global buffering of temperatures under forest canopies. *Nature Ecology & Evolution*, 3(5), 744–749. https://doi.org/10.1038/s41559-019-0842-1

De Frenne, P., Lenoir, J., Luoto, M., Scheffers, B. R., Zellweger, F., Aalto, J., ... & Hylander, K. (2021). Forest microclimates and climate change: Importance, drivers and future research agenda. *Global Change Biology*, 27(11), 2279-2297.

Di Nuzzo, L., Vallese, C., Benesperi, R., Giordani, P., Chiarucci, A., Di Cecco, V., ... & Nascimbene, J. (2021). Contrasting multitaxon responses to climate change in Mediterranean mountains. *Scientific reports*, 11(1), 4438.

Dray, S., & Legendre, P. (2008). Testing the species traits environment relationships: the fourth-corner problem revisited. *Ecology*, 89(12), 3400–3412. https://doi.org/10.1890/08-0349.1

Dray, S., & Choler, P., Dolédec, S., Peres-Neto, P.R., Thuiller, W., Pavoine, S., ter Braak, C.J.F. (2014). Combining the fourth-corner and the RLQ methods for assessing trait responses to environmental variation. *Ecology*, 95(1), 14–21. https://doi.org/10.1890/13-0196.1

Ellis, C.J. (2019). Climate change, bioclimatic models and the risk to lichen diversity. *Diversity*, 11(4), 54. https://doi.org/10.3390/d11040054

Ellis, C.J. (2020). Microclimatic refugia in riparian woodland: a climate change adaptation strategy. *Forest Ecology and Management*, 462, 118006. https://doi.org/10.1016/j.foreco.2020.118006

Ellis, C.J., & Coppins, B.J. (2017). Taxonomic survey compared to ecological sampling: are the results consistent for woodland epiphytes? *The Lichenologist*, 49(02), 141–155. https://doi.org/10.1017/S0024282917000056

Ellis, C.J., & Eaton, S. (2021). Microclimates hold the key to spatial forest planning under climate change: cyanolichens in temperate rainforest. *Global Change Biology*, 27(9), 1915–1926. https://doi.org/10.1111/gcb.15514

Ellis, C.J., Eaton, S., Theodoropoulos, M., Coppins, B.J., Seaward, M.R.D., & Simkin, J. (2014). Response of epiphytic lichens to 21st century climate change and tree disease scenarios. *Biological Conservation*, 180, 153–164. https://doi.org/10.1016/j.biocon.2014.09.046

Ellis, C.J., Geddes, H., McCheyne, N., & Stansfield, A. (2017). Lichen epiphyte response to nonanalogue monthly climates: a critique of bioclimatic models. *Perspectives in Plant Ecology, Evolution and Systematics*, 25, 45–58. https://doi.org/10.1016/j.ppees.2017.01.005

Ellis, C. J., Asplund, J., Benesperi, R., Branquinho, C., Di Nuzzo, L., Hurtado, P., ... & Giordani, P. (2021). Functional traits in lichen ecology: a review of challenge and opportunity. *Microorganisms*, 9(4), 766.

Fotheringham, A.S., Brunsdon, C., & Charlton, M. (2002). *Geographically Weighted Regression: The Analysis of Spatially Varying Relationships*. Wiley. Link

Gauslaa, Y. (2014). Rain, dew, and humid air as drivers of morphology, function and spatial distribution in epiphytic lichens. *The Lichenologist*, 46(1), 1–16. https://doi.org/10.1017/S0024282913000753

Gauslaa, Y., & Solhaug, K.A. (1998). The significance of thallus size for the water economy of the cyanobacterial old-forest lichen Degelia plumbea. *Oecologia*, 116(1–2), 76–84. https://doi.org/10.1007/s004420050565

Giordani, P., & Incerti, G. (2008). The influence of climate on the distribution of lichens: a case study in a borderline area (Liguria, NW Italy). *Plant Ecology*, 195, 257–272. https://doi.org/10.1007/s11258-007-9324-7

Giordani, P., Brunialti, G., Bacaro, G., & Nascimbene, J. (2012). Functional traits of epiphytic lichens as potential indicators of environmental conditions in forest ecosystems. *Ecological Indicators*, 18, 413–420.

Giordani, P., Malaspina, P., Benesperi, R., Incerti, G., & Nascimbene, J. (2019). Functional overredundancy and vulnerability of lichen communities decouple across spatial scales and environmental severity. *Science of the Total Environment*, 666, 22–30. https://doi.org/10.1016/j.scitotenv.2019.02.187

Giordani, P., Benesperi, R., Bianchi, E., Malaspina, P., & Nascimbene, J. (2020). Threats and conservation strategies for overlooked organisms: the case of epiphytic lichens. In *Environmental Concerns and Sustainable Development* (pp. 1–26). https://doi.org/10.1007/978-981-13-6358-0_1

Giorgi, F., & Lionello, P. (2008). Climate change projections for the Mediterranean region. *Global Planetary Change*, 63(2), 90–104. https://doi.org/10.1016/j.gloplacha.2007.09.005

Haesen, S., Lembrechts, J. J., De Frenne, P., Lenoir, J., Aalto, J., Ashcroft, M. B., ... & Van Meerbeek, K. (2021). ForestTemp–Sub-canopy microclimate temperatures of European forests. *Global Change Biology*, 27(23), 6307-6319.

Hannah, L., Flint, L., Syphard, A.D., Moritz, M.A., Buckley, L.B., & McCullough, I.M. (2014). Fine-grain modeling of species' response to climate change: holdouts, stepping-stones, and microrefugia. *Trends in Ecology & Evolution*, 29(7), 390–397. https://doi.org/10.1016/j.tree.2014.04.006

Hawksworth, D.L., & Grube, M. (2020). Lichens redefined as complex ecosystems. *New Phytologist*, 227(5). https://doi.org/10.1111/nph.16630

Hurtado, P., Prieto, M., Aragón, G., Escudero, A., & Martínez, I. (2019). Critical predictors of functional, phylogenetic and taxonomic diversity are geographically structured in lichen epiphytic communities. *Journal of Ecology.* https://doi.org/10.1111/1365-2745.13189

Hurtado, P., Prieto, M., Martínez-Vilalta, J., Giordani, P., Aragón, G., López-Angulo, J., ... & Martínez, I. (2020). Disentangling functional trait variation and covariation in epiphytic lichens along a continent-wide latitudinal gradient. *Proceedings of the Royal Society B*, 287(1922), 20192862.

Karger, D. N., Conrad, O., Böhner, J., Kawohl, T., Kreft, H., Soria-Auza, R. W., ... & Kessler, M. (2017). Climatologies at high resolution for the earth's land surface areas. *Scientific data*, 4(1), 1-20.

Keppel, G.,. & Wardell-Johnson, G.W. (2015). Refugial capacity defines holdouts, microrefugia and stepping-stones: a response to Hannah et al. *Trends in Ecology & Evolution*, 30(5), 233–234. https://doi.org/10.1016/j.tree.2015.01.008

Kranner, I., Beckett, R., Hochman, A.,. & Nash III, T.H. (2008). Desiccation-tolerance in lichens: a review. *Bryologist*, 111(4), 576–593.

Lakatos, M., Rascher, U.,. & Büdel, B. (2006). Functional characteristics of corticolous lichens in the understory of a tropical lowland rain forest. *New Phytologist*, 172(4), 679–695. https://doi.org/10.1111/j.1469-8137.2006.01871.x

Lange, O.L., Kilian, E.. &, Ziegler, H. (1986). Water vapor uptake and photosynthesis of lichens: performance differences in species with green and blue-green algae as phycobionts. *Oecologia*, 71(1), 104–110. https://doi.org/10.1007/BF00377327

Le Bagousse-Pinguet, Y., Xiao, S., Brooker, R.W., Gross, N., Liancourt, P., Straile, D.,. & Michalet, R. (2014). Facilitation displaces hotspots of diversity and allows communities to persist in heavily stressed and disturbed environments. *Journal of Vegetation Science*, 25(1), 66–76. https://doi.org/10.1111/jvs.12064

Lenoir, J., Hattab, T.,. & Pierre, G. (2017). Climatic microrefugia under anthropogenic climate change: implications for species redistribution. *Ecography*, 40(2), 253–266. https://doi.org/10.1111/ecog.02788

Lüdecke, D., Ben-Shachar, M., Patil, I.,. & Makowski, D. (2020). Extracting, computing and exploring the parameters of statistical models using R. *Journal of Open Source Software*, 5(53), 2445. https://doi.org/10.21105/joss.02445

Maclean, I.M.D.,. & Wilson, R.J. (2011). Recent ecological responses to climate change support predictions of high extinction risk. *Proceedings of the National Academy of Sciences*, 108(30), 12337–12342. https://doi.org/10.1073/pnas.1017352108

Maclean, I.M.D., Hopkins, J.J., Bennie, J., Lawson, C.R.,. & Wilson, R.J. (2015). Microclimates buffer the responses of plant communities to climate change. *Global Ecology and Biogeography*, 24(11), 1340–1350. https://doi.org/10.1111/geb.12359

Matos, P., Pinho, P., Aragón, G., Martínez, I., Nunes, A., Soares, A.M.V.M.,. & Branquinho, C. (2015). Lichen traits responding to aridity. *Journal of Ecology*, 103(2), 451–458. https://doi.org/10.1111/1365-2745.12364

Merinero, S., Hilmo, O.,. & Gauslaa, Y. (2014). Size is a main driver for hydration traits in cyano- and cephalolichens of boreal rainforest canopies. *Fungal Ecology*, 7, 59–66. https://doi.org/10.1016/j.funeco.2013.12.001

Miller, J.E.D., Villella, J., Carey, G., Carlberg, T.,. & Root, H.T. (2017). Canopy distribution and survey detectability of a rare old-growth forest lichen. *Forest Ecology and Management*, 392, 195–201. https://doi.org/10.1016/j.foreco.2017.03.007

Morelli, T. L., Barrows, C. W., Ramirez, A. R., Cartwright, J. M., Ackerly, D. D., Eaves, T. D., ... & Thorne, J. H. (2020). Climate-change refugia: Biodiversity in the slow lane. *Frontiers in Ecology and the Environment*, 18(5), 228-234.

Nascimbene, J., & Spitale, D. (2017). Patterns of beta-diversity along elevational gradients inform epiphyte conservation in alpine forests under a climate change scenario. *Biological Conservation*, 216, 26–32. https://doi.org/10.1016/j.biocon.2017.09.021

Nascimbene, J., Casazza, G., Benesperi, R., Catalano, I., Cataldo, D., Grillo, M., ... & Giordani, P. (2016). Climate change fosters the decline of epiphytic *Lobaria* species in Italy. *Biological conservation*, 201, 377-384.

Nascimbene, J., Benesperi, R., Casazza, G., Chiarucci, A., & Giordani, P. (2020). Range shifts of native and invasive trees exacerbate the impact of climate change on epiphyte distribution: the case of lung lichen and black locust in Italy. *Science of the Total Environment*, 735, 139537. https://doi.org/10.1016/j.scitotenv.2020.139537

Ovaskainen, O., Meyke, E., Lo, C., Tikhonov, G., Delgado, M. D. M., Roslin, T., ... & Shalaeva, K. (2020). Chronicles of nature calendar, a long-term and large-scale multitaxon database on phenology. *Scientific data*, 7(1), 47.

Pacifici, M., Foden, W. B., Visconti, P., Watson, J. E., Butchart, S. H., Kovacs, K. M., ... & Rondinini, C. (2015). Assessing species vulnerability to climate change. *Nature climate change*, 5(3), 215-224.

Phinney, N.H., Solhaug, K.A., & Gauslaa, Y. (2018). Rapid resurrection of chlorolichens in humid air: specific thallus mass drives rehydration and reactivation kinetics. *Environmental and Experimental Botany*, 148, 184–191. https://doi.org/10.1016/j.envexpbot.2018.01.009

Phinney, N.H., & Solhaug, K.A., & Gauslaa, Y. (2019). Photobiont-dependent humidity threshold for chlorolichen photosystem II activation. *Planta*, 250(6), 2023–2031. https://doi.org/10.1007/s00425-019-03282-4

Porada, P., & Giordani, P. (2021). Bark water storage plays key role for growth of Mediterranean epiphytic lichens. *Frontiers in Forests and Global Change*, 4. https://doi.org/10.3389/ffgc.2021.668682.

Porada, P., Weber, B., Elbert, W., Pöschl, U., & Kleidon, A. (2013). Estimating global carbon uptake by lichens and bryophytes with a process-based model. *Biogeosciences*, 10(11), 6989–7033. https://doi.org/10.5194/bg-10-6989-2013.

Porada, P., Van Stan, J.T., & Kleidon, A. (2018). Significant contribution of non-vascular vegetation to global rainfall interception. *Nature Geoscience*, 11(8), 563–567. https://doi.org/10.1038/s41561-018-0176-7.

Proctor, M.C.F., & Tuba, Z. (2002). Poikilohydry and homoihydry: antithesis or spectrum of possibilities? *New Phytologist*, 156(3), 327–349.

Rubio-Salcedo, M., Merinero, S., & Martínez, I. (2015). Tree species and microhabitat influence the population structure of the epiphytic lichen Lobaria pulmonaria. *Fungal Ecology*, 18, 1–9. https://doi.org/10.1016/j.funeco.2015.08.002.

Sadeghi, S.M.M., Gordon, D.A., & Van Stan II, J.T. (2020). A global synthesis of throughfall and stemflow hydrometeorology. In *Precipitation Partitioning by Vegetation: A Global Synthesis*, 48–69. https://doi.org/10.1007/978-3-030-29702-2_4.

Saiz, H., Dainese, M., Chiarucci, A., & Nascimbene, J. (2021). Networks of epiphytic lichens and host trees along elevation gradients: climate change implications in mountain ranges. *Journal of Ecology*, 109(3), 1122–1132. https://doi.org/10.1111/1365-2745.13538

Schall, P., & Heinrichs, S. (2020). Comment on “Forest microclimate dynamics drive plant responses to warming”. *Science*, 370(6522), eabd9920. https://doi.org/10.1126/science.abd9920.

Smith, R.J., Jovan, S., & McCune, B. (2020). Climatic niche limits and community-level vulnerability of obligate symbioses. *Journal of Biogeography*, 47(2), 382–395. https://doi.org/10.1111/jbi.13719.

Spribille, T., Tuovinen, V., Resl, P., Vanderpool, D., Wolinski, H., Aime, M. C., ... & McCutcheon, J. P. (2016). Basidiomycete yeasts in the cortex of ascomycete macrolichens. *Science*, 353(6298), 488-492.

Spribille, T., Tagirdzhanova, G., Goyette, S., Tuovinen, V., Case, R., & Zandberg, W.F. (2020). 3D biofilms: in search of the polysaccharides holding together lichen symbioses. FEMS *Microbiology Letters*, 367(5). https://doi.org/10.1093/femsle/fnaa023.

Thomas, C. D., Cameron, A., Green, R. E., Bakkenes, M., Beaumont, L. J., Collingham, Y. C., ... & Williams, S. E. (2004). Extinction risk from climate change. *Nature*, 427(6970), 145-148.

Trotsiuk, V., Hartig, F., & Forrester, D.I. (2020). R3PG – an R package for simulating forest growth using the 3-PG process-based model. *Methods in Ecology and Evolution*, 11(11), 1470–1475. https://doi.org/10.1111/2041-210X.13474.

Valente, F., David, J.S., & Gash, J.H.C. (1997). Modelling interception loss for two sparse eucalypt and pine forests in Central Portugal using reformulated Rutter and Gash analytical models. *Journal of Hydrology*, 190(1–2), 141–162. https://doi.org/10.1016/S0022-1694(96)03066-1.

Vallese, C., Nascimbene, J., Giordani, P., & Benesperi, R. (2021). Modelling range dynamics of terricolous lichens of the genus Peltigera in the Alps under a climate change scenario. *Fungal Ecology*, 49, 101014. https://doi.org/10.1016/j.funeco.2020.101014.

Van Stan II, J.T., Gutmann II, E., & Friesen II, J. (Eds.) (2020). *Precipitation Partitioning by Vegetation: A Global Synthesis.* Springer International Publishing. https://doi.org/10.1007/978-3-030-29702-2.

Violle, C., Navas, M.-L., Vile, D., Kazakou, E., Fortunel, C., Hummel, I., & Garnier, E. (2007). Let the concept of trait be functional! *Oikos*, 116(5), 882–892. https://doi.org/10.1111/j.2007.0030-1299.15559.x.

Wang, Y., Naumann, U., Eddelbuettel, D., Wilshire, J., Warton, D., Byrnes, J., ... & Silva, R.dos S. (2020). mvabund: Statistical Methods for Analysing Multivariate Abundance Data (4.1.3) [Computer software]. https://CRAN.R-project.org/package=mvabund.

Zellweger, F., De Frenne, P., Lenoir, J., Rocchini, D., & Coomes, D. (2019). Advances in microclimate ecology arising from remote sensing. *Trends in Ecology & Evolution.* https://doi.org/10.1016/j.tree.2018.12.012.

Chapter 2

Responses of three alpine lichen species to passive warming in an alpine ecosystem

Luca Di Nuzzo, Elisabetta Bianchi, Michele Carbognani, Gabriele Gheza, Paolo Giordani, Juri Nascimbene, Alessio Papini, Alessandro Petraglia, Chiara Vallese, Renato Benesperi

In preparation

Abstract

Climate change is one of the major drivers of biodiversity loss at the global level. Due to temperature warming, shifts in altitudinal ranges are expected in mountain systems. This effect could be particularly pronounced in species strongly associated with climatic conditions, such as lichens. Studies predict that lichen might shift their altitudinal distribution to follow suitable conditions. Nevertheless, experimental studies concerning whether warm-adapted lichen species can colonise higher altitudes in changed climatic conditions are scarce. To investigate this possibility and to study the impact on warming of species with different altitudinal ranges we transplanted thalli of three lichen species in Open Top Chambers and control plots at 2700m. The cryophilous *Solorina crocea* and the broad-ranging *Cetraria islandica* were collected at the same site, while the more termophilous *Peltigera malacea* was collected at lower altitudes. We monitored the photosynthetic efficiency (F_v/F_m) for two summers. The impact of warming varied among the three species and depended on the climatic conditions. All three species showed a peak of F_v/F_m at intermediate temperature values (5 – 10℃) after which values tended to decline. We found a reduction in the photosynthetic efficiency of *P. malacea*, compared with warming plots, at lower temperatures. Similarly, the thalli of *S. crocea* and *C. islandica* in the warming plots exhibited increased photosynthetic efficiency compared to those in control plots under cooler conditions. However, as temperatures rose, the F_v/F_m ratio of *S. crocea* decreased in both the warming and control plots, whereas for *C. islandica*, this decline was observed only under warming conditions. The results obtained showed that species from lower altitudes might be able to colonize high altitudes environments in a warming scenario. At the same time, even species with broad altitudinal ranges could be negatively affected by warming conditions, probably due to acclimatization to colder conditions of local populations.

2.1 Introduction

Several factors have detrimental effects on global biodiversity, encompassing habitat degradation, fragmentation and overexploitation, invasive species and, with increasing importance, climate change (Purvis et al., 2019; Jaureguiberry et al., 2022). Despite not yet reaching the same magnitude as other factors (Caro et al., 2022), this latter has already impacted various facets of biodiversity, determining shifts in phenology, alterations of physiology and biotic interaction, changes in community structure and distribution ranges (Bellard et al., 2012), thus spanning from whole individual to the ecosystem level (Scheffers et al., 2016). While the effects of climate change can be observed across almost all ecosystems, some are more vulnerable (Verrall & Pickering, 2020); for example, alpine ecosystems are considered to face more elevated risks (Theurillat & Guisan, 2001). This vulnerability arises from two primary factors: firstly, these ecosystems are characterized by highly specific climatic conditions (Körner & Paulsen, 2004; Verrall & Pickering, 2020). Secondly, their spatial distribution is altitudinally limited, as any possible shift at some point is bounded by the mountaintop (Verrall & Pickering, 2020). Moreover, high-mountain environments are predicted to have more rapid changes in temperature (Pepin et al., 2015). For the European Alps, an increase in air temperature is expected, with a significant decrease in snow cover duration and more uncertainty on precipitations (Gobiet & Kotlarski, 2020). Such changes may particularly impact organisms whose physiology is tightly coupled with climatic conditions. This is the case of poikilohydric organisms like lichens, which are profoundly influenced by climatic factors. This connection is mainly due, albeit not totally, to their inability to regulate water loss actively (Stanton et al., 2023). The water content of a lichen thallus tends to reach equilibrium with the surrounding conditions, resulting in potential fluctuations, even on a minute or hourly scale (Stanton et al., 2023). During periods of dehydration, almost all metabolic activity is suspended, allowing them to survive for long periods without water but at the cost of reducing the length of photosynthetically active periods (Aubert et al., 2007). The increasing temperature and the related increase in vapour pressure deficit (VPD) (Yuan et al., 2019; McDowell et al., 2022) could lead to a reduction in the overall active time by increasing the drying effect of the air (Chinnery & Ellis, 2023). VPD is an effective metric of air humidity. It is essential for lichen ecophysiology (Stanton et al., 2023), particularly for species like broad-lobed foliose or fruticose lichens, whose thalli predominantly interact with air. For example, previous studies showed that lichens resulted wet only when VPD was below 1 kPa (Gaio-Olivera et al., 2004) or found a positive association in the growth rate with decreasing VPD values (Rambo, 2010), or differential investment in radial or thallus thickness growth in different cumulative VPD scenarios (Chinnery & Ellis, 2023). Nevertheless, in habitats with very high humidity, lichens could be negatively affected through supersaturation (Ellis, 2020; Chinnery & Ellis, 2023). The combined effects of variations in VPD and temperature fluctuations are likely to influence more lichen ecophysiology than temperature changes alone (Stanton et al., 2023). Nevertheless, temperature could also have direct effects. In the framework of the lichen symbiotic relationship, algal gross photosynthesis and fungal respiration, which determine the majority of respired CO_2, are not strictly coupled. In fact, beyond a certain temperature, gross photosynthesis remains stable while respiration continues to grow, resulting in an optimal temperature for net photosynthesis, above which any increase in temperature decreases carbon gain (Green et al. 2008; Colesie et al., 2018). Moreover, as nitrogen fixation is temperature-limited (Antoine, 2004), increasing temperature in the colder zone of the globe would increase productivity if the duration of physiological activity remains constant in cyanolichens (Nash & Olafsen, 1995).

Both experimental simulations and field observations have shown that climate change significantly impacts lichen ecophysiology, ranging from individual thalli to entire communities. Specifically, alterations in the C and N content of the thalli (Concostrina-Zubiri et al., 2021) and changes in carbon-based secondary metabolites have been reported (Asplund et al., 2017). Similarly, it has been observed a reduction in annual biomass growth (Smith et al., 2018) and a decline in photosynthetic yield and F_v/F_m ratios due to shortened active periods (Maphangwa et al., 2011; Raggio et al., 2023; Osyczka et al., 2023). Photobiont mortality occurs in scenarios exceeding a warming threshold of approximately 2°C (Mayer et al., 2023). This could result in altered lichen communities by increasing or decreasing the presence of species with specific traits (van Herk et al., 2002; Aptroot and van Herk, 2007; Finger-Higgens et al., 2022), reducing lichen cover (Baldauf et al., 2023) and the overall diversity (Lang et al., 2012; Alatalo et al., 2017; Di Nuzzo et al., 2022). At the same time, other taxonomical groups are influenced by climate change, which results in changed interaction as in the case of increasing competition of vascular plants with terricolous lichens (Cornelissen et al., 2001; Ellis et al., 2011; Di Nuzzo et al., 2021) or decoupling the climatic overlap between epiphytic lichens and their phorophytes (Zhao et al., 2019; Nascimbene et al., 2020). Nevertheless, the response of lichens to climate change emerges as highly species-specific depending on the species characteristics, their climatic niche, their capacity to acclimatise and the future scenario considered (Ellis, 2019; Stanton et al., 2023). Not all lichen species will be affected similarly, with species being observed to be unaffected (Bokhorst et al., 2016; Nybakken et al., 2011; Osyczka et al., 2023) or even benefit from warming conditions (Bokhorst et al., 2016). In some cases, enhanced net photosynthesis production has been predicted for the future (Beltrán-Sanz et al., 2023). Certain species are anticipated to expand their geographical range, while others are projected to contract (Vallese et al., 2021; Mallen-Cooper et al., 2023). At the same time, diverse climate change scenarios, with corresponding different temperature increases and altered precipitation regimes, are anticipated to yield significantly different impacts (Mayer et al., 2023; Di Nuzzo et al., 2022).

The divergent responses between species observed in previous studies also arise from the species-specific capacity of lichens to acclimate to changed climatic conditions. As symbiotic entities, lichens may undergo acclimatization at the level of individual symbionts, tuning their physiology to new environmental conditions (Stanton et al., 2023). This acclimatization may include modifying respiration rates, altering photosynthetic performance, and adjusting traits of the symbiosis, such as specific thallus mass or the ratio between symbiotic partners (Stanton et al., 2023). Additionally, over broader time scales spanning generations, the switching of bionts has been observed (Stanton et al., 2023). Generally, species with a broad ecological niche are expected to be able to cope with temperature changes (Colesie et al., 2018). Thus, the contrasting responses observed, coupled with the different effects of climate change across the globe, depict a complex framework, making it challenging to pinpoint general impacts (Stanton et al., 2023). Accurately predicting climate change's effects on lichen diversity and ecophysiology requires considering and comparing different species, climatic conditions and ecosystems (Porada et al., 2023).

Lichens are key components of alpine ecosystems, where they have a high biodiversity and biomass (Körner, 2021). The forecasted climatic conditions will likely determine shifts in the altitudinal distribution of lichen species, even though with differences between cold- and warm-adapted species, where these latter seemed to be less able to migrate at high altitudes (Di Nuzzo et al., 2021). Whether warm-adapted lichen species can colonise higher altitudes in changed climatic conditions represents a significant gap in our current ecological knowledge. Yet, it is fundamental to understand the current dynamics of lichen communities in these ecosystems to forecast their future dynamics under the changing climate. To this aim, we investigated the impact of temperature warming on the photosynthetic efficiency of three common alpine terricolous lichen species characterised by different altitudinal ranges (considered as a proxy for their climatic preferences) by transplanting them in open-top chambers (OTCs) and monitoring them across two summers along with control transplants located outside OTCs. We expected the warming treatment to not have negative effects of the photosynthetic efficiency of the thermophilous species compared to control plots (i.e. *Peltigera malacea* (Ach.) Funck). On the contrary, we hypothesize a reduction in photosynthetic efficiency in control plots. In fact, in the plots subjected to warming, *P. malacea* experienced climatic conditions similar to its collection site whereas in control plots was exposed to colder conditions. At the same time, we expected that the warming treatment would have a negative effect on the photosynthetic efficiency of the cryophilous species (i.e. *Solorina crocea* (L.) Ach.) and show no or a little effect on the broad-ranging species (i.e. *Cetraria islandica* (L.) Ach. subsp. *islandica*). Moreover, we hypothesise that the effect of the warming treatment might vary depending on the microclimatic conditions experienced by the thalli. Finally, we investigated which climatic conditions between VPD, air and soil temperature predicted better the photosynthetic efficiency also comparing the mean conditions during the measurements, two days before and after the last measurement.

2.2 Materials & Methods

2.2.1 Study sites, species, warming treatments and microclimate

The study was carried out at Passo Gavia (province of Sondrio, N Italy) in the Rhaetian Alps (46.340459N, 10.498132E), inside the Stelvio National Park, at approximately 2,700 m a.s.l. Based on the high-resolution Koppen climate classification, the area belongs to the tundra (ET) climate (Beck et al., 2018). It is characterised by cold winters and cold summers, with liquid precipitation mainly restricted to summer. According to the Chelsa 2.1 dataset, the area's mean annual temperature and total annual precipitation are -3.05 °C and 1568 mm, respectively (Karger et al., 2017). The area remains covered by snow for 8-9 months, from early October to late May – early June. We selected three lichen species, i.e. *C. islandica*, *S. crocea* and *P. malacea*, that are frequent in the study area and differ in their altitudinal distribution. *S. crocea*, a foliose lichen with a photobiont layer divided into an upper part with green algae and a lower part with cyanobacteria, is mainly restricted to the alpine belt and occurs especially in microhabitats with a long snow-lie (Nimis & Martellos, 2023). *C. islandica*, a subfruticose lichen with green algae as photobiont, shares the same habitats as *S. crocea*, but is also found at lower altitudes reaching the montane belt. *P. malacea*, a foliose lichen with cyanobacteria as photobiont, is sometimes found in the same environments as *S. crocea*, but more frequently at lower altitudes.

Squared Open Top Chambers (OTCs) were used to simulate warming. Each OTC consisted of four 3mm thick sheets of UV transparent polymethyl methacrylate with a 90 cm base and 50 cm opening at the top. Six OTCs, and an equal number of control plots with the same basal area of OTCs, were installed in July 2021 at the study site. For each of the three lichen species, we collected 120 thalli of similar size from the surrounding area, except for thalli of *P. malacea*, which were collected approximately 500m far from the study site at 2500m a.s.l. Immediately after having been collected, the thalli were translocated inside the OTCs and the control plots. In each OTC and control plot, we randomly placed 10 thalli for each species, resulting in

30 thalli in each OTC and control plot. As the study site is inaccessible during winter and snow depth often exceeds 3m, the OTCs were kept only during summer and removed during winter: they were installed for the two subsequent field seasons from 28 July 2021 to 01 October 2021 and from 23 June 2022 to 22 September 2022. For two OTCs and two control plots, microclimatic conditions were measured throughout the whole experiment. We used Decagon EHT Temperature/RH Sensor connected to a Decagon Em50 control unit mounted at 3cm from the ground to measure air temperature and humidity. Moreover, we measured soil temperature at -3cm using a Spectrum WatchDog Soil Temperature Sensor connected to a Spectrum WatchDog 1400 Micro Station control unit. All measurements were taken with a resolution of one hour.

2.2.2 Maximum efficiency of photosystem II of the Lichen Photobiont

Every two weeks, the potential quantum yield of primary photochemistry (F_v/F_m) of the lichen thalli was investigated using the measurement of chlorophyll (Chl) a fluorescence. Before the measurements, each thallus was hydrated and dark–adapted in low light conditions (< 40 μmol m^{-2} s^{-1}, monitored through an Apogee SQ–420X sensor) for at least 20 minutes using layers of white velvet cloths mounted on a square structure closed with plastic sheets to reduce desiccation. Low light was used as it was reported to allow a better recovery of photoinhibition (Solhaug 2018). Subsequently, using a Plant Efficiency Analyzer fluorimeter (Handy PEA, Hansatech Ltd, Norfolk, UK) we recorded fluorescence emission for one second after lightening the thalli with a red light (650nm) pulse of saturating light (3000 μmol(photon) m^{-2} s^{-1}). The gain of PEA was 1.0. We took three measurements randomly in different parts of each thallus. This parameter is derived from the analysis of the OJIP transient, obtained from fluorescence measurements at the millisecond level. The measured fluorescence typically rises from F0, where all PSII reaction centres are open, to Fm, where all PSII reaction centres are closed. A polyphasic behaviour is observed if the transient is plotted on a logarithmic scale. The first part is the origin (O) which starts from F0, followed by J and I, which are intermediate levels, and ends with P, corresponding to Fm. Each phase is a marker for specific events in the photosynthetic electron transport chain (Strasser et al., 2004).

2.2.3 Data Analysis

To investigate the influence of warming treatment and its interaction with microclimatic conditions experienced by organisms in different time spans with respect to the measurement day on the potential quantum yield, we fitted a series of linear mixed models using the nlme package (Pinheiro et al. 2023). As we wanted to test the effects of microclimatic conditions in both the OTCs and the control plots during the measurements and those that occurred in the days before (time spans), we fitted three series of models. On the one hand, we used data captured between 9:00 a.m. and 4:00 p.m. on each measurement date (SD), aligning with the timeframe of data recording. On the other hand, we used data recorded during the two days before the measurements (TD) and data recorded between the measurements (~2 weeks, TW). The microclimatic variables used were mean air temperature, mean soil temperature, and mean vapour pressure deficit (VPD). VPD was calculated using measured temperature and relative humidity through the equation:

$$0.611 \exp{[17.502 \frac{T}{(T + 240.97)}]} * (1 - \frac{RH}{100})$$

where T is the air temperature and RH is the Relative Humidity. Models were built using microclimatic variables, treatment (warming vs control), lichen species, and their interactions as predictors. As the microclimatic variables vary between warming and control plots, we employed the control microclimatic variables in our model to assess the impact of treatments under diverse environmental conditions. We incorporated the microclimatic predictors as quadratic polynomial terms. This decision was based on the fact that we did expect a non–linear relationship between microclimatic factors and $\mathrm{F_v/F_m}$. We used thallus id nested inside plots as random effects to account for repeated measurements and possible similarity of thalli inside the same plot. Over the two–year measurement period, a limited number of thalli (n = 21) were lost either through wind or inundation caused by intense rainfall. Such thalli were subsequently excluded from the analyses starting from the point of their disappearance. As model diagnostics highlighted heterogeneity in the variances of the residuals in species and treatment, all models incorporated a varIdent variance structure to accommodate variations in residual error within treatment groups (warming vs control) and species. We assessed the importance of fixed effects and their corresponding interaction factors by means of a Type III ANOVA table. In order to identify the microclimatic conditions (air temperature, soil temperature or VPD) and the different periods (SD, TD, BM) that better predicted F_v/F_m values, we compared the models with the same structure but different microclimatic variables using the Akaike Information Criterion (AIC). All plots were produced using ggplot2 (Wickham, 2016), ggpubr (Kassambara, 2023) and ggeffects (Lüdecke et al., 2018) packages. All data analyses were performed using R version 4.2.3 (R Core Team, 2023).

2.3 Results

2.3.1 Microclimate

During the measurement period, the OTCs enhanced on average the air temperature by 2.04 °C and the soil temperature by 1.85 °C (Table 2.1). At the same time, the treatment had little effect on relative humidity, as it was, on average, 0.481% lower inside the OTCs, even though during different climatic conditions such difference reached peaks of 20%. VPD was higher inside the OTCs by an average of 0.105 kPa (Table 2.2).

2.3.2 Model results

A summary of the results concerning F_v/F_m values is presented in Table 2.3 and Figure 2.1. On average, *P. malacea* showed the lowest values of F_v/F_m, 0.469 – 0.501, while *S. crocea* and *C. islandica* had similar values, 0.589 – 0.607 and 0.593 – 0.600 respectively. The warming treatment did not have a permanent effect on the photosynthetic efficiency of the species over the experiment periods, but values of

		Control	Warming
AIR	**Relative Humidity (%)**		
	Mean (SD)	81.7 (14.9)	81.2 (15.8)
	Median [Min, Max]	87.9 [28.3, 96.2]	88.3 [22.4, 96.2]
	Temperature (°C)		
	Mean (SD)	8.37 (7.23)	10.4 (8.47)
	Median [Min, Max]	6.77 [-5.99, 35.8]	8.01 [-3.97, 42.3]
	VPD (kPa)		
	Mean (SD)	0.306 (0.440)	0.411 (0.650)
	Median [Min, Max]	0.105 [0.0251, 4.24]	0.108 [0.0282, 6.46]
SOIL	**Temperature (°C)**		
	Mean (SD)	8.62 (7.34)	10.5 (7.48)
	Median [Min, Max]	7.05 [-4.35, 41.2]	8.80 [-1.00, 40.8]

Table 2.1: Mean, median, maximum and minimum microclimatic data of control (n = 2) and warming (n = 2) plots recorded during the experiment.

	AIR			SOIL
	Relative humidity (%)	**Temperature (°C)**	**VPD (kPa)**	**Temperature (°C)**
Mean (SD)	-0.481 (4.51)	2.04 (1.73)	0.105 (0.235)	1.85 (1.98)
Median	-0.0895	1.44	0.00705	1.50
[Min, Max]	[-20.9, 19.4]	[-2.80, 8.24]	[-0.349, 2.22]	[-5.25, 16.5]

Table 2.2: Mean, median, maximum and minimum difference in microclimatic data between control and warming plots recorded during the experiment.

	C. islandica		**P. malacea**		**S. crocea**	
	Control	**Warming**	**Control**	**Warming**	**Control**	**Warming**
Mean (SD)	0.607 (0.062)	0.589 (0.072)	0.469 (0.103)	0.501 (0.107)	0.593 (0.069)	0.600 (0.0792)
Median	0.616	0.604	0.479	0.515	0.603	0.615
[Min, Max]	[0.372, 0.743]	[0.286, 0.731]	[0.0803, 0.673]	[0.110, 0.677]	[0.279, 0.716]	[0.312, 0.725]

Table 2.3: Summary statistics of F_v/F_m between warming and control plots in the three species monitored

F_v/F_m varied on different measurement dates (Figure 2.1).

The model considering mean soil temperature in the two days before the measurements had the lowest AIC (–10281.7), followed by mean VPD and soil temperature in the same day (–10194.2, –10157.5, Table 2.4). The model following were VPD and Air temperature two days before (–10092.0, –10042.5) and all models considering between measurements. The last model was Air Temperature on the same day.

Models considering microclimatic variables and F_v/F_m, in all the three considered time spans, resulted in a significant ($p < 0.0001$) interaction between the microclimatic

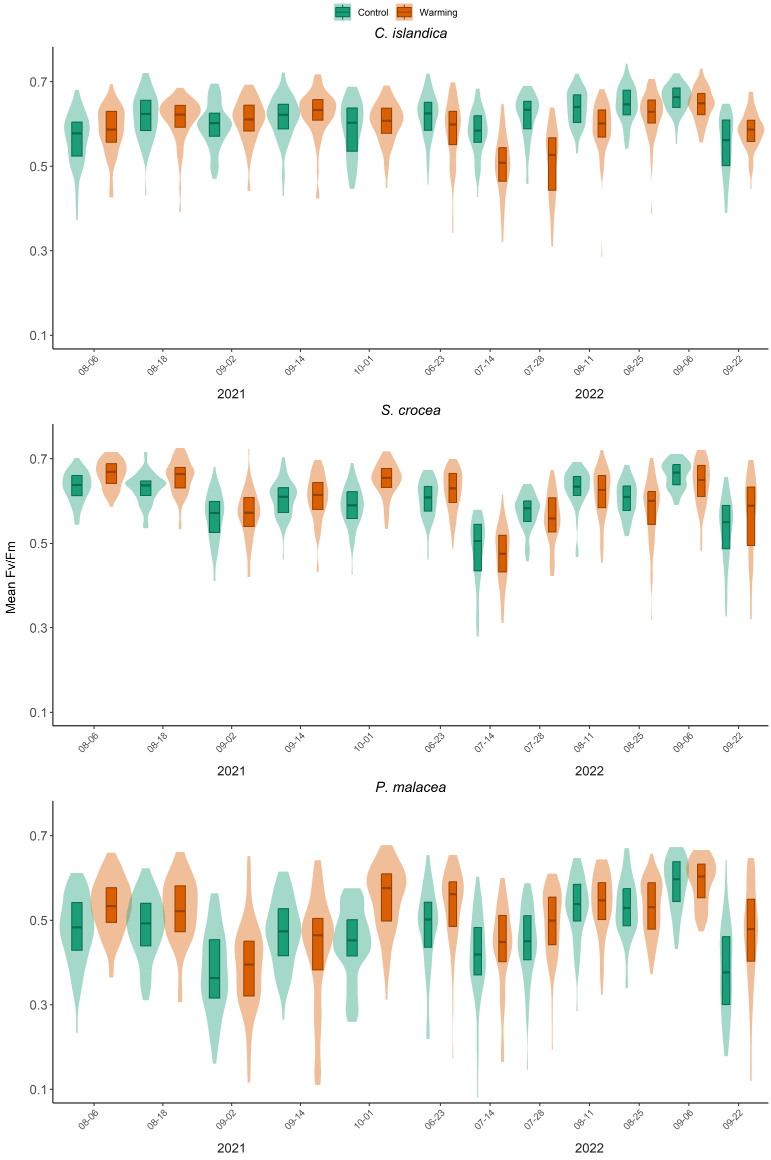


Figure 2.1: F_v/F_m values averaged between the three measurements taken on each thallus along the experimental period for the three species in the warming and control conditions.

Predictor	**AIC**	**Delta_AIC**
Soil Temperature (TD)	-10281.7	0.0
VPD (SD)	-10194.2	87.4
Soil Temperature (SD)	-10157.5	124.1
VPD (TD)	-10092.0	189.6
Air Temperature (TD)	-10042.5	239.2
Air Temperature (BM)	-9875.7	406.0
Soil Temperature (BM)	-9835.9	445.8
VPD (BM)	-9801.5	480.1
Air Temperature (SD)	-9666.4	615.2

Table 2.4: Models AIC. Time-spans abbreviations: SD: Same day, TD: two days, BM: between measurements.

variables, species and treatment. F_v/F_m was influenced by temperature differently, depending on the interaction between species and treatment (Table 2.5). When considering soil temperature TD, *P. malacea* showed higher F_v/F_m values inside the warming plots at lower temperatures ($\sim$ 0°C), while having similar values at higher temperatures ($\sim$ 14 °C). Both *C. islandica* and *S. crocea* showed higher values of F_v/F_m inside warming plots at lower temperatures (Figures 2.1, 2.2). However, the trend inverted at higher temperatures for *C. islandica*, where it showed higher values in control plots. All three species showed a peak of F_v/F_m at intermediate temperature values (5 – 10°C) after which values tended to decline (Figure 2). The model considering SD and BM time spans showed similar trends. Air temperatures showed similar trends to soil temperatures.

Model outcomes showed that at higher VPD, the F_v/F_m ratio of *C. islandica* in warming plots exhibited lower values than those in control plots. Conversely, at lower VPD values, the F_v/F_m values showed overlapping trends. *P. malacea* showed an inverse trend, with a higher value of F_v/F_m inside warming plots at lower VPD values while similar values were outside. *S. crocea*, on the contrary, showed a general decline in F_v/F_m value with increasing VPD for both warming and control plots. When considering the other two time spans, the model reported a similar trend for the three species.

		Same day (SD)			Two days before measurements (TW)			Between measurements (BM)		
		denDF	F-value	p-value	denDF	F-value	p-value	denDF	F-value	p-value
Mean Air Temperature	MAT	3841	17,99	<0.0001	3841	62,78	<0.0001	3841	42,58	<0.0001
	Species	344	195,86	<0.0001	344	214,71	<0.0001	344	214,86	<0.0001
	Treatment	10	3,43	0,0939	10	3,68	0,0839	10	3,94	0,0753
	MAT:Species	3841	10,10	<0.0001	3841	13,68	<0.0001	3841	9,80	<0.0001
	MAT:Species	3841	30,35	<0.0001	3841	62,87	<0.0001	3841	67,81	<0.0001
	Species:Treatment	344	9,56	0,0001	344	10,75	<0.0001	344	10,93	<0.0001
	MAT:Species:Treatment	3841	8,14	<0.0001	3841	10,03	<0.0001	3841	13,33	<0.0001
Mean VPD	VPD	3841	15,55	<0.0001	3841	23,48	<0.0001	3841	9,20	0,0001
	Species	344	209,20	<0.0001	344	208,86	<0.0001	344	209,02	<0.0001
	Treatment	10	3,80	0,0798	10	4,04	0,0722	10	3,91	0,0761
	VPD:Species	3841	20,02	<0.0001	3841	25,74	<0.0001	3841	13,50	<0.0001
	VPD:Species	3841	22,74	<0.0001	3841	27,35	<0.0001	3841	68,09	<0.0001
	Species:Treatment	344	10,42	<0.0001	344	10,64	<0.0001	344	10,78	<0.0001
	VPD:Species:Treatment	3841	3,46	0,008	3841	2,42	0,0460	3841	13,97	<0.0001
Mean Soil Temperature	MST	3841	37,38	<0.0001	3841	76,12	<0.0001	3841	37,46	<0.0001
	Species	344	210,98	<0.0001	344	214,03	<0.0001	344	213,94	<0.0001
	Treatment	10	3,74	0,0817	10	3,63	0,0860	10	3,94	0,0751
	MST:Species	3841	14,99	<0.0001	3841	19,48	<0.0001	3841	8,10	<0.0001
	MST:Species	3841	55,51	<0.0001	3841	65,35	<0.0001	3841	69,47	<0.0001
	Species:Treatment	344	10,57	<0.0001	344	10,61	<0.0001	344	10,92	<0.0001
	MST:Species:Treatment	3841	10,89	<0.0001	3841	10,91	<0.0001	3841	13,07	<0.0001

Table 2.5: Anova tables of the LMM models. Abbreviations: MAT: mean air temperature, VPD: vapour pressure deficit, MST: mean soil temperature

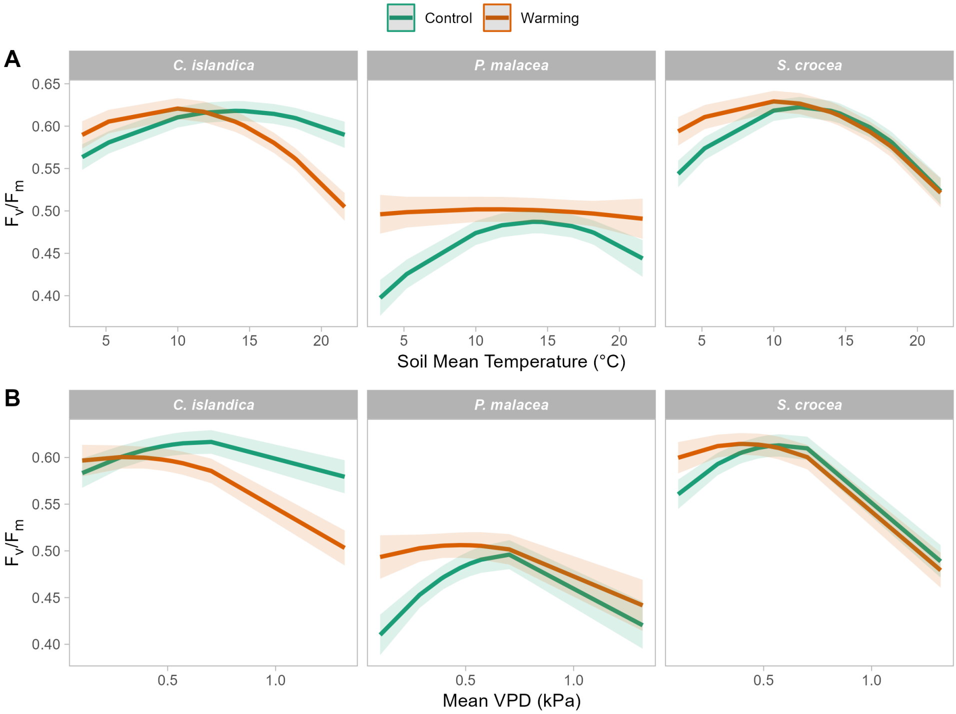


Figure 2.2: Results of the LMM models considering Soil Mean Temperature TD (A) and Mean VPD SD.

2.4 Discussion

In the context of climate change, studying the impact of warming on sensitive organisms like lichens plays a crucial role in understanding and predicting their future responses (Sancho et al., 2019). In this work, we tested the effect of warming conditions on three different lichen species characterised by different altitudinal ranges, two collected at the same site of the experiment and one at lower altitudes, hypothesizing contrasting responses to the warming treatment. The effects of the warming, despite significant, were mainly transitory, not leading to a permanent reduction in F_v/F_m. In particular, the 2°C warming tested does not seem to damage lichen's photosynthetic system permanently but highlights different stress conditions between species. Mean soil temperature in the two days before measurements was the better predictor for F_v/F_m in the studied species. Our hypothesis that the thermophilous species would not be affected in the warming plots was supported by our results, as the thermophilous *P. malacea* effectively has higher chlorophyll fluorescence in warming conditions, but this effect is observed mainly at lower temperatures. Similarly, both *C. islandica* and *S. crocea* have higher values of $\mathrm{F_v/F_m}$ at lower temperatures in warming plots, but *C. islandica* showed substantial differences at higher temperatures while *S. crocea* does not.

The model incorporating soil mean temperatures TD resulted in the best model. On one hand, the better prediction capacity of microclimatic variables over two days may reflect the small time–scale responses of the photosynthetic apparatus to temperature variation observed in other lichens (MacKenzie et al., 2004). Similar results were observed for two species belonging to the *Lasallia* genus, where the environmental conditions of the two days before the measurements were strongly correlated with $\mathrm{F_v/F_m}$ (Vivas et al., 2017). Thus, the effects we are observing are probably short–term responses to the conditions that occurred in the previous days. The absence of permanent effects in a 2°C warming scenario is consistent with previous studies that highlighted similar results. Meyer et al., (2022) reported permanent impacts of warming on the physiological functioning of the epiphytic lichen *Evernia mesomorpha* Nyl. only above 2°C warming, with smaller increases (e.g. 1.6 °C) seeming to enhance chlorophyll fluorescence slightly.

Nevertheless, albeit not permanent, our findings showed stress signs in different climatic conditions in the three species. The thermophilous species, *P. malacea*, had lower values of $\mathrm{F_v/F_m}$ in control plots, indicating a lack of acclimatization to the cooler conditions encountered following transplantation. It seems that even the increase in temperature caused by the warming treatments is sufficient to determine higher photosynthetic capacity even at lower temperatures. Cyanolichens are reported to be unable to photosynthesize at sub–zero temperatures (Green et al., 2011). The lower values of $\mathrm{F_v/F_m}$ observed in control plots for *P. malacea* outside warming plots were at 0°C, possibly indicating a stress derived from the presence of conditions close to the lower bound of suitable conditions. By contrast, there is no difference in the treatment at higher temperatures, suggesting that in future warming scenarios this species might be able to colonize higher altitude. Nevertheless, changes in other climatic factors, such as precipitations, could hinder this possibility. *S. crocea* showed a similar trend but with a smaller difference at lower temperatures. Moreover, beyond 8–9 °C photosynthetic efficiency starts to decrease. Previous studies have reported an optimum net photosynthesis between 10 and 20°C (Kallio, 1972). Such differences at higher temperatures might be related to colder acclimatization to the population of the species in our study site, which leads to negative effects at higher temperatures. Finally, in warming plots, *C. islandica* is enhanced during colder periods, but at the same time, its chlorophyll fluorescence reaches lower values in warmer periods. *C. islandica* has values F_v/F_m proximally to its maximum from 0 °C to 20 °C, and at 30°C it starts a rapid decline (Hájek et al., 2001). Hajek et al., (2001) observed minimal differences in the optimal temperature for photosynthetic processes between low (1300m) and high altitude (2000m) populations, with notable differences only in shade–adapted populations. The high–altitude population sampled by Hajek et al. 2001 was studied in an area with a mean annual air temperature of –0.8°C. The lower mean annual air temperature of –3.05°C at the study site, coupled with its higher altitude of 2700m and the collection of thalli from snow bed areas, could have determined an acclimatization to cold temperatures in our samples. Thus, despite being a species with a wide altitudinal range, the population considered seems to be not able to acclimatize to temperature increase.

Although it did not result in the best model, the VPD was a significant predictor of F_v/F_m. Thalli placed in the control plots typically experienced lower average VPD values. *C. islandica* seemed to be the most influenced by changing values in VPD, with the main differences at higher values of VPD. This is the only of the three species studied with a sub–fruticose growth form, in which the thallus is detached from the ground and is mainly in contact with the air. Thus, the observed difference between warming and control plots may be related to the stronger dependence of this species on air conditions. Furthermore, given that many chlorolichens can reactivate using air humidity (Gauslaa, 2014), variations in VPD might lead to more significant changes in their active periods than precipitation changes. Thus, at higher VPD, which are even higher in the OTC, thalli of *C. islandica* have been less active inside the warming plots, likely reducing photosynthetic efficiency. On the other hand, cyanolichens need liquid water to reactivate (Lange et al., 1993) and thus are probably less influenced by a slight variation in VPD. They are similarly subjected to reduced active time after rain or dew formation. However, they are not able to reactivate through vapour alone, leading to similar results between treatments as it did not change the amount of precipitation received by the thalli. Finally, the influence of VPD on lichen water content is probably reduced or at least partially decoupled in lichens closely attached to the ground, which might be more influenced by soil humidity and temperature. In fact, *S. crocea* resulted in no differences between warming and control plots, but only in a general reduction with higher values of VPD.

In conclusion, this work highlights the complex interactions between climate change and the physiological responses of lichens and the species–specific response to changed climatic conditions. Our work confirms the possibility of warm–adapted species, such as *P. malacea* to possibly colonize higher altitudes if temperature is the only limiting factor. On the other hand, we showed that a population of species with a wide altitudinal range might be acclimated to specific local conditions and that those populations might be negatively affected by increasing temperatures and VPD. Thus, although studied species are unlikely to extinct locally, specific populations could be negatively influenced.

2.5 Limitation and perspectives

In our study, we compared three species, two of which were collected close to the experimental area, while the third (*P. malacea*) was collected at a lower altitude. The contrasting responses of *C. islandica* to warming treatment at higher temperatures may be partially explained by a cold adaptation of the population collected at the study site. Nevertheless, a previous study (Hájek et al., 2001) showed little or no adaptation of the high–altitude population of *C. islandica.* Thus, our speculation has to be confirmed by further studies aimed at comparing the response of low–altitude and probably warm–adapted populations with those collected in the study area.

The experimental area's constraints limited our warming treatment to the snow–free period of the year. On the one hand, the lack of strong effects observed could be due to the fact that during winter both control and warming plots experienced the same temperature. Nevertheless, lichens are typically not active under snow cover (Pannewitz et al., 2003), and snow stabilises ground temperatures in winter (Winkler et al., 2000). Thus, under snowpacks, lichens would likely experience similar temperatures to the present even in warmed conditions. However, rising mean temperatures could shorten the duration of snow cover (Gobiet & Kotlarski, 2020). This not only extends vegetative periods, but also increases exposure to freezing conditions. Therefore, exploring the effects of reduced snow cover through snow–removal experiments could give insights into lichens' responses to warming scenarios.

We hypothesize that the degree of soil humidity and VPD influence depends on growth form. Similarly, we hypothesize a negative effect on photosynthetic efficiency after short periods of high VPD. While these ideas are speculative in our current study, they are crucial for understanding lichen ecophysiology in alpine ecosystems. To validate such hypotheses, long–term air and soil humidity, temperature, and thallus water content measurements should be conducted (Gaio-Oliveira et al., 2004; Leo et al., 2018). Concurrently, laboratory experiments to examine the drought resilience of the species studied would be instrumental in understanding their adaptive responses under various drought scenarios (Osyczka et al., 2023). Finally, while chlorophyll fluorescence is an essential parameter of lichen CO_2 gain and the overall vitality of the algal partner, it is still unclear how the CO_2 transferred is used by the fungal symbiont (Spribille et al., 2022). Thus, it would be strongly informative to consider, further than photosynthetic efficiency, even growth of the entire thalli, also considering the partition in radial growth and increase in biomass, which is known to have a seasonal pattern (Larsson et al., 2012) and is determined by climatic factors (Chinnery & Ellis, 2023).

Finally, we measured every two weeks the maximum efficiency of photosystem II of the lichen photobiont in optimal conditions (dark adaptation and full hydration), nevertheless, this approach could have two downsides: on the one hand, we did not have measures at finer time scales (e.g. days or hours), or in different parts of the day (e.g. late afternoon or night), thus more rapid responses or responses in specific conditions could not be detected. On the other hand, using optimal conditions may have reduced the responses to the warming treatment, by reducing the stressful conditions in the moment of measurements.

2.6 Bibliography

Alatalo, J. M., Jägerbrand, A. K., Chen, S., & Molau, U. (2017). Responses of lichen communities to 18 years of natural and experimental warming. *Annals of Botany*, 120(1), 159-170.

Antoine, M. E. (2004). An ecophysiological approach to quantifying nitrogen fixation by Lobaria oregana. *Bryologist*, 82-87.

Aptroot, A., & Van Herk, C. M. (2007). Further evidence of the effects of global warming on lichens, particularly those with Trentepohlia phycobionts. *Environmental pollution*, 146(2), 293-298.

Asplund, J., Siegenthaler, A., & Gauslaa, Y. (2017). Simulated global warming increases usnic acid but reduces perlatolic acid in the mat-forming terricolous lichen Cladonia stellaris. *The Lichenologist*, 49(3), 269-274.

Aubert, S., Juge, C., Boisson, A. M., Gout, E., & Bligny, R. (2007). Metabolic processes sustaining the reviviscence of lichen Xanthoria elegans (Link) in high mountain environments. *Planta*, 226, 1287-1297.

Aurelle, D., Thomas, S., Albert, C., Bally, M., Bondeau, A., Boudouresque, C. F., ... & Fady, B. (2022). Biodiversity, climate change, and adaptation in the Mediterranean. *Ecosphere*, 13(4), e3915.

Baldauf, S., Porada, P., Raggio, J., Maestre, F. T., & Tietjen, B. (2021). Relative humidity predominantly determines long-term biocrust-forming lichen cover in drylands under climate change. Journal of Ecology, 109(3), 1370–1385. doi: https://doi.org/10.1111/1365-2745.13563

Beck, H. E., Zimmermann, N. E., McVicar, T. R., Vergopolan, N., Berg, A., & Wood, E. F. (2018). Present and future Köppen-Geiger climate classification maps at 1-km resolution. *Scientific data*, 5(1), 1-12.

Bellard, C., Bertelsmeier, C., Leadley, P., Thuiller, W., & Courchamp, F. (2012). Impacts of climate change on the future of biodiversity. *Ecology letters*, 15(4), 365-377.

Beltrán-Sanz, N., Raggio, J., Gonzalez, S., Dal Grande, F., Prost, S., Green, A., ... & Sancho, L. G. (2022). Climate change leads to higher NPP at the end of the century in the Antarctic Tundra: Response patterns through the lens of lichens. *Science of the Total Environment*, 835, 155495.

Bokhorst, S., Convey, P., Huiskes, A., & Aerts, R. (2016). Usnea antarctica, an important Antarctic lichen, is vulnerable to aspects of regional environmental change. *Polar Biology*, 39(3), 511–521. doi: 10.1007/s00300-015-1803-z

Bozinovic, F., & Pörtner, H. O. (2015). Physiological ecology meets climate change. *Ecology and evolution*, 5(5), 1025-1030.

Caro, T., Rowe, Z., Berger, J., Wholey, P., & Dobson, A. (2022). An inconvenient misconception: Climate change is not the principal driver of biodiversity loss. *Conservation Letters*, 15(3), e12868.

Chinnery, E. V., & Ellis, C. J. (2023). Growth-dependent acclimation constrains climatic response for the lichen epiphyte Lobaria pulmonaria. *The Lichenologist*, 55(5), 401-408.

Colesie, C., Büdel, B., Hurry, V., & Green, T. G. A. (2018). Can Antarctic lichens acclimatize to changes in temperature?. *Global Change Biology*, 24(3), 1123-1135.

Concostrina-Zubiri, L., Valencia, E., Ochoa, V., Gozalo, B., Mendoza, B. J., & Maestre, F. T. (2021). Species-specific effects of biocrust-forming lichens on soil properties under simulated climate change are driven by functional traits. *New Phytologist*, 230(1), 101-115.

Cornelissen, J. H. C., Callaghan, T. V., Alatalo, J. M., Michelsen, A., Graglia, E., Hartley, A. E., ... & Aerts, R. (2001). Global change and arctic ecosystems: is lichen decline a function of increases in vascular plant biomass?. *Journal of Ecology*, 89(6), 984-994.

Di Nuzzo, L., Benesperi, R., Nascimbene, J., Papini, A., Malaspina, P., Incerti, G., & Giordani, P. (2022). Little time left. Microrefuges may fail in mitigating the effects of climate change on epiphytic lichens. *Science of the Total Environment*, 825, 153943.

Di Nuzzo, L., Vallese, C., Benesperi, R., Giordani, P., Chiarucci, A., Di Cecco, V., ... & Nascimbene, J. (2021). Contrasting multitaxon responses to climate change in Mediterranean mountains. *Scientific Reports*, 11(1), 4438. doi: 10.1038/s41598-021-83866-x

Ellis, C. J. (2019). Climate change, bioclimatic models and the risk to lichen diversity. *Diversity*, 11(4), 54.

Ellis, C. J. (2020). Microclimatic refugia in riparian woodland: A climate change adaptation strategy. *Forest Ecology and Management*, 462, 118006.

Ellis, C. J., Yahr, R., Hodkinson, T. R., Jones, M. B., Waldren, S., & Parnell, J. A. N.

(2011). An interdisciplinary review of climate change trends and uncertainties: lichen biodiversity, arctic-alpine ecosystems and habitat loss. *Climate change, ecology and systematics*, 457-489.

Finger-Higgens, R., Duniway, M. C., Fick, S., Geiger, E. L., Hoover, D. L., Pfennigwerth, A. A., ... & Belnap, J. (2022). Decline in biological soil crust N-fixing lichens linked to increasing summertime temperatures. *Proceedings of the National Academy of Sciences*, 119(16), e2120975119. Gauslaa, Y. (2014). Rain, dew, and humid air as drivers of morphology, function and spatial distribution in epiphytic lichens. *The Lichenologist*, 46(1), 1-16.

Gaio-Oliveira, G., Dahlman, L., Máguas, C., & Palmqvist, K. (2004). Growth in relation to microclimatic conditions and physiological characteristics of four Lobaria pulmonaria populations in two contrasting habitats. *Ecography*, 27(1), 13-28.

Gobiet, A., & Kotlarski, S. (2020). Future climate change in the European Alps. In *Oxford Research Encyclopedia of Climate Science.*

Green, T. A., Nash T. H. III, & Lange, O. L. (2008). “Physiological ecology of carbon dioxide exchange,” in *Lichen Biology*, ed. T. H. Nash III (Cambridge: University Press), 152–181.

Green, T. A., Sancho, L. G., Pintado, A., & Schroeter, B. (2011). Functional and spatial pressures on terrestrial vegetation in Antarctica forced by global warming. *Polar Biology*, 34, 1643-1656.

Greiser, C., Ehrlén, J., Luoto, M., Meineri, E., Merinero, S., Willman, B., & Hylander, K. (2021). Warm range margin of boreal bryophytes and lichens not directly limited by temperatures. *Journal of Ecology*, 109(10), 3724-3736.

Hájek, J., Barták, M., & Gloser, J. (2001). Effects of thallus temperature and hydration on photosynthetic parameters of Cetraria islandica from contrasting habitats. *Photosynthetica*, 39, 427-435.

IPCC, 2022: Climate Change 2022: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [H.-O. Pörtner, D.C. Roberts, M. Tignor, E.S. Poloczanska, K. Mintenbeck, A. Alegría, M. Craig, S. Langsdorf, S. Löschke, V. Möller, A. Okem, B. Rama (eds.)]. Cambridge University Press. Cambridge University Press, Cambridge, UK and New York, NY, USA, 3056 pp., doi:10.1017/9781009325844.

Jaureguiberry, P., Titeux, N., Wiemers, M., Bowler, D. E., Coscieme, L., Golden, A. S., ... & Purvis, A. (2022). The direct drivers of recent global anthropogenic biodiversity loss. *Science advances*, 8(45), eabm9982.

Kallio, P. (1974). Nitrogen fixation in subarctic lichens. *Oikos*, 194-198.

Karger, D. N., Conrad, O., Böhner, J., Kawohl, T., Kreft, H., Soria-Auza, R. W., ... & Kessler, M. (2017). Climatologies at high resolution for the earth's land surface areas. *Scientific data*, 4(1), 1-20.

Kassambara A (2023). ggpubr: 'ggplot2' Based Publication Ready Plots. R package version 0.6.0, https://CRAN.R-project.org/package=ggpubr

Körner, C (2021). *Alpine Plant Life*, 3rd ed.; Springer: Cham, Switzerland.

Körner, C., & Paulsen, J. (2004). A world-wide study of high altitude treeline temperatures. *Journal of biogeography*, 31(5), 713-732.

Lang, S. I., Cornelissen, J. H., Shaver, G. R., Ahrens, M., Callaghan, T. V., Molau, U., ... & Aerts, R. (2012). Arctic warming on two continents has consistent negative effects on lichen diversity and mixed effects on bryophyte diversity. *Global Change Biology*, 18(3), 1096-1107.

Lange, O. L., Büdel, B., Meyer, A., & Kilian, E. (1993). Further evidence that activation of net photosynthesis by dry cyanobacterial lichens requires liquid water. *The Lichenologist*, 25(2), 175-189.

Larsson, P., Solhaug, K. A., & Gauslaa, Y. (2012). Seasonal partitioning of growth into biomass and area expansion in a cephalolichen and a cyanolichen of the old forest genus Lobaria. *New Phytologist*, 194(4), 991-1000.

Leo, M., Lareo, A., Garcia-Saura, C., Hortal, J., & Medina, N. G. (2019). BtM, a low-cost open-source datalogger to estimate the water content of nonvascular cryptogams. *JoVE (Journal of Visualized Experiments)*, (145), e58700.

Lüdecke D (2018). "ggeffects: Tidy Data Frames of Marginal Effects from Regression Models." *Journal of Open Source Software*, 3(26), 772. doi:10.21105/joss.00772 <https://doi.org/10.21105/joss.00772>.

MacKenzie TDB, Johnson J and Campbell DA (2004) Environmental change provokes rapid macromolecular reallocations within the photosynthetic system in a static population of photobionts in the lichen Lobaria pulmonaria. *Lichenologist* 36, 425–433

MacKenzie, T. D., MacDonald, T. M., Dubois, L. A., & Campbell, D. A. (2001). Seasonal changes in temperature and light drive acclimation of photosynthetic physiology and macromolecular content in Lobaria pulmonaria. *Planta*, 214, 57-66.

Mallen-Cooper, M., Rodríguez-Caballero, E., Eldridge, D. J., Weber, B., Büdel, B., Höhne, H., & Cornwell, W. K. (2023). Towards an understanding of future range shifts in lichens and mosses under climate change. *Journal of Biogeography*, 50(2), 406-417.

Maphangwa, K. W., Musil, C. F., Raitt, L., & Zedda, L. (2012). Experimental climate warming decreases photosynthetic efficiency of lichens in an arid South African ecosystem. *Oecologia*, 169(1), 257-268.

McDowell, N. G., Sapes, G., Pivovaroff, A., Adams, H. D., Allen, C. D., Anderegg, W. R., ... & Xu, C. (2022). Mechanisms of woody-plant mortality under rising drought, CO2 and vapour pressure deficit. *Nature Reviews Earth & Environment*, 3(5), 294-308.

McDowell, N. G., Sapes, G., Pivovaroff, A., Adams, H. D., Allen, C. D., Anderegg, W. R., ... & Xu, C. (2022). Mechanisms of woody-plant mortality under rising drought, CO2 and vapour pressure deficit. *Nature Reviews Earth & Environment*, 3(5), 294-308.

Nascimbene, J., Benesperi, R., Casazza, G., Chiarucci, A., & Giordani, P. (2020). Range shifts of native and invasive trees exacerbate the impact of climate change on epiphyte distribution: The case of lung lichen and black locust in Italy. *Science of the Total Environment*, 735, 139537.

Nash, T. H., & Olafsen, A. G. (1995). Climate change and the ecophysiological response of arctic lichens. *The Lichenologist*, 27(6), 559-565.

Nimis P.L., Martellos S. (2023): ITALIC - The Information System on Italian Lichens. Version 7.0. University of Trieste, Dept. of Biology, (https://dryades.units.it/italic), accessed on 2023, 12, 10. When not otherwise specified, all data and items are released under a CC BY-SA 4.0 licence.

Nybakken, L., Sandvik, S. M., & Klanderud, K. (2011). Experimental warming had little effect on carbon-based secondary compounds, carbon and nitrogen in selected alpine plants and lichens. *Environmental and Experimental Botany*, 72(3), 368-376.

Osyczka, P., Kościelniak, R., & Stanek, M. (2023). Old-growth forest versus generalist lichens: Sensitivity to prolonged desiccation stress and photosynthesis reactivation rate upon rehydration. *Mycologia*, 1-13.

Pannewitz, S., Schlensog, M., Green, T. A., Sancho, L. G., & Schroeter, B. (2003). Are lichens active under snow in continental Antarctica?. *Oecologia*, 135, 30-38.

Pepin, N., Bradley, R. S., Diaz, H. F., Baraer, M., Caceres, E. B., Forsythe, N., ... & Yang, D. Q. (2015). Elevation-dependent warming in mountain regions of the world. *Nature Climate Change*, 5(5), 424-430.

Pinheiro J, Bates D, R Core Team (2023). nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-163, <https://CRAN.R-project.org/package=nlme>.

Porada, P., Bader, M. Y., Berdugo, M. B., Colesie, C., Ellis, C. J., Giordani, P., ...

& Weston, D. J. (2023). A research agenda for nonvascular photoautotrophs under climate change. *New Phytologist*, 237(5), 1495-1504.

Purvis A., Molnar Z., Obura D., Ichii K., & Willis K., (2019). Chapter 2.2: Status and trends – nature. In Global Assessment Report on Biodiversity and Ecosystem Services of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services, ed. ES Brondízio, S Díaz, J Settele, HT Ngo, pp. 201–308. Bonn, Ger.: Secr. Intergov. Sci.-Policy Platf. Biodivers. Ecosyst. Serv.

R Core Team (2023). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/

Raggio, J., Pescador, D. S., Gozalo, B., Ochoa, V., Valencia, E., Sancho, L. G., & Maestre, F. T. (2023). Continuous monitoring of chlorophyll a fluorescence and microclimatic conditions reveals warming-induced physiological damage in biocrust-forming lichens.*Plant and Soil*, 482(1–2), 261–276. doi: 10.1007/s11104-022-05686-w

Rambo, T. R. (2010). Habitat preferences of an arboreal forage lichen in a Sierra Nevada old-growth mixed-conifer forest. *Canadian journal of forest research*, 40(6), 1034-1041.

Sancho, L. G., Pintado, A., & Green, T. A. (2019). Antarctic studies show lichens to be excellent biomonitors of climate change. *Diversity*, 11(3), 42.

Scheffers, B. R., De Meester, L., Bridge, T. C., Hoffmann, A. A., Pandolfi, J. M., Corlett, R. T., ... & Watson, J. E. (2016). The broad footprint of climate change from genes to biomes to people. *Science*, 354(6313), aaf7671.

Smith, R. J., Nelson, P. R., Jovan, S., Hanson, P. J., & McCune, B. (2018). Novel climates reverse carbon uptake of atmospherically dependent epiphytes: Climatic constraints on the iconic boreal forest lichen Evernia mesomorpha. *American Journal of Botany*, 105(2), 266-274.

Solhaug K. A. (2018). Low-light recovery effects on assessment of photoinhibition with chlorophyll fluorescence in lichens. *The Lichenologist*, 50(1), 139-145.

Spribille, T., Resl, P., Stanton, D. E., & Tagirdzhanova, G. (2022). Evolutionary biology of lichen symbioses. *New Phytologist*, 234(5), 1566-1582.

Stanton, D. E., Ormond, A., Koch, N. M., & Colesie, C. (2023). Lichen ecophysiology in a changing climate. *American journal of botany*, 110(2), e16131.

Strasser, R. J., Tsimilli-Michael, M., & Srivastava, A. (2004). Analysis of the chlorophyll a fluorescence transient. In Chlorophyll a fluorescence: a signature of photosynthesis (pp. 321-362). Dordrecht: Springer Netherlands.

Theurillat, J. P., & Guisan, A. (2001). Potential impact of climate change on vegetation in the European Alps: a review. *Climatic change*, 50(1-2), 77-109.

Vallese, C., Nascimbene, J., Giordani, P., Benesperi, R., & Casazza, G. (2021). Modelling range dynamics of terricolous lichens of the genus Peltigera in the Alps under a climate change scenario. *Fungal Ecology*, 49, 101014.

van Herk, C. V., Aptroot, A., & Van Dobben, H. F. (2002). Long-term monitoring in the Netherlands suggests that lichens respond to global warming. *The Lichenologist*, 34(2), 141-154.

Verrall, B., & Pickering, C. M. (2020). Alpine vegetation in the context of climate change: A global review of past research and future directions. *Science of the Total Environment*, 748, 141344.

Vivas, M., Pérez-Ortega, S., Pintado, A., & Sancho, L. G. (2017). F_v/F_m acclimation to the Mediterranean summer drought in two sympatric *Lasallia* species from the Iberian mountains. *The Lichenologist*, 49(2), 157-165.

Wickham. H. (2016) ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York.

Winkler, J. B., Kappen L., & Schulz F. (2000). Snow and ice as an important ecological factor for the cryptogams in the maritime Antarctic. Pages 220–224 in W. Davison, C. Howard-Williams, and P. Broady, editors. *Antarctic ecosystems: models for a wider ecological understanding.* New Zealand Natural Sciences, Christchurch, New Zealand.

Yuan, W., Zheng, Y., Piao, S., Ciais, P., Lombardozzi, D., Wang, Y., ... & Yang, S. (2019). Increased atmospheric vapor pressure deficit reduces global vegetation growth. *Science advances*, 5(8), eaax1396.

Zhao, J. L., Werth, S., Ikegami, M., Gugger, P. F., & Sork, V. L. (2019). Historical interactions are predicted to be disrupted under future climate change: The case of lace lichen and valley oak. *Journal of Biogeography*, 46(1), 19-29.

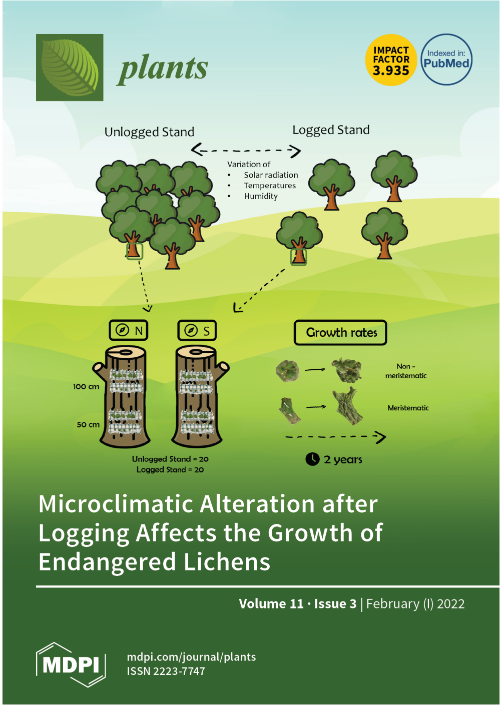
Chapter 3

Microclimatic Alteration after Logging Affects the Growth of the Endangered Lichen Lobaria pulmonaria

Luca Di Nuzzo, Paolo Giordani, Renato Benesperi, Giorgio Brunialti, Zuzana Fačkovcová, Luisa Frati, Juri Nascimbene, Sonia Ravera, Chiara Vallese, Luca Paoli and Elisabetta Bianchi

Published in *Plants*, January 2022, volume 11, issue 3 DOI: 10.3390/plants11030295 .

The article was selected to be in the front page of the issue.



Abstract

Microclimatic conditions are important in determining lichen distribution at small scale, and may determine whether the species persist when the surrounding environmental conditions have drastically changed. This is the case with forest management, since a sudden variation of microclimatic conditions (increase of solar radiation, temperature, wind and a reduction of humidity) may occur after logging. In this study, the combined effect of forest logging and microclimatic conditions on the growth probabilities and growth rates of the model species *Lobaria pulmonaria* was assessed in mixed oak stands. To this purpose, 800 fragments of *L. pulmonaria* (<1 cm) were transplanted in logged and unlogged stands for two years. Young and adult fragments were positioned on Turkey oak boles according to distance from the ground (100 and 50 cm) and aspect (north and south). The results, evaluated by generalized linear mixed models on a yearly basis, highlighted differences in growth—particularly on isolated trees in the logged stand. South-exposed samples in the logged stand showed a low probability of growth, while samples transplanted north in the unlogged stand showed higher growth probabilities. However, the highest annual growth coefficients corresponded to south-exposed samples 50 cm from the ground in the unlogged stand. In general, higher growth rates were observed in young thallus fragments when compared with adult ones. Beyond confirming the importance of microclimate for lichen ecology, these results could be implemented in conservation actions to preserve *L. pulmonaria* populations in logged forests.

3.1 Introduction

Microclimatic conditions are important in determining lichen distribution at small scale and can affect the persistence of a species when the surrounding environmental conditions have drastically changed (Ellis & Eaton, 2021a). The structural complexity of forests creates heterogeneous microclimates at a fine scale, and the link between microclimatic features and their physiological and ecological importance, as well as their consequences for biodiversity conservation, have long been recognized (De Frenne et al., 2021). Nevertheless, despite a deepening interest in how microclimatic parameters can reduce threats to understory species, there is still a gap in the consideration of microclimatic parameters when implementing conservation policies during forest management, in particular their use in maintaining suitable microclimatic refugia for target organisms (De Frenne et al., 2021, Ellis & Eaton, 2021b).

Several studies have highlighted the negative impact of intensive forest logging on lichen communities, demonstrating a reduction of biodiversity and capacity to provide ecosystem services (Otálora et al., 2011; Nascimbene et al., 2013; Whittet & Ellis, 2013; Paoli et al., 2019). Gradients in microclimatic parameters, determined by forest logging along forest edges and according to the aspect of isolated trees, can affect several ecosystem functions, including evapotranspiration, nutrient and water availability and cycling, N and CO_2 diffusion and photosynthetic processes (Redding et al., 2003; Coxson & Stevenson, 2007; Baker et al., 2013; Paoli et al., 2019), with implications on the survival and growth of various target organisms. In this regard, the important role of lichens in providing ecosystem services (habitat, shelter, food) and functions (nutrient and water cycling, metal chelation, microclimate regulation, primary colonization and soil formation) has been widely acknowledged (Zedda & Rambold, 2015).

As an example of an endangered forest lichen, *Lobaria pulmonaria* (L.) Hoffm. has been widely used as a model organism for population ecology and conservation biology. It is a tripartite foliose epiphytic species with a thallus often exceeding 20–30 cm in diameter, and has green algae as its main photobiont and nitrogen-fixing cyanobacteria within its cephalodia. It is considered a flag species for lichen conservation and also an umbrella species—a suitable sensitive indicator of forest habitats worthy of conservation and hosting other rare lichens (e.g., cyanolichens) (Scheidegger & Werth, 2009; Nascimbene et al., 2013; Brunialti et al., 2015; Paoli et al., 2019). The species has declined throughout Europe as a consequence of air pollution (especially past air pollution) and is threatened by intensive forest management, the effects of which are expected to be further exacerbated by climate change (Nascimbene et al., 2016).

On the whole, silvicultural practices may especially threaten *L. pulmonaria* (and sensitive forest lichens in general) by causing habitat fragmentation, degradation and loss, with negative consequences on local population size, structure and dynamics (Scheidegger & Werth 2009; Benesperi et al., 2018; Paoli et al., 2019). As a primary consequence of logging (beyond the loss of the substrate), forest lichens are exposed to a sudden microclimatic variation, consisting of an increase in solar radiation, temperature and wind as well as a reduction of humidity. Such drier conditions, if in excess of the ecological range of the species, may negatively affect their photosynthetic activity, and hence their overall vitality (Gauslaa & Solhaug, 1999; Larsson et al., 2014). These aspects can be exacerbated in potentially arid environments such as the Mediterranean region, where oak-dominated forests are one of the main habitats of *L. pulmonaria* populations (Rubio-Salcedo et al., 2015). The climatic niche of *L. pulmonaria* largely overlaps (>70%) with that of oak-dominated forests (Nascimbene et al., 2020); hence, it is important to maintain suitable refugia for the conservation of the model species within such habitats, also in managed forests. There is already evidence that the effective conservation-oriented management of this species should be tailored at the habitat level, and especially at the tree level, where microclimatic features are of utmost importance (Nascimbene et al., 2013; Benesperi et al., 2018). Therefore, it is essential to understand microscale dynamics under various microclimatic conditions to be able to design effective conservation strategies suitable to all stages of population development (Benesperi et al., 2018).

In a previous study (Bianchi et al., 2020), comparing young and adult *L. pulmonaria* thalli exposed for one year in logged and unlogged stands (on the northern side of Turkey oaks), a lower growth of *L. pulmonaria* was observed on isolated trees in the logged stand, especially in the case of adult thalli. Similarly, native thalli on isolated trees appeared thinner and showed lower photosynthetic performance and water holding capacity when compared with healthy samples from unlogged forests or retained forest patches (Fačkovcová et al., 2019), with consequent implications for their growth and the possibility of providing ecosystem services.

The translocation of lichen thalli (or fragments of thalli) can be regarded as a method for the in situ conservation of threatened lichen populations (Scheidegger et al., 1995). Some projects have investigated this opportunity, monitoring the performance of translocated *L. pulmonaria* thalli (Efremov & Plikina, 2018). The aim of the present work was to investigate the combined effect of forest logging and microclimatic conditions on the probability of survival of transplants of the model species. Our working hypothesis is that microclimatic alteration at the tree level after logging affects their growth, with young thalli having a higher chance of survival.

To this purpose, 800 fragments of *L. pulmonaria* were transplanted in logged and unlogged stands for two years. Fragments were positioned on oak trunks according to the distance from the ground (100 and 50 cm) and aspect (north and south). Since the viability of *L. pulmonaria* populations in relation to forest management often depends on the regenerative capacity of young and adult thalli, two types of fragments were considered: those with meristematic (young) properties, and those with non-meristematic (adult) properties. Growth rates and probabilities were assessed and modelled for each experimental condition.

3.2 Materials & Methods

3.2.1 Study Area and Experimental Design

The study area was a Mediterranean mixed oak forest stand dominated by *Quercus cerris*, *Q. pubescens* and *Q. ilex* recognized as a local hotspot for *L. pulmonaria* (Paoli et al., 2013) (Tuscany, Central Italy, WGS84: N 43.1851; E 11.3602). Oak forests in the area are managed by a coppice system with a long rotation cycle. The last cut was carried out in 2016 (the previous cut likely dates back more than 40 years). Logging reduced the density of stems from 1100 to 165 per ha, with a consequent increase of sun irradiance (from 130–1100 in the unlogged stand to 900–1550 µmol m^{-2} s^{-1} PAR, measured at noon in the logged stand) and temperature, as well as a decrease of humidity (Fačkovcová et al., 2019). Since *L. pulmonaria* is not legally protected in Italy, logging operations started without considering the presence of this relevant population, which resulted heavily impacted by logging (Paoli et al., 2019).

According to the methodology described in Bianchi et al. (2020), healthy *L. pulmonaria* thalli were randomly selected from a nearby oak forest to obtain 400 meristematic fragments and 400 non-meristematic fragments (Figure 3.1). Specifically, the first were upward-growing young lobes with intact apical meristems, and the latter were fragments of the inner sorediate or non-sorediate parts of the thallus, lacking apical growth (Giordani & Brunialti, 2002). The source habitat for collected fragments had the same characteristics as the unlogged stand, being adjacent to the study sites and extending onto a hillside with a northern slope, where *Q. cerris*, *Q. ilex* and *Q. pubescens* were the most common trees colonized by *L. pulmonaria* (Bianchi et al., 2020). In order to minimize the harvesting of material from the native population, all fragments were smaller than 1 cm. Overall, 800 fragments of *L. pulmonaria* were transplanted for two years (from March 2019 to March 2021). Thallus fragments (each representing a statistical sample) were exposed on the northern and the southern sides of the trunks of twenty randomly selected Turkey oaks (*Q. cerris*) (reciprocal distance >10 m), at about 100 cm and 50 cm from the ground, half in the logged and half in the unlogged stand: 400 meristematic and 400 non-meristematic fragments in each forest type (logged and unlogged stands). *Lobaria pulmonaria* was exposed using a specific transplant device, a ‘barella’, which was composed of a sterilized bandage supported by a plastic net (10 × 2 cm2). For each device, for practical reasons, five meristematic or five non-meristematic fragments of thalli were tied onto the bandage to avoid overlapping with each other (Bianchi et al., 2020)

3.2.2 Growth

The hydrated thallus area (A) was estimated using Photoshop CS6 Extended (Adobe Systems, San Jose, CA, USA), as suggested by Bianchi et al. (2020). To avoid folding of the lobes, each thallus fragment was fully hydrated with mineral water and carefully flattened before scanning with a Canon i−SENSYS MF4320d (Canon Inc., Tokyo, Japan). The lichen growth, comparing the same samples before (T0) and after (TF) exposure, was estimated as growth coefficient A = (area TF − area T0)/area T0 and as percentage of growth A = [(area TF − area T0)/area T0] × 100. After two years, the growth of the surface of each individual thallus was assessed by

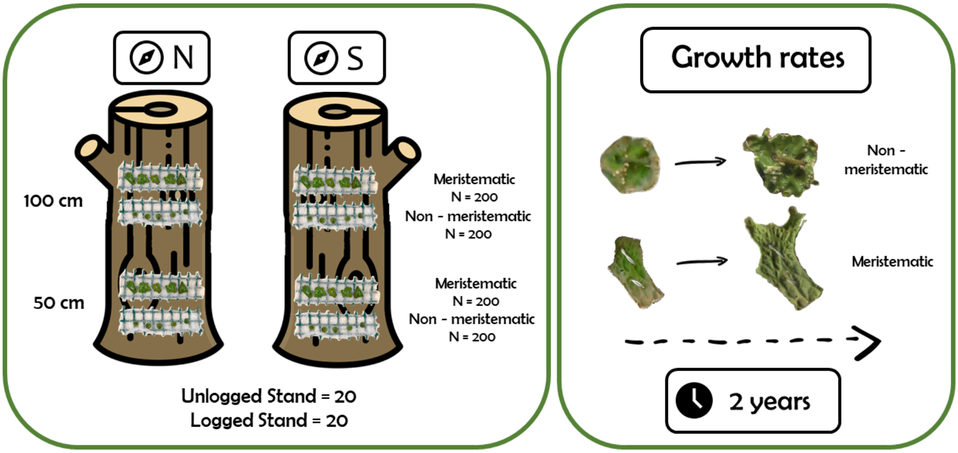


Figure 3.1: Graphical representation of the experimental design. Icons: Flaticon.com.

subtracting the initial area from the respective area at harvest. Pre-exposure and final values were considered for the growth rates.

3.2.3 Data Analysis

Each fragment was categorized as follows: (a) fragments with a positive coefficient of growth (growth coefficient >0) were defined as 'growth'; and (b) fragments with a negative coefficient of growth (<0), together with those that were completely degraded (growth coefficient $= -1$), were defined as 'no growth'. Then, the effects of forest type (logged versus unlogged), aspect (N versus S), height from the ground (50 versus 100 cm) and type of lobes (meristematic versus non-meristematic) were tested together with their possible interactions on the probability of growth. A generalized linear mixed model (GLMM) was fitted with 'tree' as a random factor to account for possible similar unmeasured conditions on the same tree and a binomial distribution with logit link function.

Subsequently, only those fragments with a positive growth coefficient were selected, and the effect of forest type, height from the ground, type of lobes and their interactions on growth coefficient were modelled. We did not include aspect as a predictive variable, since in the logged stand only nine fragments exposed to the south presented positive growth, thus making unsuitable for testing. We fitted a linear mixed model (LMM) with 'tree' as a random factor. Growth coefficients were treated with the square root to meet normality.

Finally, a model was fitted using only those fragments with positive growth coefficients in the unlogged stand. In such cases, the effects of exposure, height from the ground, type of lobes and their interactions on growth coefficient were tested. An LMM was fitted with 'tree' as a random factor, an in this case, growth coefficients were also treated with the square root.

All models were fitted using the 'glmmTMB' package (Brooks et al. 2017). The performance of the models was checked through diagnostic plots and tests using the 'DHARMa' package (Hartig, 2021).

Predictor importance was evaluated using multimodel inference through the information−theoretic approach (Anderson & Burnham, 2004). All candidate models, nested within the full model, were fitted and compared using Akaike's information criterion corrected for sample size (AICc). Models were ranked based on their difference in AICc (ΔAICc) with respect to the best−fitting model (i.e., the one with the lowest value of AICc, AICc min). Thus, ΔAICc was calculated as $\Delta \text{AIC}ci = \text{AIC}ci - \text{AICc min}$. Models that presented a $\Delta\text{AICc} < 2$ were considered plausible, and the coefficient of each predictor was averaged among this set of models. Then, the significance of the estimated coefficients was tested using a z-Wald test. For each predictor, its relative importance 'wr' was calculated by summing the AICc weights of all the models where that predictor appeared (Burnham and Anderson, 2002). AICc weight, the relative likelihood of a specific model, was calculated by dividing the likelihood of the model by the sum of likelihoods of all models. The functions dredge, model.avg and importance were used in the MuMIn package to perform the multimodel inference (Bartoń, 2021). Graphical outputs were produced using ggplot2 (Wickham, 2016) and ggdist (Kay, 2021) packages. All analyses were performed using R 4.1.1 (Core Team, 2021).

3.3 Results

3.3.1 Fragment Growth

After two years of exposure, transplanted fragments presented a wide range of growth rates, with an average growth rate of 16%. In detail, exposed fragments in the unlogged stand showed a higher growth rate (31%) when compared to exposed fragments on isolated trees in the logged stand (10%). North-exposed fragments grew more (26.9%) than those exposed to the south (14.7%). Similarly, meristematic lobes presented higher percentages of growth (25.8%) than adult fragments (16%). By contrast, only a slight difference emerged between fragments exposed at 100 cm (22%) and 50 cm (19%) from the ground. Regarding the growth of the fragments, only 44% had positive growth. South-exposed fragments in the logged stand had the lowest rate of positive growth (4%). Most of these fragments appeared progressively damaged during the transplant period, and were finally lost, having fallen or broken apart.

3.3.2 Determinants of Probability and Coefficient of Growth

Models considering the probability of growth of the fragments highlighted a lower growth probability in the logged stand and southern aspect (Figure 3.2, Table 3.1). They also revealed a significant interaction between the forest stands and their aspect (Figure 3.2, Table 3.1), with fragments exposed to the south in the logged stand showing low probability of growth. In addition, the interaction between type of lobes and height from the ground was also significant. Higher growth probabilities were associated with the unlogged stand, particularly for north-exposed transplants. Meristematic fragments exposed at 50 cm from the ground had a higher chance of growth; by contrast, non-meristematic fragments showed higher growth probability at 100 cm.

Modelling related to growth coefficients (Figure 3.3, Table 3.1) indicated a significant difference between meristematic and non-meristematic fragments, with the first having a higher growth coefficient. Considering the whole dataset, no significant

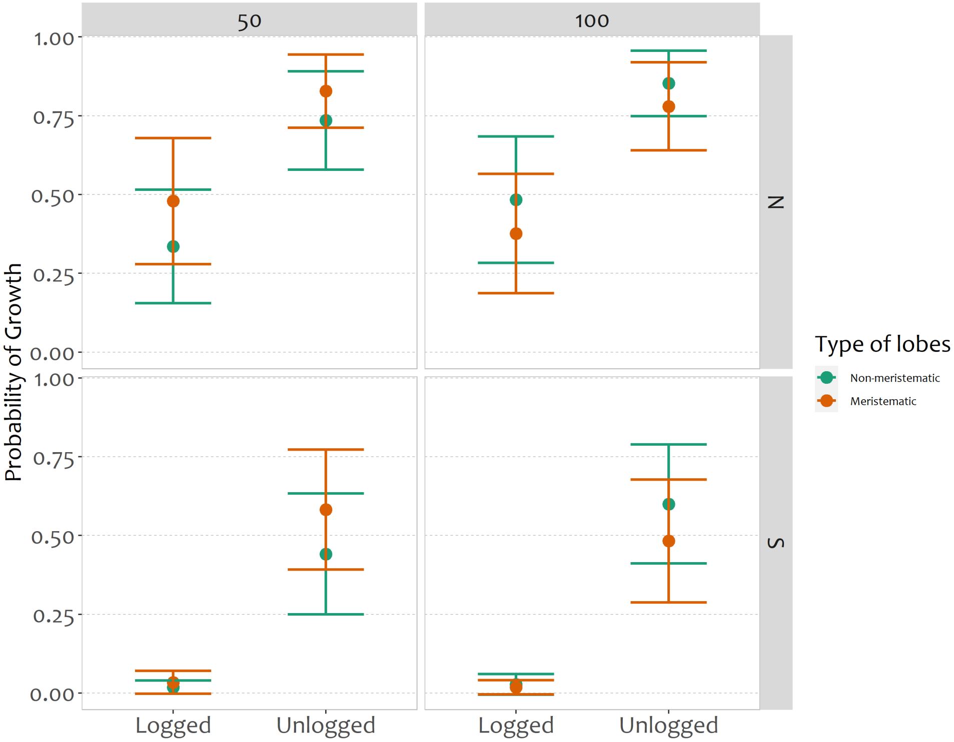


Figure 3.2: Predicted probability of growth for forest types (logged and unlogged), type of lobes (non-meristematic and meristematic), aspect (N = north, S = south) and different heights from the ground (50 and 100 cm).

correlation between forest type and distance from the ground was evident, suggesting an overall similar growth both in logged and unlogged stands, regardless of the distance from the ground (Figure 3.3, Table 3.1). However, it is important to state that such models considered only fragments that had a positive growth coefficient, not including aspect as a predictive variable, since few fragments presented a positive growth coefficient in the southern side of the trees in the logged stand.

Therefore, focusing specifically on growth coefficients in the unlogged stand, models outlined higher growth by meristematic fragments (Figure 3.4, Table 3.1) and moreover, south-exposed fragments had a higher growth coefficient than those that were north-exposed, especially at 50 cm from the ground.

Parameter	Relative Importance	Lower CI	Upper CI	Full Averaged Coefficient	p-value
Probability of growth					
Height from the ground	1.000	-0.246	0.173	-0.037	0.731
Cardinal exposure	1.000	0.927	1.413	1.170	0.000
Type of lobes	1.000	-0.214	0.150	-0.032	0.732
Forest type	1.000	-1.898	-0.884	-1.391	0.000
Height from the ground : Type of lobes	1.000	-0.442	-0.079	-0.261	0.005
Cardinal exposure : Forest type	1.000	0.282	0.763	0.523	0.000
Height from the ground : Forest type	0.346	-0.272	0.101	-0.013	0.791
Height from the ground : Cardinal exposure	0.330	-0.102	0.373	0.047	0.625
Type of lobes : Forest type	0.147	-0.366	0.114	-0.041	0.651
Cardinal exposure : Type of lobes	0.107	-0.142	0.221	0.004	0.897
Height from the ground : Cardinal exposure : Forest type	0.103	-0.425	0.072	-0.018	0.788
Coefficient of growth					
Height from the ground	1.000	-0.009	0.033	0.012	0.269
Height from the ground : Type of lobes	0.637	-0.032	0.003	-0.009	0.356
Height from the ground : Forest type	0.425	-0.038	0.004	-0.007	0.505
Type of lobes	1.000	-0.083	-0.040	-0.062	0.000
Type of lobes : Forest type	0.581	-0.039	0.001	-0.011	0.366
Forest type	0.752	-0.053	0.015	-0.014	0.403
Coefficient of growth - only Unlogged					
Height from the ground	1.000	0.010	0.048	0.029	0.003
Cardinal exposure	1.000	-0.051	-0.011	-0.031	0.002
Type of lobes	1.000	-0.068	-0.031	-0.049	0.000
Height from the ground : Cardinal exposure	1.000	-0.048	-0.010	-0.029	0.003
Cardinal exposure : Type of lobes	1.000	-0.035	0.004	-0.010	0.343
Height from the ground : Type of lobes	0.671	0.003	0.041	0.022	0.025
Height from the ground : Cardinal exposure : Type of lobes	0.371	-0.003	0.035	0.006	0.546

Table 3.1: Full averaging of generalized linear mixed model coefficients among models showing a delta AICc < 2. Significant interactions are in bold. Variables with sum >0.65 and with a significant Wald test are shown in bold.

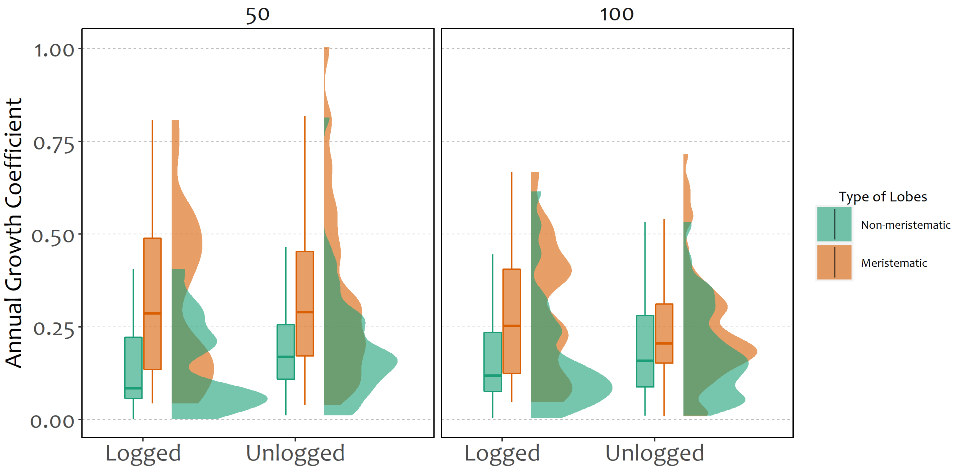


Figure 3.3: Annual growth coefficient for forest type (logged and unlogged), type of lobes (non-meristematic and meristematic) and different heights from the ground (50 and 100 cm).

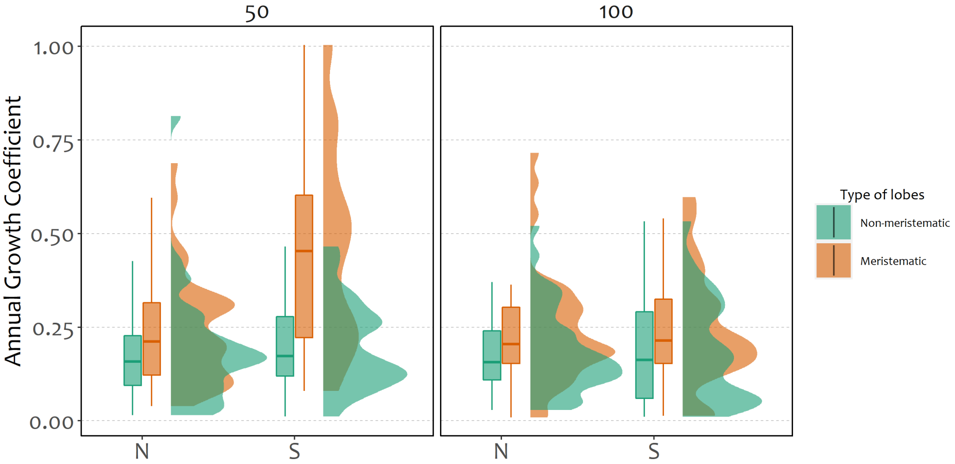


Figure 3.4: Annual growth coefficient in the unlogged stand according to type of lobes (meristematic and non-meristematic), aspect and different heights from the ground (50 and 100 cm).

3.4 Discussion

Our results support the hypothesis that microclimatic conditions are extremely important in determining the growth of *L. pulmonaria* transplants, and highlight the relevance of considering microclimatic factors when performing forest logging in areas hosting populations of *L. pulmonaria*. Preserving retained forest patches as well as isolated trees growing in favorable microclimatic conditions can maximize the possibility of *L. pulmonaria* populations persisting, even after forest logging (Fačkovcová et al., 2019). In fact, microclimatic refugia can reduce species threat if suitable climatic conditions are maintained locally, regardless of processes at the larger scale that modify environmental conditions (Ellis & Eaton, 2020).

Due to their small living space, lichens are strongly influenced by local microclimatic variations. In general, the physiological responses, abundance and diversity of smaller and less mobile organisms often more readily reflect small-scale variations in micro-environmental conditions than do the responses of organisms characterized by larger living space, size and mobility (Decocq, 2000; De Frenne et al., 2021). After forest logging, microclimatic conditions drastically change, with a strong increase of temperature, light and wind speed, and a reduction of humidity (Chen et al., 1999). Understanding the effects of the main microclimatic (Rubio-Salcedo et al., 2015) and macroclimatic (Eaton & Ellis, 2012) drivers on the survival and growth of *L. pulmonaria* is necessary in order to boost the capability of this species to recolonize fragmented or disturbed habitats and to develop proper conservation strategies. This also holds true in the case of translocations for conservation purposes, the success of which requires specific protocols (Smith, 2014) and depends on the selection of receptor sites with locally suitable microclimatic and chemical conditions that allow the long-term persistence of translocated thalli and the development of new ones (Scheidegger, 1995; Paoli et al., 2020).

In our study, fragments exposed to the south in the logged stand had an extremely low probability of growth, being progressively damaged during the transplant period and finally falling or breaking after two years. In the northern hemisphere, without considering other factors, south-exposed sites tend to receive higher amounts of light and are drier compared to north-exposed sites. This phenomenon can be exacerbated by forest logging, which, by reducing canopy cover, causes an increase in light intensity and reduces the thermal buffer capacity of the forest (Chen et al., 1999).

Lobaria pulmonaria is susceptible to high light stress, particularly in a desiccated state (Gauslaa & Solhaug 1996, 1999, 2000). Moreover, the combined effect of high temperature and irradiance can be particularly deleterious to the thalli (Gauslaa & Solhaug, 1999). In our study, the microclimatic conditions of the fragments exposed to the south in the logged stand likely exceeded the ecological range of conditions suitable for acclimation. The combination of high temperature and irradiance, as well as limited water availability, especially characterizes open south-facing sites during the summer in Mediterranean areas (Paoli et al., 2010). Solar radiation strongly influences fragment surface temperature, enhancing water loss and reducing the total hydration period (Gauslaa, 2014). Hence, south-exposed fragments could only find limited favorable periods for photosynthesis and growth, leading to the survival of almost no fragments after two years. Nevertheless, like many other organisms, *L. pulmonaria* possesses different mechanisms for acclimation, which, within a certain range, allows it to withstand changes in microclimatic conditions after forest logging. As an example, the species is able to adapt to high light stress through the melanisation of the thallus (Mafole et al., 2017), as already observed as a response to logging, or in transplant experiments (Coxson & Stevenson, 2007b; Fačkovcová et al., 2019; Bianchi et al., 2020). *Lobaria pulmonaria* synthesizes melanins when exposed to high solar radiation; this melanisation requires sufficient hydration of the thallus to occur and may effectively reduce high light stress by increasing energy dissipation and reducing photosynthetic activity (Mafole et al., 2017). Furthermore, by increasing thallus thickness the lichen can prolong hydration periods and can also prolong photobiont protection (Gauslaa, 2006). Acclimation strategies can be observed at different levels and involve gene expression, for example, to acclimate to thermal shifts (Chavarria-Pizarro et al., 2021), or during water dehydration cycles (Cernava et al., 2019).

In our study, the coefficient of growth of those fragments still attached after two years highlighted only minor differences between the logged and the unlogged stands. These results suggest that in the logged stand, certain microclimatic conditions can resemble those present in the retained forest, allowing fragment growth. Specifically, north-exposed fragments in the logged stand received far lower amounts of light than south-exposed fragments, with the first benefitting from the shading effect of the tree. Forest gaps and clear-cuts are characterized by frequent dew events (Geiger, 1950; Gauslaa, 2014), which often occur during the night, especially at the northern side, so north-exposed thalli can benefit from them, prolonging their hydration periods. Hence, the trees' shading effect combined with these dew events could explain the lack of difference in terms of growth coefficients between north-exposed fragments in the logged stand and fragments in the unlogged stand. A hydrated state of the thallus is essential for surface growth in lichens, as turgor pressure is required for hyphal expansion (Money, 2008).

Previous studies in boreal forests have reported better growth of lichens in sheltered clear-cuts than in intact old forests (Gauslaa et al., 2006; Larsson et al., 2012). However, in boreal forests, clear-cuts receive less solar radiation overall and reach lower temperatures compared with Mediterranean oak forests, where high irradiance is accompanied by a rise in temperature and an overall decrease of moisture. Consequently, in our study sites, north-exposed fragments on isolated trees (despite finding favorable conditions to grow compared to south-exposed fragments), were not in better conditions than those in mixed unlogged stands. Considering the results from the unlogged stand, aspect seemed to play a relevant role in combination with distance from the ground. South-exposed fragments could have received slightly more light than those exposed to the north. Unlike isolated trees, this increase in the amount of light could have enhanced lichen growth.

The distance from the ground seemed to influence growth coefficients only in the unlogged stand, while showing negligible effects in the logged stand. This result is consistent with a previous study (Merinero et al., 2015), which reported that in open Mediterranean forests, the fast growth of *L. pulmonaria* near the ground is likely boosted by nocturnal cooling forming temperature profiles, and hence, humidity gradients. Thus, the effect of height on lichen growth rates depends highly on climate and forest type (Merinero et al., 2015) and in our case, a fine combination of increased southerly light together with sufficient humidity in the unlogged stand led to an overall higher growth coefficient, especially for south-exposed fragments at 50 cm from the ground.

The type of lobes was a relevant factor in determining *L. pulmonaria* growth rates. Meristematic fragments grew on average more than non-meristematic ones, regardless of aspect. Similarly, our previous study highlighted that north-exposed meristematic fragments measured after one year had higher growth rates (0.16–0.18 cm^2) than non-meristematic ones (0.02–0.06 cm^2), irrespective of forest management (i.e., comparing logged versus unlogged stands) (Bianchi et al., 2020). On the other hand, the results also point out the capacity of both meristematic and non-meristematic tissues to regenerate and grow, even after severe damage, as previously observed for other lichen species (Armstrong et al., 2011).

In this sense, small fragments of *L. pulmonaria* that remain after logging could be a potential source of new propagules which, when favorable conditions occur, could allow the development of new populations, with faster growth of young (meristematic) thalli. Hence, in Mediterranean oak forests, retaining trees where *L. pulmonaria* colonize the northern side can greatly enhance the possibility of those thalli persisting even after logging. Nevertheless, taking a long-term view, the spatial distribution of retained trees also has to be considered, as the vegetative dispersal capacity of *L. pulmonaria* has a low distance (Walser et al. 2001; Juriado et al. 2011).

As concluding remarks, since the transplant technique, in the case of *L. pulmonaria*, can be used for conservation purposes via translocation of the thalli (Scheidegger, 1995; Paoli et al., 2020), we should consider the following in relation to Mediterranean oak forests:

- The translocation on isolated trees (e.g., in unlogged stands) better supports the survival of thalli on the northern side of the boles, given the stronger sun irradiance and lower water availability at the southern side.

- The translocation on trees within forest patches or unlogged stands offers higher growth performance on the southern side of the trees, thanks to the fine combination of increased light on the southern side and sufficient humidity within the forest.

Constraints such as microclimate are fundamental considerations when dealing with the ecology of lichens. They open up the opportunity to protect valuable habitats and local populations, and in general, to adapt forest management practices for the conservation of threatened species.

Acknowledgements. We are grateful to Associazione Culturale di Murlo (www.murlocultura.com 16 December 2021) for hosting the meeting of the Working Group for Ecology of the Italian Lichen Society held in Murlo (Siena), 10–12 February 2020. We are also grateful to Michelle Abajan (USA) for language revision.

3.5 Bibliography

Armstrong, R. A., & Bradwell, T. (2011). Growth of Foliose Lichens: A Review. *Symbiosis*, 53(1), 1–16. doi:10.1007/s13199-011-0108-4

Baker, S. C., Spies, T. A., Wardlaw, T. J., Balmer, J., Franklin, J. F., & Jordan, G. J. (2013). The harvested side of edges: effect of retained forests on the re-establishment of biodiversity in adjacent harvested areas. *Forest Ecology and Management*, 302, 107–121. doi:10.1016/j.foreco.2013.03.024

Barton, K. (2021). MuMIn: Multi-Model Inference, R Package Version 1.43. 17. Available online: https://CRAN.R-project.org/package= MuMIn (accessed on 13 December 2021).

Benesperi, R., Nascimbene, J., Lazzaro, L., Bianchi, E., Tepsich, A., Longinotti, S., & Giordani, P. (2018). Successful conservation of the endangered forest lichen Lobaria pulmonaria requires knowledge of fine-scale population structure. *Fungal Ecology*, 33, 65–71. doi:10.1016/j.funeco.2018.01.006

Bianchi, E., Benesperi, R., Brunialti, G., Di Nuzzo, L., Fačkovcová, Z., Frati, L., ... & Vallese, C. (2020). Vitality and Growth of the Threatened Lichen Lobaria pulmonaria (L.) Hoffm. in Response to Logging and Implications for Its Conservation in Mediterranean Oak Forests. *Forests*, 11(9), 995. doi:10.3390/f11090995

Brooks, M.E., Kristensen, K., van Benthem, K.J., Magnusson, A., Berg, C.W., Nielsen, A., Skaug, H.J., Machler, M., Bolker, B.M. GlmmTMB Balances speed and flexibility among packages for Zero-Inflated Generalized Linear Mixed Modeling. *R Journal* 2017, 9, 378–400.

Brunialti, G., Frati, L., & Ravera, S. (2015). Structural variables drive the distribution of the sensitive lichen Lobaria pulmonaria in Mediterranean Old-Growth forests. *Ecological Indicators*, 53, 37–42. doi:10.1016/j.ecolind.2015.01.023

Burnham, K. P., & Anderson, D. R. (2004). Multimodel Inference: Understanding AIC and BIC in Model Selection. *Sociological Methods & Research*, 33(2), 261–304. doi:10.1177/0049124104268644.

Burnham, K. P., & Anderson, D. R. (2002). Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach (2nd ed.). Springer. ISBN 978-0-387-95364-9.

Cernava, T., Aschenbrenner, I. A., Soh, J., Sensen, C. W., Grube, M., & Berg, G. (2019). Plasticity of a holobiont: desiccation induces fasting-like metabolism within the lichen microbiota. *ISME Journal*, 13(3), 547–556. doi:10.1038/s41396-018-0286-7

Chavarria-Pizarro, T., Resl, P., Janjic, A., & Werth, S. (2022). Gene expression responses to thermal shifts in the endangered lichen Lobaria pulmonaria. *Molecular Ecology*, 31, 839–858. doi:10.1111/mec.16281

Chen, J., Saunders, S. C., Crow, T. R., Naiman, R. J., Brosofske, K. D., Mroz, G. D., ... & Franklin, J. F. (1999). Microclimate in forest ecosystem and landscape ecology − variations in local climate can be used to monitor and compare the effects of different management regimes. *BioScience*, 49(4), 288–297. doi:https://doi.org/10.2307/1313612

Coxson, D. S., & Stevenson, S. K. (2007). Growth rate responses of Lobaria pulmonaria to canopy structure in even-aged and old-growth cedar–hemlock forests of central-interior British Columbia, Canada. *Forest Ecology and Management*, 242(1–3), 5–16. doi:10.1016/j.foreco.2007.01.031

Coxson, D. S., & Stevenson, S. K. (2007). Influence of high-contrast and low-contrast forest edges on growth rates of Lobaria pulmonaria in the inland rainforest, British Columbia. *Forest Ecology and Management*, 253(1–3), 103–111. doi:10.1016/j.foreco.2007.07.008

De Frenne, P., Lenoir, J., Luoto, M., Scheffers, B. R., Zellweger, F., Aalto, J., ... & Decocq, G. (2021). Forest microclimates and climate change: importance, drivers and future research agenda. *Global Change Biology*, 27(11), 2279–2297. doi:10.1111/gcb.15569

Decocq, G. (2000). The ‘masking effect’ of silviculture on substrate-induced plant diversity in oak-hornbeam forests from northern France. *Biodiversity and Conservation*, 9(11), 1467–1491. doi:10.1023/A:1008919027221

Eaton, S., & Ellis, C. J. (2012). Local experimental growth rates respond to macroclimate for the lichen epiphyte Lobaria pulmonaria. *Plant Ecology and Diversity*, 5(3), 365–372. doi:10.1080/17550874.2012.728640

Efremov, E., & Plikina, N. (2018). Translocation of bryocaulon and lung lichens in the north of Sakhalin Region. In Global Reintroduction Perspectives: 2018 (pp. 276–281). IUCN/SSC Reintroduction Specialist Group, Gland, Switzerland and Environment Agency, Abu Dhabi, UAE.

Ellis, C., & Eaton, S. (2021a). Climate change refugia: landscape, stand and tree-scale microclimates in epiphyte community composition. *The Lichenologist*, 53(2), 135–148. doi:10.1017/S0024282920000523

Ellis, C. J., & Eaton, S. (2021b). Microclimates hold the key to spatial forest planning under climate change: cyanolichens in temperate rainforest. *Global Change Biology*, 1–12. doi:10.1111/gcb.15514

Fačkovcová, Z., Guttová, A., Benesperi, R., Loppi, S., Bellini, E., Sanità di Toppi, L., ... & Paoli, L. (2019). Retaining unlogged patches in Mediterranean oak forests may preserve threatened forest macrolichens. *iForest – Biogeosciences and Forestry*, 12, 187. doi:10.3832/ifor2917-012

Gauslaa, Y. (2014). Rain, dew, and humid air as drivers of morphology, function and spatial distribution in epiphytic lichens. *The Lichenologist*, 46(1), 1–16. doi:10.1017/S0024282913000753

Gauslaa, Y., & Solhaug, K. A. (1996). Differences in the susceptibility to light stress between epiphytic lichens of ancient and young boreal forest stands. *Functional Ecology*, 10(3), 344–354. doi:10.2307/2390282

Gauslaa, Y., & Solhaug, K. A. (1999). High-light damage in air-dry thalli of the old forest lichen Lobaria pulmonaria —Interactions of irradiance, exposure duration and high temperature. *Journal of Experimental Botany*, 50(334), 697–705. doi:10.1093/jxb/50.334.697

Gauslaa, Y., & Solhaug, K. A. (2000). High-light-intensity damage to the foliose lichen Lobaria pulmonaria within natural forest: the applicability of chlorophyll fluorescence methods. *The Lichenologist*, 32(3), 271–289. doi:10.1006/lich.1999.0265

Gauslaa, Y., Lie, M., Solhaug, K. A., & Ohlson, M. (2006). Growth and ecophysiological acclimation of the foliose lichen Lobaria pulmonaria in forests with contrasting light climates. *Oecologia*, 147(3), 406–416. doi:10.1007/s00442-005-0283-1

Giordani, P., & Brunialti, G. (2002). Anatomical and histochemical differentiation in lobes of the lichen Lobaria pulmonaria. *Mycological Progress*, 1(2), 131–136. doi:10.1007/s11557-006-0011-1.

Geiger, R. (1950). *The Climate Near the Ground.* Cambridge: Harvard University Press.

Hartig, F. (2021). DHARMa: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models (Version 0.4.3). Retrieved from https://CRAN.R-project.org/package=DHARMa

Jüriado, I., Liira, J., Csencsics, D., Widmer, I., Adolf, C., Kohv, K., & Scheidegger, C. (2011). Dispersal ecology of the endangered woodland lichen Lobaria pulmonaria in managed hemiboreal forest landscape. *Biodiversity and Conservation*, 20(8), 1803–1819. doi:10.1007/s10531-011-0062-8.

Kay, M. (2021). Ggdist: Visualizations of Distributions and Uncertainty. R Package Version 3.0.0.

Larsson, P., Solhaug, K. A., & Gauslaa, Y. (2012). Seasonal partitioning of growth into biomass and area expansion in a cephalolichen and a cyanolichen of the old forest genus Lobaria. *New Phytologist*, 194(4), 991–1000. doi:https://doi.org/10.1111/j.1469-8137.2012.04130.x

Larsson, P., Solhaug, K. A., & Gauslaa, Y. (2014). Winter – the optimal logging season to sustain growth and performance of retained epiphytic lichens in boreal forests. *Biological Conservation*, 180, 108–114. doi:10.1016/j.biocon.2014.09.045

Mafole, T. C., Chiang, C., Solhaug, K. A., & Beckett, R. P. (2017). Melanisation in the old forest lichen Lobaria pulmonaria reduces the efficiency of photosynthesis.

Fungal Ecology, 29, 103–110. doi:10.1016/j.funeco.2017.07.004

Merinero, S., Martínez, I., Rubio-Salcedo, M., & Gauslaa, Y. (2015). Epiphytic lichen growth in Mediterranean forests: effects of proximity to the ground and reproductive stage. *Basic and Applied Ecology*, 16(3), 220–230. doi:10.1016/j.baae.2015.01.007

Money, N. P. (2008). Insights on the mechanics of hyphal growth. *Fungal Biology Reviews*, 22(3–4), 71–76. doi:10.1016/j.fbr.2008.05.002

Nascimbene, J., Benesperi, R., Casazza, G., Chiarucci, A., & Giordani, P. (2020). Range shifts of native and invasive trees exacerbate the impact of climate change on epiphyte distribution: the case of lung lichen and black locust in Italy. *Science of the Total Environment*, 735, 139537. doi:10.1016/j.scitotenv.2020.139537

Nascimbene, J., Casazza, G., Benesperi, R., Catalano, I., Cataldo, D., Grillo, M., ... & Giordani, P. (2016). Climate change fosters the decline of epiphytic Lobaria species in Italy. *Biological Conservation*, 201, 377–384. doi:10.1016/j.biocon.2016.08.003

Nascimbene, J., Thor, G., & Nimis, P. L. (2013). Effects of forest management on epiphytic lichens in temperate deciduous forests of Europe – A Review. *Forest Ecology and Management*, 298, 27–38. doi:10.1016/j.foreco.2013.03.008

Otálora, M. G., Martínez, I., Belinchón, R., Widmer, I., Aragón, G., Escudero, A., & Scheidegger, C. (2011). Remnants fragments preserve genetic diversity of the old forest lichen Lobaria pulmonaria in a fragmented mediterranean mountain forest. *Biodiversity and Conservation*, 20(6), 1239–1254. doi:10.1007/s10531-011-0025-0

Paoli, L., Benesperi, R., Fačkovcová, Z., Nascimbene, J., Ravera, S., Marchetti, M., ... & Bianchi, E. (2019). Impact of forest management on threatened epiphytic macrolichens: evidence from a Mediterranean mixed oak forest (Italy). *iForest - Biogeosciences and Forestry*, 12, 383. doi:10.3832/ifor2951-012

Paoli, L., Guttová, A., & Anselmi, B. (2013). Osservazioni sui licheni di Crevole [Observations on the Lichens of Crevole]. *MurloCultura* 16, 8–10.

Paoli, L., Guttová, A., Sorbo, S., Lackovičová, A., Ravera, S., Landi, S., ... & Vannini, A. (2020). Does air pollution influence the success of species translocation? Trace elements, ultrastructure and photosynthetic performances in transplants of a threatened forest macrolichen. *Ecological Indicators*, 117, 106666. doi:10.1016/j.ecolind.2020.106666

Paoli, L., Pisani, T., Munzi, S., Gaggi, C., & Loppi, S. (2010). Influence of sun irradiance and water availability on lichen photosynthetic pigments during a Mediterranean summer. *Biologia* (Bratislava), 65(5), 776–783. doi:10.2478/s11756-010-0087-6

R Core Team. (2021). R: A language and environment for statistical computing (Version 4.1.1). Vienna, Austria: R Foundation for Statistical Computing.

Redding, T. E., Hope, G. D., Schmidt, M. G., & Fortin, M.-J. (2004). Analytical methods for defining standclearcut edge effects demonstrated for N mineralization. *Canadian Journal of Forest Research*, 34(5), 1018–1024. doi:10.1139/x03-282

Rubio-Salcedo, M., Merinero, S., & Martínez, I. (2015). Tree species and microhabitat influence the population structure of the epiphytic lichen Lobaria pulmonaria. *Fungal Ecology*, 18, 1–9. doi:10.1016/j.funeco.2015.08.002

Scheidegger, C., & Werth, S. (2009). Conservation strategies for lichens: insights from population biology. *Fungal Biology Reviews*, 23(2–3), 55–66. doi:10.1016/j.fbr.2009.10.003

Scheidegger, C., Frey, B., & Zoller, S. (1995). Transplantation of symbiotic propagules and thallus fragments: methods for the conservation of threatened epiphytic lichen populations. *Mitteilungen Eidgenössische Forschungsanstalt für Wald Schnee Landschaft*, 70, 41–62.

Smith, P. L. (2014). Lichen translocation with reference to species conservation and habitat restoration. *Symbiosis*, 62(1), 17–28. doi:10.1007/s13199-014-0269-z

Walser, J.-C., Zoller, S., Büchler, U., Scheidegger, C. (2010). Species-Specific detection of Lobaria pulmonaria (Lichenized Ascomycete) diaspores in litter samples trapped in snow cover. *Molecular Ecology*, 10, 2129–2138, doi:10.1046/j.1365-294X.2001.01353.x.

Whittet, R., & Ellis, C. J. (2013). Critical tests for lichen indicators of woodland ecological continuity. *Biological Conservation*, 168, 19–23. doi:10.1016/j.biocon.2013.09.011

Wickham, H. (2016). Ggplot2: Elegant graphics for data analysis.

Zedda, L., & Rambold, G. (2015). The diversity of lichenised fungi: ecosystem functions and ecosystem services. In Recent Advances in Lichenology: Modern Methods and Approaches in Lichen Systematics and Culture Techniques, Volume 2 (pp. 121–145). Springer India. ISBN 978-81-322-2235-4.

Chapter 4

Life-stage dependent response of the epiphytic lichen Lobaria pulmonaria to climate

Luca Di Nuzzo*, Giulia Canali*, Paolo Giordani, Juri Nascimbene, Renato Benesperi, Alessio Papini, Elisabetta Bianchi, Philipp Porada

Published in *Frontiers in Forests and Global Change*, October 2022, volume 5 DOI: 10.3389/ffgc.2022.903607. * These authors have contributed equally to this work

Abstract

Lichens are poikilohydric organisms, whose internal water content tends to reflect external humidity conditions. After drying, they can reactivate their metabolic activity through water vapor uptake or liquid water input. Thus, lichen water-related functional traits are important as they are involved in the duration of the hydration period. Models predicting the effect of environmental conditions on lichens are based mainly on the presence or absence of adult thalli. Nevertheless, ecological conditions required by lichens might vary during their life cycle, for example during propagule establishment or in the first stages of thallus development. Little is known about the different ecological requirements at the different development stages in lichens. In this work, we measured water holding capacity (WHC) and specific thallus mass (STM) of adult and juvenile thalli of the model species *Lobaria pulmonaria* along a climatic gradient to constrain the process-based model LiBry. The LiBry model allows accounting for the productivity of lichens with different physiological strategies under various environmental conditions. We simulated the activity and performance of adult and juvenile thalli in 9 regions of Italy and Corsica. The model was used to test if adult thalli of *L. pulmonaria* have a higher survival probability due to their higher aerodynamic resistance. In the current climatic condition, the LiBry model predicts a higher survival probability of adults with decreasing absolute survival rates of both life stages with increasing temperature. Adult thalli also result in having higher active time, STM, and relative growth rate (RGR). We discuss the main implications of our simulation outputs, provide future perspectives and possible implementations of the LiBry model.

4.1 Introduction

Human-induced atmospheric CO_2 rising has led to temperature increase together with more frequent and intense drought events (Astigarraga et al., 2020). Numerous negative effects have already been reported to potentially affect biodiversity, for instance species distributional shift, phenological changes, and variation in population dynamics (Bellard et al., 2012). However, not all organisms are affected in the same way: different ecological and physiological characteristics determine their sensitivity to warming (Pörtner and Farrell, 2008; Paaijmans et al., 2013). Lichens, for example, being poikilohydric organisms, are not able to regulate their water content, which tends to reflect external conditions (Proctor & Tuba, 2002). Consequently, both water availability and temperature are fundamental in determining the duration of their hydrated periods, influencing their physiology and, as a consequence, their growth (Gauslaa, 2014). In general, lichen ecophysiological performance depends on external environmental conditions that regulate their photosynthetic and respiratory activities (Green & Lange, 1995; Green et al., 2008). This strong dependence on external climatic factors makes them susceptible to climate change (Nash & Olafsen, 1995). This effect is exacerbated in the Mediterranean region, where a reduction of precipitation and warming are expected (Giorgi & Lionello, 2008), thus determining a climate change hotspot (Tuel & Eltahir, 2020).

Lichens cannot actively control water loss but they can recover their metabolic activity at low water potentials, through water vapor uptake or liquid water input via dew and fog (Gauslaa, 2014). In this framework, identification of key water-related traits in lichens could help determine their susceptibility to environmental factors mainly related to water uptake and loss (Phinney et al., 2018; Phinney, 2019). Water-related traits are involved in the duration of hydrated periods and mediate the response to different sources of water (Gauslaa & Coxson, 2011; Gauslaa, 2014; Di Nuzzo et al., 2022a). A high intraspecific variability has been observed in such traits, reflecting their fundamental role in acclimation to local conditions of water availability (Merinero et al., 2014; Longinotti et al., 2017; Wan & Ellis, 2020). Increasing specific thallus mass (STM), for example, which strongly drives water holding capacity (WHC), has been observed to increase in dry and high light conditions or in the drier part of the year (Larsson et al., 2012). Intraspecific variability in STM and WHC is also associated with thallus size and age (Merinero et al., 2014), leading to different ecological requirements at different life stages as observed, for example, for *Lobaria pulmonaria* (L.) Hoffm. (Benesperi et al., 2018; Ignatenko et al., 2020). The model species *L. pulmonaria* is a foliose green-algal lichen (Figure 4.1) with cyanobacteria in small internal cephalodia. It is mainly restricted to humid old-growth forests characterized by ecological continuity, and it is often associated with other rare species. During the last centuries, its presence has declined due to forest management and air pollution (Scheidegger & Werth, 2009; Paoli et al., 2019). This negative trend is expected to be worsened by both direct and indirect effects of climate change. Directly, by a reduction of its climatic suitability in the geographical space (Nascimbene et al., 2016) and indirectly through climatic-induced loss of suitable substrates, such as host trees (Nascimbene et al., 2020). Thus, different environmental filters will shape the adult population, to such an extent that even the fine-scale location of the juvenile thalli may determine their survival and development success (Benesperi et al., 2018). It is, therefore, crucial to consider population structure and development stages when testing the effect of climate change on lichens (Benesperi et al., 2018; Bianchi et al., 2020; Di Nuzzo et al., 2022b).

Predicting species responses to climate change can be a challenging task, yet it is fundamental to provide effective management policies to counteract the negative effects of rising temperature (Bellard et al., 2012). Correlative models use the currently recognized niche of a species, comparing it with future climatic conditions (Pacifici et al., 2015). By contrast, mechanistic simulation models can be valuable complementary tools because they allow projections to be made in time and space by analyzing underlying physiological processes that lead to the observed effects of climate change on organisms (Pacifici et al., 2015). Mechanistic modeling through detailed small-scale information offers the possibility to define niche estimates to reflect the specific niche characteristics of locally adapted individuals (Peterson et al., 2015). Moreover, this approach allows for accounting for both population structure and possible differences in ecological requirements at different life stages. So far, this has mostly been done for vascular vegetation, such as forest stands, for instance (Fisher et al., 2018). As mechanistic models are often more time-consuming in their development and require a large amount of observational data for evaluation, in ecology, they are less used than correlative models. One of the advantages that we want to highlight of a mechanistic model, is the possibility of switching processes on and off, and examining them separately, to gain an understanding of their role and relevance. Such a modeling approach indeed requires making basic assumptions

Figure 4.1: Life stages of *Lobaria pulmonaria* as considered in this study: **(A)** adult thallus with downward growing lobes that tend to overlap leaving an air space between the different levels; **(B)** juvenile thalli at the beginning of development. The differentiation between ascending and descending lobes is still in the early stages, but the lobes are not yet overlapping; **(C)** adult thallus in the dehydrated state with convoluted apex of the lobes; **(D)** detail of an adult thallus in the hydrated state in a natural situation after heavy rain. The accumulation of external water favored by the spoon-shaped conformation of the lobes is visible.

that facilitate the link between environmental conditions, available resources, and ecological interactions (Pontarp et al., 2019).

Regarding lichens, previous studies mainly modeled ecological niches and patterns taking into account adult thalli (Nascimbene et al., 2020; Ellis & Eaton, 2021; Vallese et al., 2021). Thus, less is known on the effects of climatic conditions on different life stages in lichens. Moreover, different ecological requirements at different life stages are fundamental to determining the survival of a population. Some steps of the life cycle are more affected by climatic conditions and are extremely critical for population development, for instance, dispersion and establishment of propagules. In fact, some traits of the organisms could change during development. For example, the WHC could be different at different life cycle points determining a non-homogeneous probability of occurrence of a thallus along the whole life cycle.

This study aims at testing the hypothesis that adult *Lobaria pulmonaria* thalli better survive under given climatic conditions than juveniles, due to slower water loss rate related to higher aerodynamic resistance. This advantage may overcompensate the benefit of a low STM in juveniles, which means that juveniles may require less carbon assimilation to achieve the same given relative growth rate (RGR).

We tested this hypothesis using the process-based non-vascular vegetation model LiBry (Porada et al., 2013). The LiBry model accounts for the productivity of lichens in many physiological strategies under a broad range of environmental conditions (Porada et al., 2013).

4.2 Materials & Methods

4.2.1 Study area and sampling design

For the purpose of this work, we have established some operational definitions, which are described below:

- Region: each of the 9 ERA5 grid cells located in different areas of Italy and selected for this study. Each region contains a number of sites ranging from 1 to 14, depending on the local abundance of *L. pulmonaria*.

- Sites: areas within the regions where biological populations of *L. pulmonaria* are present.
- Plot: each of the 30 × 30 m areas located within each site and used to statistically select samples of *L. pulmonaria*.
- Population: the biological population of *L. pulmonaria* present at each of the selected sites.

Sites were selected randomly among all localities known to host *L. pulmonaria* in Italy and Corsica (Nascimbene et al., 2016). The selection was done trying to represent all different ecological conditions (climate, substrata, habitat) in which *L. pulmonaria* is present in Mediterranean environments. We selected 50 sites in 9 regions across Italy and Corsica (Figure 4.2). In accordance with Benesperi et al. (2018), we selected 2 life stages of the thalli: (i) juvenile thalli (with an area ≤ 1 cm^2, lacking sexual or vegetative diaspores) and (ii) adult thalli (with sexual or vegetative diaspores). In each site, we randomly selected a 30 × 30 m plot in which at least 3 trees with a minimum of 10 adult thalli of *L. pulmonaria* occurred. In each plot, the number of adult thalli was counted. We considered a thallus an individual if it was completely separated from the closest thallus ($>$ 2 cm). We then randomly collected 5–15 lobes, proportionally to the overall number of adult thalli. To avoid damage to *L. pulmonaria* populations, we collected only the last 5 cm of each lobe. In order to have a variable range of measurements without harming the population, we also collected 4–5 samples of juvenile thalli of *L. pulmonaria* in a subset of populations where it was possible (i.e., where the abundance of juveniles was higher enough so that the collection would not cause a threat to the population).

4.2.2 Water-related traits

The measurement of water-related traits was performed according to Longinotti et al. (2017). The upper surface of collected thalli was sprayed with deionized water reaching full hydration, i.e., continuing spraying would not induce wet mass gain. The water mass (WM) was then measured in three subsequent steps. Thalli were

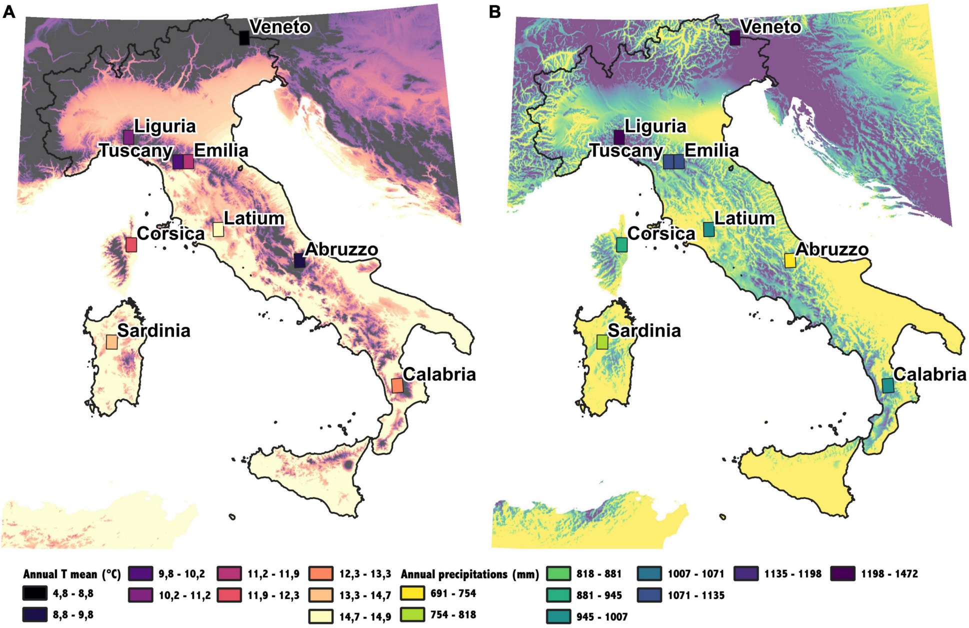


Figure 4.2: Climatic conditions in the studied area. Annual mean temperature **(A)** and Annual precipitation **(B)**. The rectangles indicate ERA5 pixels used for each site, the color inside rectangles is the mean value of temperature and precipitation.

measured when (i) fully water-saturated (WM_{max}), (ii) after being gently shaken until no more drops were falling ($WM_{shaking}$), and after being blotted with dry filter paper ($WM_{blotting}$). The hydrated thalli were then scanned with a Canon i-SENSYS MF4320d (Canon Inc., Tokyo, Japan). Images obtained from scansion were processed digitizing the thallus outline using Photoshop CS6 Extended (Adobe Systems, San Jose, CA, USA) to measure the projected area (A_{wet}). Thalli were dried at environmental conditions and weighted to measure dry mass (DM).

STM was calculated as DM/A_{wet}. In order to calculate WHCmax, $WHC_{shaking}$ and $WM_{blotting}$ we used respectively, WM_{max}, $WM_{shaking}$ and $WM_{blotting}$ as WM in the equation (WM-DM)/A_{wet}. Finally, we calculated the external WHC (WHC_{ext}) as $WHC_{shaking}$ -$WHC_{blotting}$, while WHC internal (WHC_{int}) was assumed to be equal to $WHC_{blotting}$. Moreover, we calculated percent water content (WC) as (WM-DM)/DM both after shaking and blotting.

Thallus thickness was estimated by 3 measures on a thin section of the thallus under a standard light microscope.

For a subset of lobes, we measured the $\mathrm{WM}_{\mathrm{blotting}}$ loss halftime (T50). All lobes were hydrated for 24 h in distilled water. Lobes were then left dehydrated at air temperature and humidity and weighted with a time interval of 5 min on an analytical scale until the desiccation at room temperature was reached. The evaporation process could be described as,

$$\frac{dRWC\%}{dt} = -k_{wl}t_{\frac{1}{2}}$$

Where k_{wl} is water loss rate $\mathrm{t}_{\frac{1}{2}}$ and water loss halftime. Samples k_{wl} and $\mathrm{t}_{\frac{1}{2}}$ were calculated following the exponential function which better approximates the experimental points. The dehydration curve was constructed interpolating 10 subsequent weights.

4.2.3 The model: LiBry

To test our hypothesis, we apply here a mechanistic computer simulation model of lichens, bryophytes, terrestrial algae, and cyanobacteria, called LiBry. This model was developed as a dynamic global vegetation model (DGVM) which, in contrast to most other DGVMs, focuses on non-vascular vegetation, while trees, shrubs, and grasses are only included as a relatively static environment which influences the growth of non-vascular organisms (Porada et al., 2013). The original purpose of the LiBry model was to assess the impact of non-vascular vegetation on global biogeochemical cycles, such as cycles of carbon, water, and nitrogen, and it has been applied to various research questions in this regard (Porada et al., 2013, 2014, 2018, 2019).

The model, however, is not limited to biogeochemical processes alone, it also accounts for factors that shape the physiological diversity of non-vascular communities. Hence, impacts of environmental factors, such as climate, on community composition or on individual species can be assessed using LiBry (e.g., Porada et al., 2019; Baldauf et al., 2021). This is possible through the explicit representation of the large physiological and morphological diversity of non-vascular communities in the model.

To this end, LiBry simulates a large number (thousands) of different strategies at the same time in a given location. Each of these strategies is characterized by a unique combination of values of 11 physiological and morphological traits. This means that real non-vascular species can be described by the model to the level of the phenotype. Thereby, one strategy may match individuals from more than one species in the real world, since these may be highly similar in their trait values. Moreover, individuals from the same species may correspond to different strategies in the model, in case of high intra-specific variation in trait values. The traits which are considered in LiBry include STM, WHC, thallus thickness, optimum temperature of photosynthesis, or photosynthetic capacity.

At the beginning of a LiBry simulation, the ranges of possible trait values, which are based on literature, are randomly sampled to create a set of initial physiological strategies (see Porada et al., 2013 for details). Subsequently, the long-term carbon balance of each strategy under given climatic conditions is computed by the model. Differences in trait values usually result in a divergence of the carbon balance values of the simulated non-vascular community at a given location. Consequently, the carbon balance is used as a selection criterion for the success of each strategy: Those strategies which show a negative carbon balance in the long term are removed from the simulation, and the remaining ones are weighted according to a scheme, which is based on growth and other properties, and which determines their relative abundance and, thus, the community composition at the respective location. It should be noted that WHC values reported here ($\mathrm{WHC}_{\mathrm{Libry}}$) do not correspond to the WHC of a single individual, but represent the community mean WHC weighted by relative abundances of the simulated strategies. Hence, to compare $\mathrm{WHC}_{\mathrm{Libry}}$ to observations, the measured samples should be representative of the entire community in a given location.

The biomass of each strategy in the LiBry model corresponds to their accumulated long-term carbon balance. Biomass dynamics depend on Net Primary Production (NPP) and mortality, where the latter is based on tissue turnover and disturbance. Moreover, biomass translates into the relative cover fraction of a strategy in the community, depending on its STM. Thereby, the RGR of the cover of a strategy corresponds to the difference between NPP and tissue turnover. In a steady state of the cover, the RGR is balanced by disturbance. It should be pointed out that RGR is a spatially averaged property that integrates processes such as dispersal and establishment. It cannot be compared to the growth rate of an individual thallus, it is rather the average expansion of a population of thalli which share the same trait values. NPP is computed as the difference between photosynthesis and respiration. The former is estimated as a function of light, CO_2, and temperature according to Farquhar and von Caemmerer (1982), and the latter is derived from a Q10-relation to temperature. While these basic processes are similar in vascular and non-vascular organisms, the LiBry model also explicitly accounts for physiological properties which only occur in non-vascular vegetation. These include poikilohydry, which means a lack of active control on water loss, the exchange of water via the thallus surface, due to the absence of roots and stomata, the ability to deactivate metabolism upon desiccation, and the reduction of CO_2 diffusion at high water saturation of the thallus. Thereby, simulated metabolic activity of the strategies in LiBry is based on the (dark) respiration rate which increases with water saturation. Usually, full activity is reached before the thallus is saturated with water.

Furthermore, several physiological processes in LiBry are connected via trade-offs, which are characteristic for non-vascular organisms and which have a large influence on carbon balance, and, consequently, success in the simulated selection. These trade-offs include a positive correlation between photosynthetic capacity and specific respiration rate, or the negative correlation between metabolic activity and CO_2 diffusivity, mediated by water content (see Porada et al., 2013; and Porada & Giordani, 2021 for a more detailed description).

4.2.4 Adaptation of LiBry to *Lobaria pulmonaria*

To represent both adult and juvenile thalli of *L. pulmonaria* in the LiBry model, the representation of (a) water pools in the lichen thallus and (b) the morphology of the thallus was extended for this study, compared to the latest published LiBry version (Porada & Giordani, 2021). Furthermore, the initial strategies in LiBry were

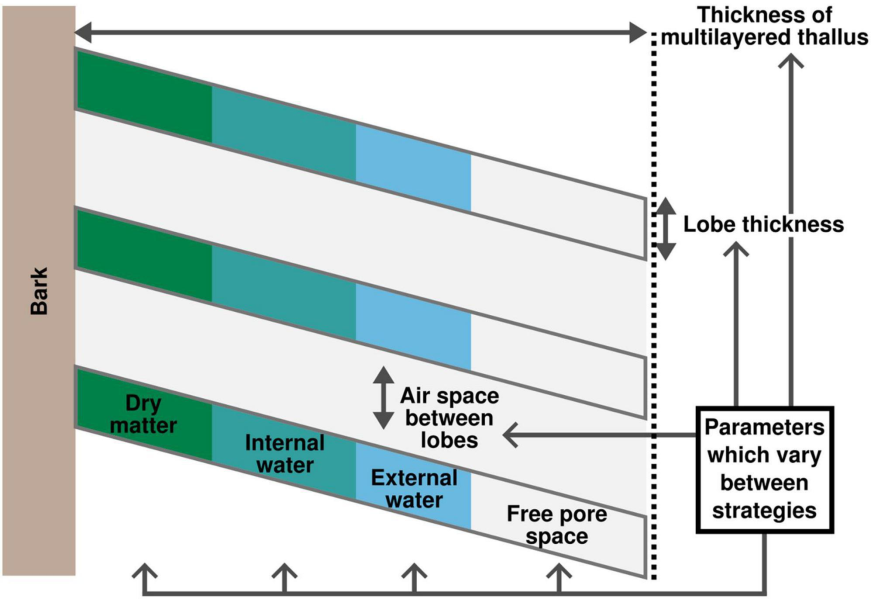


Figure 4.3: Overview on the morphological representation of *L. pulmonaria*: in the LiBry model. The individual morphological properties (fractions of dry matter, internal/external water, air in the free pore space inside lobes and air between lobes, lobe thickness, and total thallus height) vary between the strategies in the model. The ratio of dry matter to internal water, however, is constant across strategies.

constrained by observational data.

In LiBry, water in any part of the thallus can be stored in two pools: (1) inside the cells (internal), which expand upon water uptake, or (2) between the cells (external), either in the pore space or attached to the surface of the thallus (see Figure 4.3). The remainder of the thallus part is filled with air. Under natural conditions, a certain fraction of the thallus (free pore space) always remains air-filled due to either hydrophobicity or pore size effects. In lichens the free pore space usually corresponds to parts of the medulla. Since LiBry simulates a large variety of different physiological strategies, the relative fractions of dry matter of the cells, storage capacities for internal and external water, and free pore space vary largely between the strategies with which the model is initialized. Thereby, however, a fixed relation between the amount of cell dry matter and the amount of internal water is assumed, which means that the cells are limited in their capacity to expand. This is supported by various studies on the relation of STM to $\mathrm{WHC_{int}}$ (e.g., Gauslaa and Arsenault, 2020) (see Figure 4.4). Furthermore, in the LiBry version that is applied here, the transition point to full metabolic activity corresponds to the saturation of the internal water pool. Once all cells are fully turgid, activity is assumed to remain constant.

To account for the characteristic lobe structure of *L. pulmonaria*, an additional scaling relation was introduced in LiBry, which prescribes a minimum of air space between lobes, and which leads to relatively more air space in multilayered thalli with a higher thickness. Furthermore, thicker thalli are slightly more efficient in storing water in this model version due to the more complex surface structure (Figure 4.3).

Several further constraints were applied to LiBry in order to represent *L. pulmonaria*: First, the value of the fixed ratio between cell dry matter and internal water was set similar to that found for *L. pulmonaria* by Gauslaa et al. (2021). It was, however, adapted subsequently to observations from the sites used in this study (see Figure 4.2 and Table 4.1). Thereby, adult thalli have a higher fraction of $\mathrm{WHC_{int}}$ compared to juvenile thalli for the same STM. This is due to the prescribed lower efficiency of water storage per STM for smaller thalli in the model, which was motivated by the observed lower ratio of internal to $\mathrm{WHC_{ext}}$ in juvenile thalli (Table 4.1). In this simplified scheme, it is possible that juvenile thalli have STM values similar to adult thalli in the LiBry model, since a small strategy (low overall thickness) may have a high fraction of dry matter, and a tall strategy a substantially lower dry matter fraction. While it is likely that juvenile thalli have a lower STM on average than adult ones, the low number of measurements of STM of juveniles does not allow for a definite distinction between juveniles and adults in this regard. To test the implications of this simplification, we run a sensitivity analysis (see below).

Secondly, the thickness of the lobes (Figure 4.3) was constrained to the range of 200-1,000 μm, based on observations from the sites (Table 4.1), and the maximum thickness of the total multilayered thallus, meaning the distance between the bark surface and the outer tips of the lobes, was set to 10 cm, which means that all strategies differed in their thickness, but could not exceed 10 cm. Finally, initial strategies which had an STM outside the range of 4–40 $\mathrm{mg/cm^2}$ were excluded from the model, and those strategies which had a ratio of external/internal WHC outside the range of 0.21–2.7 were excluded, too. These ranges were based on median values of observations from the sites, using half the minimum and twice the maximum value, except for max. STM, which would have increased the average STM of the initial strategies too strongly.

According to our hypothesis (see above), the taller structure of adult thalli may lead to an increased resistance against evaporation of water compared to juvenile thalli. This was tested and confirmed in the laboratory using a subset of sampled *L. pulmonaria* thalli. Subsequently, we used the evaporation scheme built into the LiBry model to reproduce the observed drying dynamics. For the adult and juvenile thalli, we set WHC to 14 and 7.6 mg/cm^2, respectively, and the observed T50 time was 24 min for adults and 12 min for juvenile thalli (median values). Environmental conditions for the evaporation model were set to those of the laboratory (20°C and 60% RH, and the surface resistance of the thalli was used as the calibration parameter to match the observed difference in T50 time.

It was found that the difference in WHC alone could not explain the longer T50 time of adult thalli. Instead, it was required to set the resistance of the juvenile thalli to 0.3 times that of the adult thalli, all other conditions being equal, to match the observations. Therefore, we then used the thallus height Z of a strategy in the LiBry model to define its surface resistance, prescribing an exponential scaling from the minimum height z_{MIN} of 5 mm to the maximum z_{MAX} of 10 cm:

$$r_s = \frac{log(\frac{Z}{Z_{MIN}})}{log(\frac{Z_{MAX}}{Z_{MIN}})} r$$

with $r_{\mathrm{S\_MAX}} = 200$ s/m. This simple relation resulted in median values of the juvenile initial strategies which were roughly 0.3 times that of the adult initial strategies.

4.2.5 Simulation setup

To differentiate between adult and juvenile individuals of *L. pulmonaria* strategies in the LiBry model, we used only the thickness of the multilayered thallus. Individuals which were smaller than 1 cm were assigned to the juvenile group, and the remainder to the adult group. Figure 4.5 shows the distributions of STM, WHC$_{\mathrm{int}}$, and the

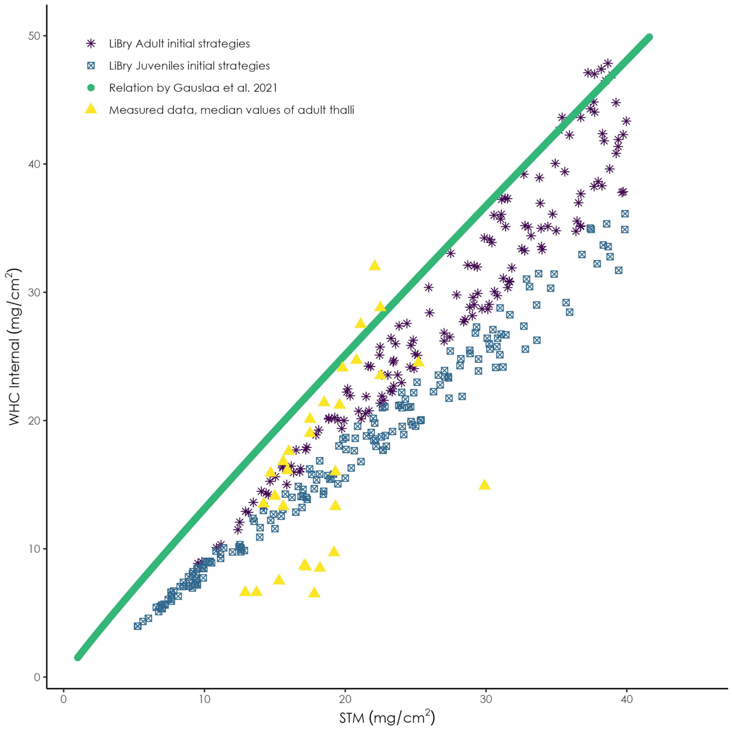


Figure 4.4: Relation between STM and internal WHC for the initial adult and juvenile strategies in the LiBry model and of measured adult thalli. The scatter in the strategies results from the free variation of several morphological parameters in the model, such as thallus height and the relative fractions of water and air in different parts of the thallus.

$WHC_{ext/int}$ ratio for the initial strategies in the LiBry model, and also for the observational data from Table 4.1.

Two simulations were run with the LiBry model, which only differed with regard to the group of initial strategies (juvenile vs. adult). The simulations were carried out for 9 regions across Italy (Figure 4.2) and the model was driven by climate data (years 1979–2019) from the ERA5 data set (Hersbach et al., 2020). Those grid cells were cut out from the global data set which corresponds roughly to the center of the individual sites in the 9 respective regions.

Leaf area index was derived from site observations, by imposing a seasonal variation (monthly resolution) on median LAI values for each region. The extent

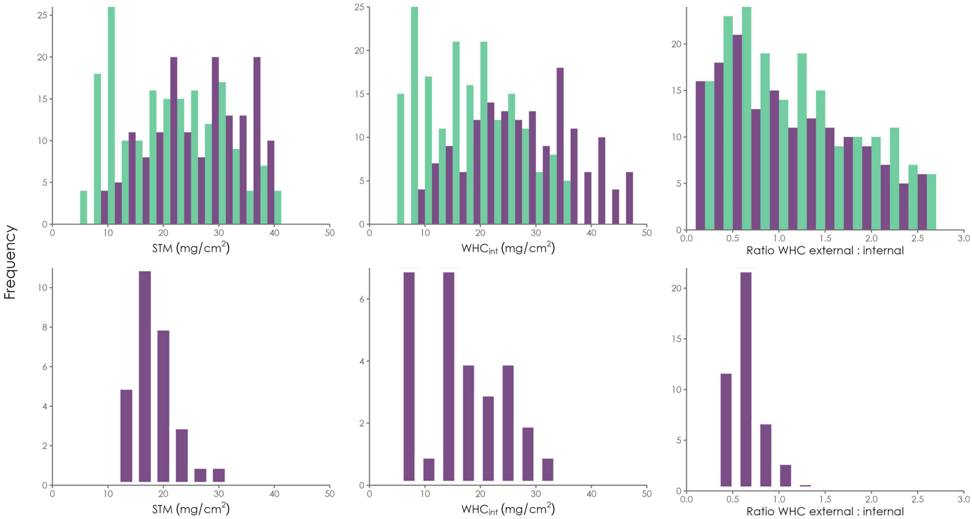


Figure 4.5: The initial LiBry strategies are separated into juvenile thalli (light green) and adult ones (dark purple). Frequency distributions of STM, internal WHC, and the ratio of external to internal WHC, both of the initial strategies in the LiBry model (Upper row), and also of the observed adult thalli (Lower row) of which only median values are shown.

of the variation was based on a previous study carried out in Sardinia (Porada & Giordani, 2021). Stem area index was set to a constant value of 0.1, also based on the study by Porada & Giordani (2021). Note that the SAI in the LiBry model is used to obtain correct estimates for ecosystem level extensive properties, such as biomass or productivity, since per-area growth needs to be scaled up spatially. Since we focus in this study on differences in the energy and water balance between juvenile and adult individuals of *L. pulmonaria*, the exact area which is available for growth on the stems is not of crucial relevance here.

The simulations were run using 3,000 initial strategies, out of which 632 remained after constraining their properties to characteristics of *L. pulmonaria*. The juvenile and adult groups consisted of 359 and 273 strategies, respectively. The simulation was run for 300 years to ensure equilibrium with regard to community composition, thereby repeating the 41-year climate data. At the end of each simulation, the number of surviving strategies and their community-averaged properties were analyzed. Thereby, all strategies were assigned equal weights in the averaging. Finally, a sensitivity analysis was carried out to test the effects of different uncertain morphological and physiological properties and environmental conditions on the outcome of our study. This run is carried out for one site only with the same setup as used in a preceding publication (Porada & Giordani, 2021), but applying the new model version.

We want to point out here that LiBry is a deterministic model, which means that each of the strategies is simulated as an individual, consisting of dynamic pools of carbon and water, which are changing based on the state of the individual and the driving environmental conditions. Hence, for the same set of initial strategies and climate data, the model will always calculate the same output values. When comparing differences between the simulations for adult and juvenile strategies, it is thus not appropriate to test if these differences are significant, since the model estimates cannot be interpreted as draws from a statistical population. It is rather the generation of the initial strategies itself which corresponds to statistical sampling. Here, it is important to test if the sample size of strategies is large enough to ensure consistent results. This is done in the last part of our sensitivity analysis.

Region	**STM_adult (mg/cm^2)**	**WHC$_{int}$_adult (mg/cm^2)**	**Ratio WCext/int adult**	**Ratio WCext/int recruit**	**Thallus thickness adult (µm)**	**Thallus thickness recruit (µm)**
Abruzzo [1]	20.8	24.7	0.55	-	500	-
Calabria [14]	18.2	16.0	0.69	-	490	-
Corsica [2]	18.4	20.4	0.64	-	536	-
Emilia Romagna [2]	22.3	30.4	0.62	-	650	-
Liguria [10]	14.8	11.1	0.64	0.81	602	325
Sardegna [14]	13.8	14.9	0.67	0.79	455	340
Toscana [5]	19.1	23.5	0.51	-	582	-
Veneto [2]	14.7	14.5	0.84		507	-

Table 4.1: Median of measured values for each region. In brackets the number of sample populations in each region.

4.3 Results

4.3.1 Simulation output

The results of our simulations with the LiBry model confirmed our overall hypothesis. Adult strategies of *L. pulmonaria* had a 32% higher survival rate than juveniles (comparison of the median values of all regions, see also Figure 4.6). Adult strategies

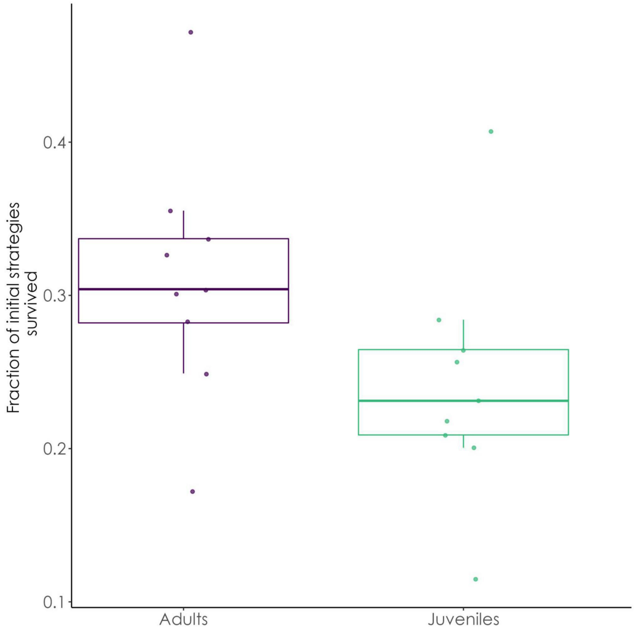


Figure 4.6: Boxplots of the fraction of initial strategies which survived the simulation, adult (Left) vs. juvenile thalli (Right). The boxplots contain 9 values each, corresponding to the different regions of Italy analyzed here.

show a higher fraction of active time compared to juveniles (Act.Frac.). They also have a higher ($\mathrm{WHC_{LiBry}}$), and a higher RGR, see also Table 4.4.

When the 9 regions are sorted along climatic gradients, certain patterns in the ratios of survival rates and physiological functioning of adult vs. juvenile strategies can be noted (Figure 4.7 top panels). Increasing total amount of rainfall (range: 691–1,472 mm/a) does not seem to result in a clear pattern in the ratios of survival rate or the physiological functions (WHC, Act.Frac., RGR), and also absolute survival rates of adults and juveniles show no clear trend (Figure 4.7 middle panels). Instead, survival rates seem to be lowest at intermediate amounts of rainfall. Increasing mean air temperature (range: 4.8–14.9°C), however, seems to lead to an increase in the relative survival rate of the adult strategies compared to juvenile ones, and to a decrease in the difference in WHC. RGR seems to be connected to survival rate, while active time fraction does not show a clear pattern or a strong correlation to other ratios. For high air temperature, absolute survival rates show a decrease for

		Adult	Recruit
Control			
	%S	20.9	15.6
	WHC (mg/cm^2)	39.7	25
	Act.Frac.	0.83	0.78
	RGR[1/a]	0.23	0.18
(1) No difference in surface resistance			
	%S	24.2	21.5
	WHC (mg/cm^2)	37.6	24.4
	Act.Frac.	0.87	0.84
	RGR[1/a]	0.27	0.26
(2) With resaturation respiration			
	%S	17.6	11.4
	WHC (mg/cm^2)	39.7	27.9
	Act.Frac.	0.83	0.78
	RGR[1/a]	0.23	0.15
(3) With bark water supply?			
	%S	24.5	23.1
	WHC (mg/cm^2)	36.9	23
	Act.Frac.	0.9	0.85
	RGR[1/a]	0.34	0.42
(4) With constrained dry matter fraction			
	%S	21.5	18.8
	WHC (mg/cm^2)	39.7	16.7
	Act.Frac.	0.83	0.78
	RGR[1/a]	0.24	0.18

Table 4.2: Summary of sensitivity analysis

both adult and juvenile thalli. Moreover, it should be pointed out that the ratio of adult to juvenile survival is always larger than one, which means that the simulated advantage of adult thalli is consistent throughout the study region.

	Control	**#2**	**#3**	**#4**	**#5**
Adult	20.9	23.7	26.2	23.1	24.8
Recruit	15.6	13.5	14.8	15.4	14.3
ratio A:B	1.34	1.76	1.77	1.5	1.73

Table 4.3: Percentage of surviving strategies for 5 different drawings of 3,000 initial strategies from the pool of potential strategies, and the ratio of adult: juvenile survival.

When considering adult and juvenile thalli separately, and comparing the shift in their mean properties (due to the selection in the LiBry model), it becomes clear that the ratio of external to internal WHC in juveniles exhibits the strongest shift from initial to final mean properties (Figure 4.7 bottom panels). This means that juvenile strategies are selected for higher external: internal WHC ratio during the simulation, but not adult thalli. Another consistent pattern is the selection for slightly higher resistance against evaporation in adult thalli, but not in juveniles. Other properties, such as STM and $\mathrm{WHC}_{\mathrm{Libry}}$, show a slight decrease in the mean values, but no clear pattern along the climatic gradients. Another feature seems to be the increase in $\mathrm{WHC}_{\mathrm{Libry}}$ for juveniles in regions with the lowest survival rates.

4.3.2 Sensitivity analysis

The sensitivity analysis confirmed our assumptions about the direction of the impacts of physiological and environmental properties on the survival of strategies in the LiBry model:

1. When the physiological disadvantage of juvenile strategies due to the reduced resistance against evaporation is switched off in the model, and the maximum resistance value $\mathrm{r}_{\mathrm{S\_MAX}}$ is used for every strategy, the survival rates increase overall and the adult strategies have only a 13% higher survival rate than the juvenile ones. This suggests that more than half of the advantage in the survival of adult vs. juvenile thalli depends on their higher resistance against water loss, and the remainder may be due to their higher WHC per area.
2. It has been observed that lichens show a burst of respiration upon rewetting from the dry state, with magnitude varying between individuals and species (Smith & Molesworth, 1973). In the standard version of LiBry, this resaturation respiration is not included, due to a lack of data on potential trade-offs which may explain the variation in the magnitude of respiration. Hence, we here test the effect of a simple parameterization of resaturation respiration, where a 50% increase in respiration is assumed for 2 h after each rewetting event. As expected, overall survival rates declined compared to the control run. Moreover,

the advantage of the adult strategies increases and they show 54% more survival than the juvenile ones.

3. It has been shown that the bark may act as an important water reservoir for epiphytic lichens (Porada & Giordani, 2021), and utilizing a bark water reservoir in the LiBry model increases survival rates, as expected. The conditions improve to an extent that almost removes the difference between adult and juvenile strategies (6
4. We additionally constrained the dry matter fraction in the model to 5% or less. In this way, juvenile thalli cannot compensate their lower stature (less than 1 cm in the model) by a high fraction of dry matter in the thallus, and thus automatically have a lower STM on average. We thereby account for a potential systematic lower STM of juveniles thalli which is not captured by our simplified model. The modified set of initial strategies does not change substantially the outcome of our simulation (see Tables 4.3.1, 4.3.1). The WHC of the juveniles is lower, which can be explained by the now prescribed low amount of dry matter that stores water for each strategy. Also, the survival rate of the juveniles is slightly higher now, since those strategies with high STM values were already removed in the initialization of the model, due to the new constraint. In the control version, the average STM of juveniles declined only during the simulation due to selection, leading to fewer survivors compared to the number of initial strategies.
5. The advantage of adult thalli compared to juvenile ones regarding survival rate is consistent across 5 drawings of 3,000 initial random strategies in the LiBry model, which suggests that this effect is not a random result due to insufficient sample size.

4.4 Discussion

The results of our simulations depict for the first time a different survival probability of life stages of *L. pulmonaria*. The LiBry model highlights a consistently higher

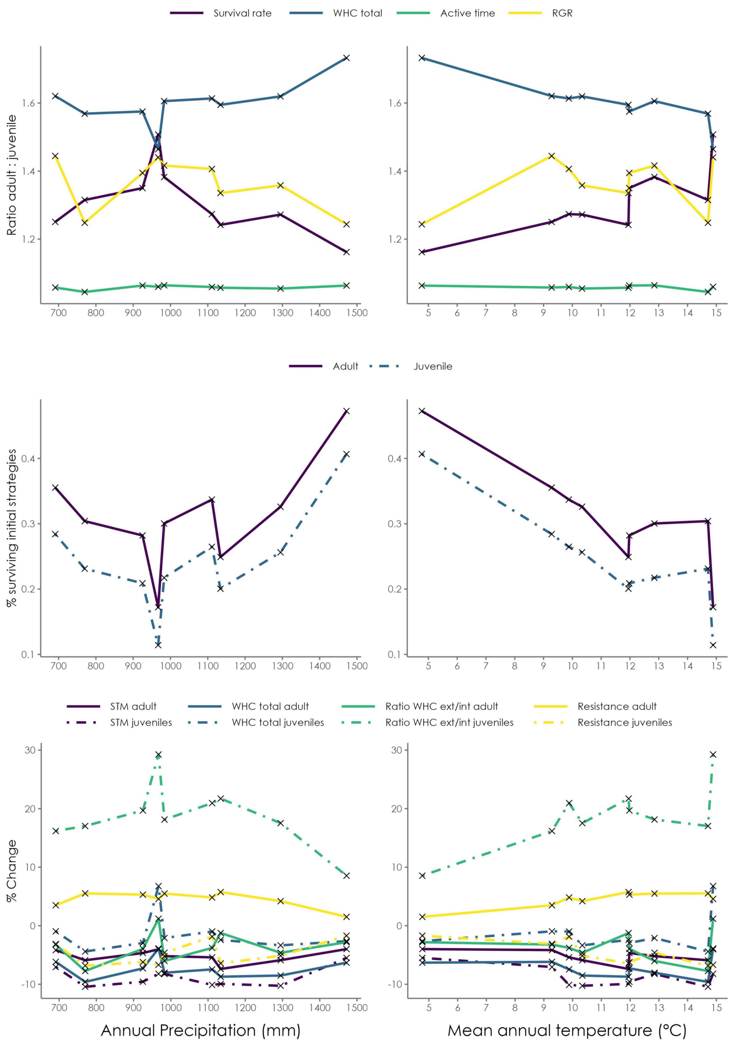


Figure 4.7: **(Top)** Simulated ratios of adult to juvenile survival rate (purple), Water holding capacity—WHC$_{\text{Libry}}$ (blue), Active fraction—Act.Frac. (yellow) and Relative Growth Rate—RGR (yellow) for the 9 regions in Italy examined here. The climate variable increases from left to right, total rainfall (Left), and average air temperature (Right). **(Middle)** survival rates of adult (purple) and juvenile (blue) thalli. **(Bottom)** Shift in the community mean values of four hydration traits from the initial strategies to those surviving at the end of the simulation; STM (purple), WHC$_{\text{Libry}}$ (blue), the ratio of external to internal WHC (green), and resistance against evaporation (yellow) are shown separately for adult thalli (solid) and juvenile ones (dashed).

	Adult	**Recruit**
%S	30.4	23.1
WHC (mg/cm^2)	56.564	35.470
Act.Frac.	0.68	0.64
RGR[1/a]	0.33	0.23

Table 4.4: Median values of the 9 simulated regions. %S corresponds to the percentage of surviving strategies at the end of the simulation, Act.Frac., corresponds to the fraction of hours of the simulation in which the strategies showed metabolic activity. RGR corresponds to the relative growth rate per year at the end of the simulation.

probability of adult thalli to survive and more active time. Our simulations set the basis for a better understanding of how climatic gradients affect different age stages of *L. pulmonaria*, revealing complex population dynamics. *L. pulmonaria* is known to be influenced by both spatial (Eaton & Ellis, 2012) and seasonal climatic gradients (Larsson et al., 2012). More oceanic climates seem to enhance biomass gain and reduce the age-at-reproduction (Eaton & Ellis, 2014 and references therein). Nevertheless, also microclimatic and microhabitat conditions, nested inside macroclimatic gradients, influence the growth rate and the overall survival of *L. pulmonaria* populations (Benesperi et al., 2018; Ellis, 2020; Di Nuzzo et al., 2022b). For example, the same distance from watercourses in two different areas could lead to different growth rates (Ellis, 2020).

A consistent outcome of the simulation of *L. pulmonaria* along climatic gradients is that the advantage of adult thalli compared to juvenile ones, regarding survival, increases with decreasing absolute survival rates of both life stages, which can be interpreted as stressful conditions. It is interesting that adult and juvenile thalli seem to become more similar with respect to $\mathrm{WHC_{Libry}}$ under these conditions (Figure 4.7 top panels). A more detailed analysis shows that the relative increase in $\mathrm{WHC_{Libry}}$ in juvenile thalli compared to adult ones seems to be accompanied by a selection of juvenile strategies toward higher ratios of external to internal WHC (Figure 4.7 bottom panels). This increase in external WHC can be interpreted as a means to maintain active time and, thus, growth under stressful conditions, at lower carbon cost facilitated by a lower fraction of dry matter (which is related to internal WHC). Alternatively, the reduced fraction of internal WHC compared to external one may be driven by the advantage of earlier activation, since only internal WHC is relevant for the level of metabolic activity in LiBry. Usually, the increased external WHC would also have a negative effect on growth via the associated decrease in CO_2 diffusivity (Lange & Green, 1996; Lange et al., 1999). However, it could be possible that this effect is not relevant in warm and dry climatic conditions which seldom lead to full saturation, or where evaporation rapidly decreases the external water. In contrast to juvenile strategies, adults do not show a shift toward higher WHC, but they instead have slightly increased their resistance against evaporation. A plausible explanation for these contrasting patterns is the limitation of juvenile thalli to a height of 1 cm in the model. Since we parameterized resistance against evaporation as a function of height, juvenile strategies are constrained in their ability to increase surface resistance. The lack of simulated increase in external: internal WHC ratio in adult thalli as an additional means to increase active time may be explained by their larger overall WHC. Due to this, periods of higher water saturation after rainfall may be prolonged compared to juveniles and they may be more often limited by CO_2 diffusion under these conditions if water is stored externally. This may also explain why the external: internal ratio only increases at high temperature for adult thalli, since they may be more frequently dry then. Merinero et al. (2014) demonstrated that the preferred source of water between younger and adult thalli shift from air humidity to liquid water. The increment of STM in adult thalli increases the amount of water necessary to achieve full saturation, making them more dependent on liquid water. At the same time, thicker thalli could prolong the hydrated periods prolonging the photosynthetic activity.

Finally, the slight decrease in STM and WHC_{Libry} for both adult and juvenile strategies may be explained by the general disadvantage of high STM under stressful conditions in the model. This is due to the fact that, for the same amount of lateral expansion, a strategy with a high STM has to assimilate more carbon than one with a low STM. Expansion, however, is crucial for survival in LiBry since it needs to compensate for the loss of cover due to disturbance and turnover. In the standard version of LiBry, thallus height, which is associated with high STM under otherwise equal trait values, is connected to a competitive advantage, and thus results in higher relative cover of the surviving tall strategies in the simulated community (Porada et al., 2019). Here, however, we treated all strategies as equally competitive since we focused on ecophysiological processes, and not on potential interactions between different life stages in the same population. The latter process is poorly known, since data on competition between adults and juveniles on single trees are currently not available. We thus wanted to avoid an overestimation of the advantage of adults based on their growth height in the model.

The simulation outputs obtained from LiBry led to two main implications: (1) Warming due to climate change may be harmful to the survival of juvenile thalli. In fact, harsher conditions could exacerbate the difference in terms of survival between adults and juveniles. Moreover, an overall lower active time could lead to delaying the age-at-reproduction, reducing the dispersal capability of such a population. Such effects of warming on *L. pulmonaria* may be studied through forcing LiBry by climate change scenarios. (2) A monitoring of the metabolic activity of *L. pulmonaria* thalli of different ages under field conditions along a climatic gradient, combined with laboratory analysis of their water relationships could be used to evaluate in better detail the findings predicted by the LiBry model. In particular, analysis of mean morphological traits at the community level for different locations along the climate gradient could be used to assess the selection process in LiBry.

The present implementation of LiBry does not account for either intra-and interspecific competition and facilitation processes. Nevertheless, these dynamics could be extremely important over long time periods. In the case of *L. pulmonaria*, interaction with bryophytes could play a facilitation role at an early stage of development, as bryophytes could provide supplementary water enhancing photosynthetic active periods (Benesperi et al., 2018). This is supported by our sensitivity analysis with regard to the bark water reservoir, which may have a similar effect. A possible further application of the LiBry model could be to test the competition and facilitation dynamics at a community level, by setting different interaction schemes and then comparing them to observed community compositions of both lichens and bryophytes. These interactions are probably mediated by different functional traits. For instance, foliose aerohygrophytic chlorolichens, such as *L.*

pulmonaria (Larsson et al., 2012) could benefit from bryophytes species with a growth form that reduces evaporation of water but at the same time could be overgrown by these latter. Crustose lichen species could be outcompeted by fast-growing bryophyte or other lichen species. As demonstrated for Alpine plant communities, complex interactions of facilitation and competition might support higher biodiversity (Losapio et al., 2021).

Populations of *L. pulmonaria* have strongly declined during the last century, mainly due to anthropogenic disturbance. Moreover, future climate change is predicted to worsen and reduce suitable conditions (Nascimbene et al., 2016, 2020). The results discussed here strengthen the importance of management practices focused on the preservation of specific conditions aimed at enhancing the buffer potential of forests (De Frenne et al., 2021). The temperature under the forest canopy could be up to 2 degrees lower compared to free-air temperature. At the same time, forest structure also influences the quantity and time of different types of water sources (Gauslaa, 2014; Di Nuzzo et al., 2022a). Also, topography could play an important role in buffering harsher climatic conditions, for example protecting forests close to watercourses could be important for providing more humidity (Ellis, 2020). In harsher conditions, where juveniles seem to have a lower possibility of survival, preserving forests with favorable temperature and water conditions could enhance the development of larger populations of *L. pulmonaria.*

4.5 Bibliography

Astigarraga, J., Andivia, E., Zavala, M. A., Gazol, A., Cruz-Alonso, V., Vicente-Serrano, S. M., & Ruiz-Benito, P. (2020). Evidence of non-stationary relationships between climate and forest responses: Increased sensitivity to climate change in Iberian forests. *Global Change Biology*, 26(9), 5063-5076.

Baldauf, S., Porada, P., Raggio, J., Maestre, F. T., & Tietjen, B. (2021). Relative humidity predominantly determines long-term biocrust-forming lichen cover in drylands under climate change. *Journal of Ecology*, 109(3), 1370-1385.

Bellard, C., Bertelsmeier, C., Leadley, P., Thuiller, W., & Courchamp, F. (2012). Impacts of climate change on the future of biodiversity. *Ecology letters*, 15(4), 365-377.

Benesperi, R., Nascimbene, J., Lazzaro, L., Bianchi, E., Tepsich, A., Longinotti, S., & Giordani, P. (2018). Successful conservation of the endangered forest lichen *Lobaria pulmonaria* requires knowledge of fine-scale population structure. *Fungal Ecology*, 33, 65-71.

Bianchi, E., Benesperi, R., Brunialti, G., Di Nuzzo, L., Fačkovcová, Z., Frati, L., ... & Paoli, L. (2020). Vitality and growth of the threatened lichen Lobaria pulmonaria (L.) Hoffm. in response to logging and implications for its conservation in Mediterranean oak forests. *Forests*, 11(9), 995.

De Frenne, P., Lenoir, J., Luoto, M., Scheffers, B. R., Zellweger, F., Aalto, J., et al. (2021). Forest microclimates and climate change: Importance, drivers and future research agenda. *Global Change Biology*, 27, 2279–2297.

Di Nuzzo, L., Benesperi, R., Nascimbene, J., Papini, A., Malaspina, P., Incerti, G., et al. (2022a). Little time left. Microrefuges may fail in mitigating the effects of climate change on epiphytic lichens. *Science of The Total Environment*, 825:153943.

Di Nuzzo, L., Giordani, P., Benesperi, R., Brunialti, G., Fačkovcová, Z., Frati, L., et al. (2022b). Microclimatic alteration after logging affects the growth of the endangered lichen *Lobaria pulmonaria. Plants*, 11:295.

Eaton, S., & Ellis, C. J. (2012). Local experimental growth rates respond to macroclimate for the lichen epiphyte *Lobaria pulmonaria. Plant Ecology and Diversity*, 5, 365–372.

Eaton, S., & Ellis, C. J. (2014). High demographic rates of the model epiphyte *Lobaria pulmonaria* in an oceanic hazelwood (western Scotland). *Fungal Ecology*, 11, 60–70.

Ellis, C. J. (2020). Microclimatic refugia in riparian woodland: A climate change adaptation strategy. *Forest Ecology and Management*, 462:118006.

Ellis, C. J., & Eaton, S. (2021). Microclimates hold the key to spatial forest planning under climate change: Cyanolichens in temperate rainforest. *Global Change Biology*, 27, 1915–1926.

Farquhar, G. D., & von Caemmerer, S. (1982). "Modelling of Photosynthetic Response to Environmental Conditions," in *Physiological Plant Ecology II: Water Relations and Carbon Assimilation*, eds O. L. Lange, P. S. Nobel, C. B. Osmond, and H. Ziegler (Berlin: Springer), 549–587.

Fisher, R. A., Koven, C. D., Anderegg, W. R., Christoffersen, B. O., Dietze, M. C., Farrior, C. E., ... & Moorcroft, P. R. (2018). Vegetation demographics in Earth System Models: A review of progress and priorities.*Global Change Biology*, 24(1), 35-54.

Gauslaa, Y. (2014). Rain, dew, and humid air as drivers of morphology, function and spatial distribution in epiphytic lichens. *The Lichenologist*, 46, 1–16.

Gauslaa, Y., & Arsenault, A. (2020). The cyanolichens *Erioderma pedicellatum* and *Coccocarpia palmicola* need much more than a dewfall to fill their water holding capacity. *Flora*, 269:151648.

Gauslaa, Y., & Coxson, D. (2011). Interspecific and intraspecific variations in water storage in epiphytic old forest foliose lichens. *Botany*, 89, 787–798.

Gauslaa, Y., Mikulec, M. M., & Solhaug, K. A. (2021). Short-term growth experiments – A tool for quantifying lichen fitness across different mineral settings. *Flora*, 282:151900.

Giorgi, F., & Lionello, P. (2008). Climate change projections for the Mediterranean region. *Global and Planetary Change* 63, 90–104.

Green, T. G. A., & Lange, O. L. (1995). “Photosynthesis in Poikilohydric Plants: A Comparison of Lichens and Bryophytes,” in *Ecophysiology of Photosynthesis* Springer Study Edition, eds E.-D. Schulze and M. M. Caldwell (Berlin: Springer), 319–341.

Green, T. G. A., Nash, T. H. III, & Lange, O. L. (2008). “Physiological ecology of carbon dioxide exchange,” in *Lichen Biology*, ed. T. H. Nash III (Cambridge, UK: Cambridge University Press), 152–181.

Hersbach, H., Bell, B., Berrisford, P., Hirahara, S., Horányi, A., Muñoz-Sabater, J., ... & Thépaut, J. N. (2020). The ERA5 global reanalysis. *Quarterly Journal of the Royal Meteorological Society*, 146(730), 1999-2049.

Ignatenko, R. V., Tarasova, V. N., & Markovskaya, E. F. (2020). Ontogenesis of the Lichen *Lobaria pulmonaria* (L.) hoffm. in plant communities of the boreal zone. *Russian Journal of Developmental Biology* 51, 115–124.

Lange, O. L., & Green, T. G. A. (1996). High thallus water content severely limits photosynthetic carbon gain of central European epilithic lichens under natural conditions. *Oecologia* 108, 13–20.

Lange, O. L., Green, T. G. A., & Reichenberger, H. (1999). The Response of Lichen photosynthesis to External CO_2 concentration and its interaction with thallus water-status. *Journal of Plant Physiology*, 154, 157–166.

Larsson, P., Solhaug, K. A., & Gauslaa, Y. (2012). Seasonal partitioning of growth into biomass and area expansion in a cephalolichen and a cyanolichen of the old forest genus Lobaria. *New Phytologist* 194, 991–1000. doi: 10.1111/j.1469-8137.2012.04130.x

Longinotti, S., Solhaug, K. A., & Gauslaa, Y. (2017). Hydration traits in cephalolichen members of the epiphytic old forest genus Lobaria (s. lat.). *The*

Lichenologist, 49, 493–506.

Losapio, G., Schöb, C., Staniczenko, P. P., Carrara, F., Palamara, G. M., De Moraes, C. M., ... & Bascompte, J. (2021). Network motifs involving both competition and facilitation predict biodiversity in alpine plant communities. *Proceedings of the National Academy of Sciences*, 118(6), e2005759118.

Merinero, S., Hilmo, O., & Gauslaa, Y. (2014). Size is a main driver for hydration traits in cyano- and cephalolichens of boreal rainforest canopies. *Fungal Ecology* 7, 59–66. doi: 10.1016/j.funeco.2013.12.001

Nascimbene, J., Benesperi, R., Casazza, G., Chiarucci, A., & Giordani, P. (2020). Range shifts of native and invasive trees exacerbate the impact of climate change on epiphyte distribution: The case of lung lichen and black locust in Italy. *Science of Total Environment*, 735:139537.

Nascimbene, J., Casazza, G., Benesperi, R., Catalano, I., Cataldo, D., Grillo, M., ... & Giordani, P. (2016). Climate change fosters the decline of epiphytic Lobaria species in Italy. *Biological conservation*, 201, 377-384.

Nash, T. H., & Olafsen, A. G. (1995). Climate change and the ecophysiological response of arctic lichens. *The Lichenologist*, 27, 559–565. Paaijmans, K. P., Heinig, R. L., Seliga, R. A., Blanford, J. I., Blanford, S., Murdock, C. C., & Thomas, M. B. (2013). Temperature variation makes ectotherms more sensitive to climate change. *Global Change Biology*, 19(8), 2373-2380.

Pacifici, M., Foden, W. B., Visconti, P., Watson, J. E., Butchart, S. H., Kovacs, K. M., ... & Rondinini, C. (2015). Assessing species vulnerability to climate change. *Nature Climate Change*, 5(3), 215-224.

Paoli, L., Benesperi, R., Fackovcova, Z., Nascimbene, J., Ravera, S., Marchetti, M., ... & Guttova, A. (2019). Impact of forest management on threatened epiphytic macrolichens: Evidence from a Mediterranean mixed oak forest (Italy). *IForest*, 12(4), 383-388.

Peterson, A. T., Papeş, M., and Soberón, J. (2015). Mechanistic and correlative models of ecological niches. *European Journal of Ecology*, 1, 28–38.

Phinney, N. H. (2019). 3D modelling of thallus topography of *Lobaria pulmonaria* facilitates understanding of water storage pools. *The Lichenologist*, 51, 89–95. doi: 10.1017/S0024282918000531

Phinney, N. H., Solhaug, K. A., and Gauslaa, Y. (2018). Rapid resurrection of chlorolichens in humid air: Specific thallus mass drives rehydration and reactivation kinetics. *Environmental and Experimental Botany*, 148, 184–191. doi: 10.1016/j.envexpbot.2018.01.009

Pontarp, M., Bunnefeld, L., Cabral, J. S., Etienne, R. S., Fritz, S. A., Gillespie, R., et al. (2019). The latitudinal diversity gradient: Novel understanding through mechanistic eco-evolutionary models. *Trends in Ecology & Evolution*, 34, 211–223. doi: 10.1016/j.tree.2018.11.009

Porada, P., & Giordani, P. (2021). Bark water storage plays key role for growth of mediterranean epiphytic lichens. *Frontiers in Forests and Global Change*, 4:668682. doi: 10.3389/ffgc.2021.668682

Porada, P., Tamm, A., Raggio, J., Cheng, Y., Kleidon, A., Pöschl, U., & Weber, B. (2019). Global NO and HONO emissions of biological soil crusts estimated by a process-based non-vascular vegetation model. *Biogeosciences*, 16(9), 2003-2031.

Porada, P., Van Stan, J. T., & Kleidon, A. (2018). Significant contribution of non-vascular vegetation to global rainfall interception. *Nature Geoscience*, 11, 563–567. doi: 10.1038/s41561-018-0176-7

Porada, P., Weber, B., Elbert, W., Pöschl, U., & Kleidon, A. (2013). Estimating global carbon uptake by lichens and bryophytes with a process-based model. *Biogeosciences* 10, 6989–7033.

Porada, P., Weber, B., Elbert, W., Pöschl, U., & Kleidon, A. (2014). Estimating impacts of lichens and bryophytes on global biogeochemical cycles. *Global Biogeochemical Cycles*, 28, 71–85.

Pörtner, H. O., & Farrell, A. P. (2008). Physiology and climate change. *Science* 322, 690–692.

Proctor, M. C. F., & Tuba, Z. (2002). Poikilohydry and homoihydry: Antithesis or spectrum of possibilities? *New Phytologist*, 156, 327–349. doi: 10.1046/j.1469-8137.2002.00526.x

Scheidegger, C., & Werth, S. (2009). Conservation strategies for lichens: Insights from population biology. *Fungal Biology Reviews*, 23, 55–66.

Smith, D. C., & Molesworth, S. (1973). Lichen Physiology Xiii. effects of rewetting dry lichens. *New Phytologist*, 72, 525–533.

Tuel, A., & Eltahir, E. A. B. (2020). Why is the mediterranean a climate change hot spot? *Journal of Climate*, 33, 5829–5843.

Vallese, C., Nascimbene, J., Giordani, P., Benesperi, R., & Casazza, G. (2021). Modelling range dynamics of terricolous lichens of the genus Peltigera in the Alps under a climate change scenario. *Fungal Ecology*, 49:101014.

Wan, S., and Ellis, C. J. (2020). Are lichen growth form categories supported by continuous functional traits: Water-holding capacity and specific thallus mass? *Edinburgh Journal of Botany*, 77, 65–76. doi: 10.1017/S0960428619000209

Conclusions

The studies presented in this thesis underscore the importance of small-scale conditions and climatic factors in understanding the ecology and ecophysiology of lichens. Our findings were achieved through the study of lichen responses to changes in environmental factors from the thallus level, considering photosynthesis, growth and different life stages, to broader scales by including the growth form at the community level. The studies conducted were designed to broaden our understanding of the impacts of climate change and human disturbances on lichens also in a perspective to use this organisms as biological indicators of global environmental changes.

Microclimate is a key driver of lichen distribution

The results obtained in different chapters of this thesis (**Chapters I**, **II** and **III**) confirm the importance of microclimatic conditions in influencing lichen distribution. Using different aspects of lichen ecophysiology, such as growth and photosynthesis, and considering growth form at a community level, we aimed to study the influence of variation in microclimatic and thallus-scale environmental conditions. We found that variations at tree trunks significantly alter lichen performances. In **Chapters I** we showed that sub-canopy climatic factors of precipitation and temperature significantly influence the frequency of lichen growth forms. Simultaneously, results in **Chapter III** revealed differences in the annual growth coefficient of thalli based on their exposure on different sides of the tree trunk. This difference was further exacerbated by the decrease in tree density due to logging. Finally, in **Chapter II** spatially close thalli but subjected to a warming treatment of approximately 2°C had different photosynthetic efficiency. In addition to the implications for understanding the effects of microclimate on lichens, the results of this thesis highlight the need for considering microclimatic conditions when studying lichens both at the level of individual organisms and at the level of communities. Despite the importance of microclimate in influencing lichens has long been recognised (Pearson & Lawrence, 1965), large-scale studies often use coarse climatic predictors to study lichen distribution. Although these studies undoubtedly have their value in highlighting large-scale patterns, they may fail to capture the actual climatic niche of lichens. For example, when comparing the microclimatic conditions during which lichen are active, the differences between two macroclimatic different areas (i.e. continental Antarctic and a Spain desert) become less pronounced (Green et al., 2007; Green et al., 2008). Results from **Chapters II** and **III** also evidence the importance of transplant experiments in understanding the effects of microclimatic variations on lichens. These kinds of experiments allow testing the responses of changed conditions effectively experienced by organisms. While they are often conducted on lichens (Mallen-Cooper & Cornwell, 2020), transplants over a larger scale are scarce (Mallen-Cooper, 2022; Williams et al., 2017; Gaio-Oliveira et al., 2004). Coupled with model prediction, these models could allow testing of complex hypotheses regarding microclimatic variations and climate change (Mallen-Cooper, 2022).

Contrasting responses to climate change in lichens

Lichens are considered to be threatened by changes in climatic conditions (Porada et al., 2023) due to their high dependence on environmental factors. Nonetheless, the extent of the impact of climate change on lichens can differ, influenced by a range of elements. On the one hand, specific species characteristics mediate the effects of climate change on epiphytic lichens in Mediterranean ecosystems (**Chapter I**). We found that while all growth forms are negatively affected by all climatic scenarios, those already associated with drier conditions are predicted to have a lower decrease in relative abundance in the future (**Chapter I**), except in the case of the worst scenario. Similarly, when comparing three alpine species, the more thermophilous species resulted in being less influenced, or even favoured, by temperature enhancement (**Chapter II**). These results, though not directly comparable as they consider species in different substrates (epiphytic vs. terricolous) and distinct climatic components (various factors vs. solely temperature), corroborate the diverse responses among lichen species (**Chapters I** and **II**). Previous studies have shown that even within the same habitat, species exhibit varied coverage responses—some decrease, while others increase (Bokhorst et al., 2016). Furthermore, predictions indicate that species will experience changes in range dimensions, either contracting or expanding, under future climate scenarios (Mallen-Cooper et al., 2023). On the other hand, life stages could influence the response to climate change. However, often species distribution models are conducted on the supposed realized climatic niche of adults. Nevertheless, life stages could have differential capacities to withstand extreme conditions or have slightly not overlapping climatic optima. Thus, even smaller changes may lead to reduced survival capacity or growth in different life stages. In the model species *L. pulmonaria*, life stages are differentially affected by increasing temperatures (**Chapter IV**) and in general by environmental changes (**Chapter III**). Even effects that immediately do not lead to a strong reduction of lichen performances (**Chapter II**) could determine, if continued over long periods, to a reduction of growth or, in worst cases to thallus death. For example, a period of unusually high water availability in a single autumn may have led to a reduction in lichen diversity not recovered after 19 years (Gauslaa, 2023). Finally, the difference in response might be also influenced by the existing climate of the region considered. While it is widely accepted that cold and high-altitude environments will be highly impacted by climate change due to the cold adaptation of species that live therein, even low-altitude thermal-adapted species could be negatively affected. We investigated a Mediterranean landscape predicting an overall decrease of lichen diversity in all growth forms considered in all future climate change scenarios (**Chapter I**). As global warming will change climatic conditions in distinct ways, the effects on lichens could be significantly different. For example, we attributed the strong reduction of lichen diversity in future conditions to the predicted lower amount of precipitation and higher temperature (**Chapter I**). By contrast, in specific conditions, lichens could be negatively affected even by increasing precipitation (Gauslaa, 2023). These results depict a complex framework in which many levels of complexity must be taken into account to gain accurate predictions of the effect of climate change on the future of lichen biodiversity. In particular, having a detailed knowledge of the ecophysiology and species-specific responses of individual species can reveal unpredicted responses to climate change (Kimball et al., 2009).

Through this knowledge is possible to scale the effects observed at higher levels of biological organization to gain insight into changes occurring over time.

Climate change mitigation is the foundation for preserving lichen diversity in the face of upcoming climate change

Studying the response of organisms to climate change is aimed at protecting biodiversity by developing effective management strategies and prioritizing resources on specific targets. At the land scale, understanding how local factors determine deviations of microclimatic conditions from macroclimate is increasingly being investigated for its possible use in managing climate change impacts (Dobrowski, 2011; Ellis & Eaton, 2021). Local factors, including for example vegetation and topography, can lead to large differences between local and large-scale climatic conditions. In this way, these specific microclimates can be buffered or even decoupled from the macroclimate, resulting in areas with more favourable climatic conditions for certain species. Additionally, these specific microclimatic conditions may continue to persist even if the climate in the surroundings has changed, functioning as microrefuges. Thus, understanding which are the factors that contribute most to determining favourable conditions, and to which species are important, allows to actively manage specific areas in order to extend, or recreate, such conditions (Greiser et al., 2021; Ellis & Eaton, 2021). Indeed, often it is not enough to simply maintain current conditions, which may not be sufficient to conserve some of the most vulnerable species, but it is important to actively manage habitats where favourable conditions for survival and fitness can be restored. For example, microrefugia are predicted to not be sufficient to avoid the decline of sensible epiphytic species in a Mediterranean landscape (**Chapter I**). Here, probably the trees studied were already at the limit of their capacity to maintain suitable microclimatic conditions for those species and are not able to sustain the same diversity in the future (**Chapter I**). It should be noted that the simulations used for the future did not take into account the growth of the trees themselves, which could have a positive or negative effect on its buffering capacity. Nevertheless, this means, for example, that favourable conditions need to be recreated by expanding woodlands to those areas where, through the combination with other factors, a higher buffering capacity could be achieved (Ellis, 2019; Ellis & Eaton, 2021a). For example, by exploiting already present topographic heterogeneity or distance from water courses (Ellis & Eaton, 2021b) At the same time, it would be important to create and maintain a high habitat complexity, to increase the possibility of finding microhabitat conditions suitable for species survival (Ellis & Eaton, 2021b). For example, as already mentioned, in forest ecosystems even variability at the trunk scale could determine strong variation in terms of survival chances and growth (**Chapter III**).

The results obtained in this thesis on the one hand confirm and expand the current knowledge on the effects of microclimate on lichens, by studying different aspects of lichen physiology and considering a wide range of microclimatic factors. On the other hand, it showed that climate change is an important driver for lichen distribution in the future, but the effect is dependent on the climate of the area considered and on the degree of climatic variations. We also showed that microrefuges could not be sufficient to maintain lichen diversity in future scenarios and active measures are needed to create conditions to buffer the effect of changing conditions. Finally, this work gives important insights into the ecology of lichens and their conservation.

Bibilography

Carlile, D. W., Skalski, J. R., Batker, J. E., Thomas, J. M., & Cullinan, V. I. (1989). Determination of ecological scale. Landscape Ecology, 2, 203-213.

De Frenne, P., Lenoir, J., Luoto, M., Scheffers, B. R., Zellweger, F., Aalto, J., ... & Hylander, K. (2021). Forest microclimates and climate change: Importance, drivers and future research agenda. *Global Change Biology*, 27(11), 2279-2297.

Decocq, G. (2000). The ‘masking effect’ of silviculture on substrate-induced plant diversity in oak-hornbeam forests from northern France. *Biodiversity and Conservation*, 9, 1467–1491.

Dobrowski, S. Z. (2011). A climatic basis for microrefugia: the influence of terrain on climate. *Global Change Biology*, 17(2), 1022-1035.

Ellis, C. J. (2020). Microclimatic refugia in riparian woodland: A climate change adaptation strategy. *Forest Ecology and Management*, 462, 118006.

Ellis, C. J., & Eaton, S. (2021a). Microclimates hold the key to spatial forest planning under climate change: Cyanolichens in temperate rainforest. *Global Change Biology*, 27(9), 1915-1926.

Ellis, C. J., & Eaton, S. (2021b). Climate change refugia: Landscape, stand and tree-scale microclimates in epiphyte community composition. *The Lichenologist*, 53(1), 135-148.

Gauslaa, Y. (2023). Recovery kinetics of epiphytic lichen diversity after dieback during a continuously wet season. *Fungal Ecology*, 66, 101299.

Gaio-Oliveira, G., Dahlman, L., Máguas, C., & Palmqvist, K. (2004). Growth in relation to microclimatic conditions and physiological characteristics of four Lobaria pulmonaria populations in two contrasting habitats. *Ecography*, 27(1), 13-28.

Green, T. A., Schroeter, B., & Sancho, L. G. (2007). Plant life in Antarctica. In Functional plant ecology (pp. 389-434). CRC press.

Green, T. G. A., Nash T. H. III, and Lange, O. L. (2008). "Physiological ecology of carbon dioxide exchange," in *Lichen Biology*, ed. T. H. Nash III (Cambridge: University Press), 152–181.

Greiser, C., Ehrlén, J., Meineri, E., & Hylander, K. (2020). Hiding from the climate: Characterizing microrefugia for boreal forest understory species. *Global Change Biology*, 26(2), 471-483.

Kimball, S., Angert, A. L., Huxman, T. E., & Venable, D. L. (2010). Contemporary climate change in the Sonoran Desert favors cold-adapted species. *Global Change Biology*, 16(5), 1555-1565.

Mallen-Cooper, M. (2022). Range limit dynamics of biocrusts in a changing climate (Doctoral dissertation, UNSW Sydney).

Mallen-Cooper, M., Rodríguez-Caballero, E., Eldridge, D. J., Weber, B., Büdel, B., Höhne, H., & Cornwell, W. K. (2023). Towards an understanding of future range shifts in lichens and mosses under climate change. *Journal of Biogeography*, 50(2), 406-417.

Mallen-Cooper, M., & Cornwell, W. K. (2020). A systematic review of transplant experiments in lichens and bryophytes. *The Bryologist*, 123(3), 444-454.

Pearson, L. C., & Lawrence, D. B. (1965). Lichens as microclimate indicators in northwestern Minnesota. *American Midland Naturalist*, 257-268.

Phinney, N. H., Solhaug, K. A., & Gauslaa, Y. (2018). Rapid resurrection of chlorolichens in humid air: specific thallus mass drives rehydration and reactivation kinetics. Environmental and Experimental Botany, 148, 184-

191.

Porada, P., Bader, M. Y., Berdugo, M. B., Colesie, C., Ellis, C. J., Giordani, P., ... & Weston, D. J. (2023). A research agenda for nonvascular photoautotrophs under climate change. *New Phytologist*, 237(5), 1495-1504.

Chinnery, E. V., & Ellis, C. J. (2023). Growth-dependent acclimation constrains climatic response for the lichen epiphyte Lobaria pulmonaria. *The Lichenologist*, 55(5), 401-408.

Renhorn, K. E., Esseen, P. A., Palmqvist, K., & Sundberg, B. (1996). Growth and vitality of epiphytic lichens: I. Responses to microclimate along a forest edge-interior gradient. *Oecologia*, 109, 1-9.

Canters, K. J., Schöllerj, H., Ott, S., & Jahns, H. M. (1991). Microclimatic influences on lichen distribution and community development. *The Lichenologist*, 23(3), 237-252.

Pintado. A., Sancho, L. G., & Valladares, F. (2001). The influence of microclimate on the composition of lichen communities along an altitudinal gradient in the maritime Antarctic. *Symbiosis*, 31(1-3), 69-84.

Williams, L., Colesie, C., Ullmann, A., Westberg, M., Wedin, M., & Büdel, B. (2017). Lichen acclimation to changing environments: Photobiont switching vs. climate-specific uniqueness in Psora decipiens. *Ecology and Evolution*, 7(8), 2560-2574.

Co-author affiliation

Renato Bensperi, Alessio Papini (Chapters 1-4), Elisabetta Bianchi (Chapters 2-4)
Department of Biology, University of Florence, Florence, Italy

Paolo Giordani (Chapters 1-4)
Department of Pharmacy (DIFAR), University of Genoa, Genoa, Italy

Juri Nascimbene (Chapters 1-4), Chiara Vallese (Chapters 2,3), Gabriele Gheza (Chapter 2)
BIOME Lab, Department of Biological, Geological and Environmental Sciences, Alma Mater Studiorum - University of Bologna, Via Irnerio 42, 40126 Bologna, Italy

Phillip Porada (Chapter 4)
Department of Biology, Ecological Modeling, University of Hamburg, Hamburg, Germany

Paola Malaspina (Chapter 1), Giulia Canali (Chapter 4)
Department of Earth Sciences, Environment and Life (DISTAV), University of Genoa, Genoa, Italy

Giorgio Bruniali, Luisa Frati (Chapter 3)
TerraData Environmetrics, Spin-Off Company of the University of Siena, 58025 Monterotondo Marittimo, Italy

Luca Paoli (Chapter 3)
Department of Biology, University of Pisa, 56126 Pisa, Italy

Sonia Ravera (Chapter 3)
Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo

Zuzana Fačkovcová (Chapter 3)
Plant Science and Biodiversity Centre, Slovak Academy of Sciences, 84523 Bratislava, Slovakia

Guido Incerti (Chapter 1)
Department of Agri-Food, Animal and Environmental Sciences, University of Udine, 33100 Udine, Italy

Michele Carbognani, Alessandro Petraglia (Chapter 2)
Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, Parco Area delle Scienze 11/A, 43124 Parma, Italy

Overall activities

Publications presented in this thesis

Di Nuzzo, L., Benesperi, R., Nascimbene, J., Papini, A., Malaspina, P., Incerti, G., & Giordani, P. (2022). Little time left. Microrefuges may fail in mitigating the effects of climate change on epiphytic lichens. Science of the Total Environment, 825, 153943.

Di Nuzzo L. Bianchi E., Carbognani M., Gheza G., Giordani P., Nascimbene J., Papini A., Petraglia A., Vallese C. & Benesperi R. [*In preparation*]. Responses of three alpine lichen species to passive warming in an alpine ecosystem.

Di Nuzzo, L., Giordani, P., Benesperi, R., Brunialti, G., Fačkovcová, Z., Frati, L., ... & Bianchi, E. (2022). Microclimatic alteration after logging affects the growth of the endangered lichen Lobaria pulmonaria. Plants, 11(3), 295.

Di Nuzzo, L., Canali, G., Giordani, P., Nascimbene, J., Benesperi, R., Papini, A., ... & Porada, P. (2022). Life-stage dependent response of the epiphytic lichen Lobaria pulmonaria to climate. Frontiers in Forests and Global Change, 5, 903607.

Other publications

Canali, G., **Di Nuzzo, L.**, Benesperi, R., Nascimbene, J., & Giordani, P. (2024). Functional traits of non-vascular epiphytes influence fine scale thermal heterogeneity under contrasting microclimates: insights from sub-Mediterranean forests. *Botanical Journal of the Linnean Society*, boad063.

Gheza, G., **Di Nuzzo, L.**, Giordani, P., Chiarucci, A., Benesperi, R., Bianchi, E., ... & Nascimbene, J. (2023). Species–area relationship in lichens tested in protected areas across Italy. *The Lichenologist*, 55(5), 431-436.

Ravera, S., Benesperi, R., Bianchi, E., Brunialti, G., **Di Nuzzo, L.**, Frati, L., ... & Paoli, L. (2023). *Lobaria pulmonaria* (L.) Hoffm.: The multifaceted suitability of the Lung lichen to monitor forest ecosystems. *Forests*, 14(10), 2113.

Ciani, F., Fornasaro, S., Benesperi, R., Bianchi, E., Cabassi, J., **Di Nuzzo, L.**, ... & Rimondi, V. (2023). Mercury accumulation efficiency of different biomonitors in indoor environments: The case study of the Central Italian Herbarium (Florence, Italy). *Environmental Science and Pollution Research*, 1-13.

Di Nuzzo, L., Masoni, A., Frizzi, F., Bianchi, E., Castellani, M. B., Balzani, P., ... & Benesperi, R. (2022). Red wood ants shape epiphytic lichen assemblages in montane silver fir forests. *iForest-Biogeosciences and Forestry*, 15(1), 71.

Gheza, G., **Di Nuzzo, L.**, Nimis, P. L., Benesperi, R., Giordani, P., Vallese, C., & Nascimbene, J. (2022). Towards a Red List of the terricolous lichens of Italy. *Plant Biosystems-An International Journal Dealing with all Aspects of Plant*

Biology, 156(3), 824-825.

Vallese, C., Di Musciano, M., Muggia, L., Giordani, P., Francesconi, L., Benesperi, R., ... **Di Nuzzo, L.** ... & Nascimbene, J. (2022). Water-energy relationships shape the phylogenetic diversity of terricolous lichen communities in Mediterranean mountains: Implications for conservation in a climate change scenario. *Fungal Ecology*, 60, 101189.

Di Nuzzo, L., Vallese, C., Benesperi, R., Giordani, P., Chiarucci, A., Di Cecco, V., ... & Nascimbene, J. (2021). Contrasting multitaxon responses to climate change in Mediterranean mountains. *Scientific reports*, 11(1), 1-12.

Ellis, C. J., Asplund, J., Benesperi, R., Branquinho, C., **Di Nuzzo, L.**, Hurtado, P., ... & Giordani, P. (2021). Functional Traits in Lichen Ecology: A Review of Challenge and Opportunity. *Microorganisms*, 9(4), 766.

Gheza, G., **Di Nuzzo, L.**, Vallese, C., Barcella, M., Benesperi, R., Giordani, P., ... & Assini, S. (2021). Morphological and Chemical Traits of Cladonia Respond to Multiple Environmental Factors in Acidic Dry Grasslands. *Microorganisms*, 9(2), 453.

Gheza, G., **Di Nuzzo, L.**, Vallese, C., Benesperi, R., Bianchi, E., Di Cecco, V., ... & Nascimbene, J. (2021). The lichens of the Majella National Park (Central Italy): an annotated checklist. *MycoKeys*, 78, 119.

Ravera, S., Bianchi, E., Brunialti, G., Ciotti, R., **Di Nuzzo, L.**, Isocrono, D., ... & Stentella, G. (2021). Studia Lichenologica in Italy. I. New records of red-listed species. *Borziana*, 2: 87-108.

Mugnai, M., Lazzaro L., **Di Nuzzo L.**, Foggi, B., Viciani D., & Ferretti G. (2021). Synopsis of Euphorbia section Anisophyllum (Euphorbiaceae) in Italy, with an insight on variation of distribution over time in Tuscany. *Phytotaxa*, 485(1), 1-65.

Bianchi, E., Benesperi, R., Brunialti, G., **Di Nuzzo L.**, Fačkovcová, Z., Frati, L., ... & Paoli, L. (2020). Vitality and Growth of the Threatened Lichen *Lobaria pulmonaria* (L.) Hoffm. in Response to Logging and Implications for Its Conservation in Mediterranean Oak Forests. *Forests*, 11(9), 995.

Cecconi, E., Fortuna, L., Benesperi, R., Bianchi, E., Brunialti, G., Contardo, T., **Di Nuzzo L.**, Frati, L., Monaci, F., Munzi, S., Nascimbene, J., Paoli, L., Ravera, S., Vannini, A., Giordani, P., Loppi, S., Tretiach, M. (2019). New Interpretative Scales for Lichen Bioaccumulation Data: The Italian Proposal. *Atmosphere* 2019, 10, 136.

Gheza, G., **Di Nuzzo, L.** & Nascimbene, J. (2020). The lichen genus Cladonia in Monte Ceceri (Tuscany, Central Italy). — *Borziana* 1: 5-13.

Congresses

International congresses

Di Nuzzo L., Bianchi E., Benepseri R., Brunialti G., … &; Ravera S. (2023) Conservation of the threatened lichen *Lobaria pulmonaria*: hints from field experiments. *Prima Conferenza di Biologia della Conservazione per ECR* – Libro dei Riassunti: 97 - **Poster**

Di Nuzzo L., Benesperi R., Nascimbene J., Papini A., Malaspina P., Incerti G., Giordani P. (2022) No place to hide. Climate change may overrun the mitigating effect of microrefuges on epiphytic lichens. *XXIII Simposio Internacional de Botánica Criptogámica*, pp. 116 - **Oral Presentation**

Paoli, L., Bianchi, E., Benesperi, R., Brunialti, G., **Di Nuzzo, L.**, Fačkovcová, Z., Frati, L., Giordani, P., Guttová, A. & Ravera, S. (2022). Forest management and conservation of the threatened lichen *Lobaria pulmonaria* in Mediterranean oak forests. *XXIII International Symposium of Cryptogamic Botany.* Valencia 2022. pag. 55 - **Oral Presentation**

Giordani, P., Canali, G., **Di Nuzzo, L.**, Benesperi, R. & Nascimbene, J. (2022). Relationships between cryptogamic functional traits and thermal heterogeneity at tree scale: preliminary investigations and perspectives. *XXIII International Symposium of Cryptogamic Botany.* Valencia 2022. pag. 115 - **Oral Presentation**

Di Nuzzo L., Vallese C., Benesperi R., Giordani P., Chiarucci A., Di Cecco V., Di Martino L., Di Musciano M., Gheza G., Lelli C., Spitale D., Nascimbene J. (2020) Risposte contrastanti di comunità vascolari e non vascolari al cambiamento climatico lungo un gradiente altitudinale nel massiccio della Majella. *115°Congresso della Società Botanica Italiana* - **Oral Presentation**

Di Nuzzo L., Benesperi R., Incerti G., Nascimbene J., Giordani P. (2021) Small-Scale Conditions For Small-Scale Organisms: The Effects Of Microclimate On Lichen Growth Form. *International Association for Lichenology 9th Symposium (IAL 9)*, pp. 194 - **Poster**

National congresses

Di Nuzzo L., Prieto M, (2023). An overlooked treasure of biodiversity: a preliminary study on the lichen genus Endocarpon Hedw. in the Iberian Peninsula. *Notiziario Della Società Lichenologica Italiana.* vol. 36, pp. 20-20 - **Oral presentation**

Di Nuzzo L., Del Greco N., Gheza G., Pistocchi C., Benesperi R., Giordani P., Petraglia A., Carbognani M., Nascimbene J. (2023). Multitaxon dynamics in high elevation vegetation of the Stelvio National Park (Lombardy). *Notiziario Della Società Lichenologica Italiana.* vol. 36, pp. 40-40 - **Poster**

Di Nuzzo L., Bianchi E., Carbognani M., Gheza G., Giordani P., Nascimbene J. Petraglia A., Vallese C., Benesperi R. (2022). Effetto del riscaldamento passivo su trapianti di tre licheni terricoli. *Notiziario Della Società Lichenologica Italiana.* vol. 35, pp. 43-43 - **Poster**

Di Nuzzo L., Giordani P., Bianchi E., Canali C., Nascimbene J., Benesperi R. (2022). ArduTherm: a new promising tool for monitoring lichen surface temperature in the field? *Notiziario Della Società Lichenologica Italiana.* vol. 35, pp. 44-44 - **Poster**

Paoli L., Bianchi E., Brunialti G., **Di Nuzzo L.**, Fačkovcová Z., Frati L., Giordani P., Isocrono D., Ravera S., Vasta C., Benesperi R. (2022). Diversità lichenica e briofitica in lobarieti invasi da Robinia pseudacaciaì. *Notiziario Della Società Lichenologica Italiana.* vol. 35, pp. 54-54 - **Poster**

Gheza G., **Di Nuzzo L.**, Nimis P.L., Benesperi R., Giordani P., Vallese C., Nascimbene J. (2022). Verso una Lista Rossa dei licheni terricoli d'Italia. *Notiziario Della Società Lichenologica Italiana.* vol. 35, pp. 22-22 - **Oral presentation**

Vallese C., **Di Nuzzo L.**, Giordani P., Benesperi R., Francesconi L., Gheza G., Nascimbene J.(2022). Substrate type drives diversity of alpine terricolous lichen communities in the Alps.*Notiziario Della Società Lichenologica Italiana.* vol. 35, pp. 30-30 - **Oral presentation**

Di Nuzzo L., Benesperi R., Bianchi E., Brunialti G., Fačkovcová Z., Frati L., Giordani P., Nascimbene J., Ravera S., Vallese C., Paoli L. (2021). Influenza di fattori microclimatici e della gestione forestale sulla crescita di *Lobaria pulmonaria* in un ceduo mediterraneo. Notiziario Della Società Lichenologica Italiana. vol. 34, pp. 22-22 - **Oral Presentation**

Nascimbene J, Vallese C., **Di Nuzzo L.** Benesperi R., Chiarucci A., Gheza G., Giordani P., Di Cecco V., Di Martino L., Di Musciano M., Lelli C., Mair P., Spitale D. (2020) Patterns of multitaxon diversity along elevational gradients in the Alps and in the Majella massif. *11° Convegno «Ricerca zoologica e botanica in Alto Adige.* Bolzano, September 03-04, 2020 - **Oral Presentation**

Courses and schools

Microclimates: physical bases, modelling and characterization in ecology – **Held by:** AnaEE France – **2023/09**

Advanced R for Ecology and Evolutionary Biology – **Held by:** cE3c – Centre for Ecology, Evolution and Environmental Changes – **2022/05**

Until death do us apart: living in a symbiotic world – **Held by:** cE3c – Centre for Ecology, Evolution and Environmental Changes – **2022/01**

Corso introduttivo con R per il Dottorato su Maximum Likelihood, Modelli Misti e Statistica Bayesiana – **Held by:** University of Parma **- 2021/06**

Acknowledgments

Premetto che non sono bravo a esprimere i miei sentimenti a parole. Il primo e grande ringraziamento va a Renato, Paolo e Juri, perché senza di loro tutto questo non sarebbe stato possibile. Dai primi momenti fino a questi ultimi giorni mi hanno sempre supportato in tutte le parti di questo lavoro. Un grande grazie va a Gabriele, per tutte le escursioni lichenologiche, per tutte le ore passate a chiacchierare, per avermi sempre supportato, anche nei momenti più difficili e per essere sempre stato d'ispirazione per continuare a fare quello che stavo facendo e migliorarmi ogni volta. Un altro grande grazie va a Michele, per esserci sempre quando ho avuto bisogno e per essere sempre pronto a discutere e confrontarci su qualsiasi cosa. Vorrei ringraziare Chiara, per tutto il tempo passato insieme, per tutta l'ospitalità e per tutti i disagi affrontati insieme (Acme). Vorrei ringraziare Elisabetta, per tutti gli anni di condivisione e per tutte le avventure e sventure passate insieme in questi anni. Vorrei ringraziare Lorenzo per tutte le volte che sono andato a bussare alla sua porta chiedendo consigli sulla statistica e per tutte le risposte che ho ricevuto. Spero di non aver esagerato. Vorrei inoltre ringraziare Luana e Chiara perché è stato davvero bello poter condividere del tempo con voi. Un ringraziamento va a Giorgio e Luisa per tutto quello che abbiamo condiviso quest'ultimo anno e per la meravigliosa Stelvio che mi hanno regalato :D. Un grande grazie va anche a tutti i colleghi della Società Lichenologica Italiana, per aver creato una casa accogliente dove è possibile confrontarsi e discutere con serenità. Credo che avere a disposizione un ambiente del genere abbia un valore inestimabile per un giovane ricercatore. Spero che possa essere così anche negli anni a venire. Un agradecimiento va a Clara, Manu, Clara y Jesùs porque por todo el tiempo que he pasado en Madrid me han hecho sentir como en casa. Os hecho de menos. Vorrei anche ringraziare i miei amici Andrea e Kika per avermi coinvolto nelle loro avventure e avermi reso una persona migliore (spero). Un grande, grande grazie va Ivan, per essere da sempre il mio faro e approdo sicuro, e per volermi bene nonostante tutte le mie assenze. Se c'è una cosa su cui sono sicuro (e sono poche) è che senza di te non sarei quello che sono adesso. Un grazie va ai miei genitori, Cristina e Mario, per avermi lasciato libero di scegliere la strada che

volevo. Per aver sopportato i miei malumori e cambi di rotta, ma soprattutto per averi supportato con forza e costantemente in tutti questi anni. Un grazie a mio fratello Lorenzo, per essere la persona che è e per volermi bene, anche se a modo suo. Infine, un immenso grazie a Valentina, per essere stata una compagna meravigliosa.

Appendices

Appendix A

Supplementary Material Chapter 1

A.1 Modelling throughfall and stemflow

To model the throughfall and stemflow we refer to the original Gash analytical model as reported in Valente et al. 1997. As such, we calculated the component of both values as follow:

Components of the equations were calculated as follow:

A.1.1 Rainfall-related parameters - R, q

To calculate the Gross rainfall intensity (R), as we did not have collected the climatic data on the field, we decided to use data from the closest weather station to the point. Rainfall data were obtained from the website sardegna-clima.it. Data from the weather stations reported daily rainfall (mm) from 1922 to 2009. We decided to use only the last 30 years, keeping data only after 1980. For each weather station, we calculate annual mean precipitation by summing daily rainfall for each year and averaging for the last 30 years. As several plots were distant from the closest weather station, we re-proportioned each value to modelled values of the CHELSA BIO12 (Annual precipitation) bioclimatic variable. Firstly, we measured the distance of

Amount of gross rainfall necessary to saturate the canopy ($\mathrm{P_g}$’):	$-\frac{R}{E}S\ln[1-\frac{E}{(1-p-p_t)}]R$
Interception loss from the canopy:	
For m small storms insufficient to saturate the canopy ($\mathrm{P_g} < \mathrm{P_g}$’):	$(1-p-p_t)\sum_{j=1}^{m} P_{j,i}$
For n storms large enough to saturate the canopy: ($\mathrm{P_g} \geq \mathrm{P_g}$’):	$(1-p-p_t)P'_g + \frac{E}{R}\sum_{j=1}^{m}(P_{g,j} - P'_g)$
Interception loss from the trunks:	
For q storms that saturate the trunks ($\mathrm{P_g} \geq \mathrm{S_t}/\mathrm{p_t}$):	qSt
For m + n − q storms that do not ($\mathrm{P_g} < \mathrm{S_t}/\mathrm{p_t}$)	$p_t \sum_{j=1}^{m+n-q} (P_{g,j})$
Stemflow	$p_t\sum_{j=1}^{q}(P_{g,j} - qSt)$
Throughfall	$p\sum_{j=1}^{m+n} P_{g,j}[(1-p-p_t) - \frac{E}{R}]\sum_{j=1}^{n}(P_(g,j) - P'_g)$

Symbol	Component	Unit	Calculation
R	Gross rainfall intensity	mm/h	Using weather stations data
S	Canopy storage capacity	mm	Literature
E	Evaporation Rate	mm/h	From TerraClimate “AET”
pt	Stemflow partitioning		Calculated based on literature values
St	Trunk storage capacity	L	Calculated on measured values
p	Free throughfall coefficient		Measured
q	Number of storms that saturate the trunks		Using weather stations data

the plot from the closest weather station, then we calculated the ratio between BIO12 values and annual precipitation calculated from the weather stations. To re-proportioned we multiply these ratios with the daily measured values. Subsequently, to calculate R, we selected all the daily precipitation greater than 0.4 and divided it to 24 hours, to obtain a mm/h intensity. Using the same dataset, we calculated the number of events (q) selecting the storms (considered daily) that saturate the canopy and subsequently the trunk as required in the model of Valente et al. 1997.

A.1.2 Evaporation rate - E

The evaporation rate was obtained from the TerraClimate database (Abatzoglu et al. 2018) with a 4-km resolution using the AET (actual evapotranspiration). The data were obtained as monthly mean for the years 1981-2010. To obtain a single value for each plot we averaged all values to an annual value. Thus, the annual value was divided by hour in a year (8760) obtaining the mm/h value.

A.1.3 Free throughfall coefficient and Light - p

To estimate the throughfall coefficient we considered it as LAI (Leaf Area Index). For each tree in each plot a hemispherical photo was taken at each exposition where the lichen diversity was measured (see the Sampling paragraph in the text). Photos were taken using a Canon EOS 350D reflex digital camera with a SIGMA 15 mm F. 2.8 fisheye lens. Through a tripod, the camera was positioned at 30cm above the subplot, set to the zenith and magnetic Nord. ISO was set to 400. Images captured had a resolution of 4272×2848 and were converted in greyscale. To calculate LAI and the direct light we used Gap Light Analyzer software (Frazer et al., 1999). The software allows, after a registration process designed to identify the geographic orientation and circular extent of the image, to set a threshold to discriminate between canopy and clear sky pixels and calculate gap light parameters. Firstly, the cloudiness index (Iqbal 1983) was calculated as Kt =H/H0, where H0 is the extra-terrestrial radiation calculated through the software setting latitude, longitude, elevation, slope, aspect and position of magnetic north at the date of valuation. H is the global spectral radiation incident on a horizontal surface on the specified period. In our case H was the monthly averages retrieved from direct measurements of the closest meteorological station over the period 1990-2005 (SAR 2008). Subsequently, the beam fraction (Hb/H) was calculated, with Hb being the same as H but direct on the horizontal surface, as:

$$\frac{H_b}{H} = [1 - e^{-(3.044K_t^{2.436})}]$$

Moreover, in order to perform a better estimation of photosynthetically active radiation we calculated the spectral fraction Rp/Rs. This latter is the fraction of global radiation global solar radiation incident on a horizontal surface at ground level that falls within a limited range of the electromagnetic spectrum.

$$\frac{R_p}{R_s} = [1 - e^{-(0.499K_t^{-0.219})}]$$

To calculate more accurate coefficients, we set correction parameters for sky-region brightness, the clear-sky transmission coefficient (T) which was set at 0.77, as reported for the Tyrrhenian area in Bellocchi et al. (2002). Afterwards, we calculate the Leaf Area Index (LAI 4 Ring in the software) and the quantity of direct solar radiation transmitted by the canopy (Trans Direct). S1.4 Stemflow partitioning - pt Stemflow partitioning was calculated differently for different tree species as the data availability did not allow us to perform the same procedure. However, all the methods used are based on Sadeghi et al. 2020. As we had only one Fraxinus observation, we used data of throughfall and stemflow from Turner et al. 2019. A linear regression was fitted between rainfall and stemflow and the slope regression coefficient was used as partition coefficient.

A.1.4 Stemflow partitioning - pt

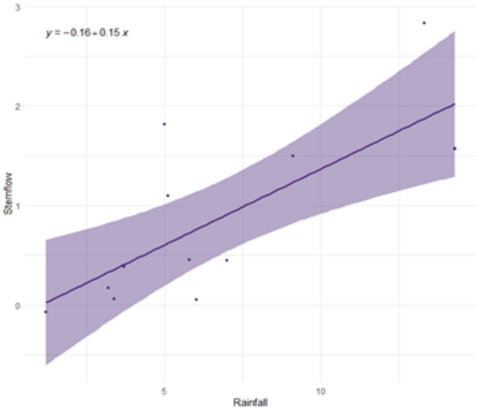
Stemflow partitioning was calculated differently for different tree species as the data availability did not allow us to perform the same procedure. However, all the methods used are based on Sadeghi et al. 2020. As we had only one Fraxinus observation, we used data of throughfall and stemflow from Turner et al. 2019. A linear regression was fitted between rainfall and stemflow and the slope regression coefficient was used as partition coefficient.

Regarding Quercus suber we retrieve H:W values (the ratio between the height and the width of the canopy) from Xiao et al. 2000. This H:W was associated with a pt value. Thus, we re-proportioned the pt value based on the H:W ratio of the survived trees. In the case of Quercus ilex, Limousin et al. 2008 presented a value for canopy height. We fit a relation between H:W ratio and Canopy Height (H) based on our data. Thus, we calculated the estimated value of H:W for data in Limousin et al. 2008 and then re-proportioned the pt value from Limousin et al. 2008 as done with Q. suber. For Olea we used the same procedure retrieving data from Gomez et al. 2001. In the same way the pt value was calculated for Prunus, Malus and Pyrus from Wang & Wang 2019. Similarly, to Fraxinus, for Eucalyptus we used data from Valente et al. 1997

A.1.5 Trunk storage capacity

To calculate the trunk water storage capacity (St), we measured the dry and wet weight of a 2 cm^2 bark sample (see below) and calculated the water content (mL).

	Species	**Rainfall**	**Stemflow**
1	Ash	14.30266	1.56864047
2	Ash	13.32795	2.8352035
3	Ash	9.103135	1.49906727
4	Ash	14.30333	1.57491521
6	Ash	7.005139	0.4459299
7	Ash	5.795017	0.4539853
8	Ash	6.022161	0.0495195
9	Ash	5.113285	1.09519503
10	Ash	5.00582	1.81912097
11	Ash	3.701339	0.3830978
12	Ash	3.38788	0.0575749
13	Ash	3.196408	0.17098643
14	Ash	1.202898	-0.069234



A specific water content was calculated taking the ratio between water content and cm^2, obtaining the water content per cm^2 (mL/cm^2). This value was then multiplied with trunk area, obtaining the whole trunk water storage capacity (mL, transformed in L). Trunk area was calculated by multiplying trunk circumference with tree height, simplifying trunk shape as a cylinder. Trunk interception was calculated as the sum of precipitation for q storms that saturate the trunks ($Pg \geq St/pt$) and For m + n − q storms that do not ($Pg < St/pt$) (Paragraph S1).

A.1.5.1 Measuring bark water holding capacity

Following Hauck et al. 2006, we collected for each tree in each plot a 2mm thick sample of bark. All the samples were subsequently cleaned from epiphytes such as lichens and bryophytes. The samples were wetted with distilled water for 24 hours, weighted after removing the external water with a paper towel to measure the wet bark weight (fwmax). Afterwards, the samples were dried at 80°C for 48h and weighted again to measure to dry bark weight (dw). Bark water holding capacity was than calculated as follow:

$$WHC_b = \frac{fwmax - dw}{dw}$$

A.1.6 Canopy Storage Capacity

Species	Value	References	Note
Eucalyptus sp.	0.21	Valente et al. 2017	
Fraxinus excelsior L.	0.27	Sadeghi et al. 2018	The article estimate S for Fraxinus rotundifolia, however, being the only values found for the genus Fraxinus, we decided to use this value
Malus sp.	1	Xiao et al. 2000	We assumed that values of S for Malus, Pyrus and Prunus are similar
Olea europaea L.	2.7	Gómez et al. 2001	We used the mean of the S value reported in the work
Olea sp.	2.7	Gómez et al. 2001	We used the mean of the S value reported in the work
Opuntia sp.	0.1		We did not find any values of S for Opuntia. The value reported is estimated as leaves of Opuntia can't retain almost any water which then flows to the stem.
Pinus halepensis Mill.	1	Link et al. 2004	We did not find an explicit S valu e for Pinus halepensis thus we used the value estimated for Pinus nigra
Pinus pinea L.	0.41	Valente et al. 1997	
Pinus sp.	1	Link et al. 2004	We decided to use the same value of Pinus halepensis as this is probably the more similar species.
Prunus sp.	1	Xiao et al. 2000	We assumed that values of S for Malus, Pyrus and Prunus are similar
Pyrus sp.	1	Xiao et al. 2000	We assumed that values of S for Malus, Pyrus and Prunus are similar
Quercus ilex L. subsp. ilex	2.6	Limousin et al. 2008	
Quercus pubescens Willd. subsp. pubescens	3.4	Oerlemans (2010)	We used the mean of all values of Q. pubescens collected in the work
Quercus sp.	3.4	Oerlemans (2010)	
Quercus suber L.	2	Xiao et al. 2000	

A.2 Potential NH_3 emission

For each plot, we estimate the potential NH3 emission based on census data of intensity of local breeding (Ribeiro et al. 2013). In Italy, local data for each census section about the amount of cattle, swine and ovine breaded is available (ISTAT 2003). Census sections are defined by ISTAT and are available on https://www.istat.it/it/archivio/104317. For each species, the number of animals present were multiplied with a specific NH3 emission factor (European Monitoring and Evaluation Programme, EMEP 2002). For each section, the total amount of potential emission (kg ha-1 year) was then spatially disaggregated into areas which could bear pasturing, based on Corine Land Cover 2.3.1 (Pastures, meadows and other permanent grasslands under agricultural use), 2.4 (Heterogeneous agricultural areas) and 3.2.1. (Natural grassland). This procedure, albeit its limits, allows estimates of emission for surface units. Subsequently, to calculate more accurate values, the percentage of cover of Corine categories within a 100m radius was calculated and then multiplied for the emission factor for surface units of census section. Buffer dimension was selected based on previous studies on NH3 dispersion (Sutton et al. 1998) and impacts on epiphytic lichens (Pinho et al. 2009). All analyses were performed with Statistica 8.0.

A.3 Measure of tree characteristics

Tree characteristics were measured for each tree in each plot. Canopy height was calculated firstly by measuring the Lowest Canopy Height at each tree exposition, averaging these values and subtracting them to the tree height. Canopy width was calculated by measuring at each exposition the maximum distance of the canopy from the trunk. All the measurements were performed with Leica DISTO A5.

A.4 Bark Buffer pH and pH

In order to finely describe the micro-stational condition experienced by the lichen communities, bark pH and buffer pH was measured for each tree in each plot. To measure bark pH, a modified protocol based on Johnsen & Søchting (1973) was used. This method uses a suspension of ground bark on which to measure the acidity value. In each bark sample, after ground 250 mg of bark, 10 ml of distilled water was added, and the solution was stored for 8 h. We used a CRINSON BASIC 20 (pH measurement scale: -2 ÷ 16; resolution 0,01; precision ± 0,01) with combine electrode (pH measurement scale: 0 ÷ 14; ceramic diaphragm; reference system: Ag/AgCl). Calibrations were performed with buffer solution a pH=4.01 and pH=7.01. As control we measure 10ml of distilled water. Similarly to pH, we followed Johnsen & Søchting (1973) to measure bark pH buffer. In particular this latter was calculated as:

$$\beta = \frac{dC_B}{dpH} = -\frac{dC_A}{dpH}$$

where β is pH buffer, CA and CB are acid and base concentration in suspension. Solution used for measuring bark pH ($\mathrm{pH_I}$) were stored for 12-16 h at air temperature in agitation with 2 ml of NaOH 0.1 N. Afterwards, samples pH was directly measured in solution ($\mathrm{pH_{II}}$). Mean buffer pH (β_{mean}) is inversely proportional to the difference between $\mathrm{pH_{II}}$ and $\mathrm{pH_I}$ (ΔpH), following the formula:

$$\beta_{\mathrm{mean}} = \frac{1}{\Delta pH}\int_{I}^{II} \beta dpH = -\frac{C_B(II) - C_B(I)}{\Delta pH} = \frac{0.0002}{\Delta pH}$$

Mean bark pH buffer could be expressed as the ratio between the number of equivalent base added to the suspension (2 x 10^{-4} eq) and measured pH variation. As our $\mathrm{pH_I}$ data were always higher than 3.5 and $\mathrm{pH_{II}}$ lower than 11, water buffer capacity was irrelevant.

A.5 Bark Microstructure

To describe bark microstructure, which could influence lichen colonization and water dynamics, we describe each square by three ordinal scale characteristics on three levels: bark texture depth, width, density and orientation. In particular, bark texture depth describes bark furrows depth, width their width. Bark texture density represents how frequent furrows were. Additionally, furrows orientation was described by means of four levels nominal scale (horizontal, vertical, oblique, grid). Moreover, holes and number and decortication cover were recorded for each square. In the end, at reticule level bark exfoliation cover was recorded. Similarly to what was done for canopy effect, in order to quantify a bark microstructure effect, a PCA was used to resume all these measures. We used the loadings of each tree on the dimension associated with increasing bark complexity to calculate a bark effect for each tree.

A.6 Bark water loss halftime – T50

To measure the bark water loss halftime, firstly we expressed the water amount in each sample proportional to dry weight, which is the Relative Water Content (RWC). Evaporation process could be described by a differential equation:

$$\frac{dRWC\%}{dt} = -k_{wl}t_{\frac{1}{2}}$$

where k_{wl} is water loss rate $\mathrm{t}_{\frac{1}{2}}$ and water loss halftime. To measure the bark water loss halftime, firstly samples were oven dried for 24 h at 80°C, afterwards dry weight was measured (dw). Subsequently, after samples were stored in distilled water for 24 h, wet bark weight (fwmax oven) was measured. Weight measures were then taken at specific time intervals, keeping samples at environmental humidity. Time intervals, which were different for different tree species, were determined in preliminary experiments. After calculating RWC% (as in A.1.5.1) and knowing time intervals between each weight, through the least square method it is possible to interpolate correspondent points. Samples Kwl and t1/2 were calculated following the exponential function which better approximated experimental point distribution where the regression was significant. Dehydration curves for each sample were interpolated on 10 subsequent weights, which, based on dry weight, corresponded to RWC% values between 130% and 20%.

Species	**Time interval**
Q. pubescens , Q.ilex , Prunus sp., Olea sp., Fraxinus sp., Eucaliptus sp.	16
Pinus sp.	8
Q. suber	5

A.7 Lichen species and growth forms

Abbreviations: Frut (Fruticose), Fol.large (Foliose large), Fol.b (Foliose broad-lobed), Fol.gel.swo (Foliose gelatinous), Sq (squamulose), Cr.co (Crustose conspicuous), Cr.in (Crustose inconspicuous).

Names	**Growth Forms**
Acolium inquinans (Sm.) A. Massal.	Cr.in
Acrocordia gemmata (Ach.) A. Massal. var. gemmata	Cr.in
Alyxoria varia (Pers.) Ertz Tehler	Cr.in
Amandinea punctata (Hoffm.) Coppins Scheid.	Cr.co
Anaptychia ciliaris (L.) Flot.	Frut
Arthonia albopulverea Nyl.	Cr.in
Arthonia apatetica (A.Massal.) Th.Fr.	Cr.in
Arthonia atra (Pers.) A. Schneid.	Cr.in
Arthonia didyma Körb.	Cr.in
Arthonia glaucella Nyl.	Cr.in
Arthonia granosa B. de Lesd.	Cr.in
Arthonia hypobela Nyl.	Cr.in
Arthonia melanophthalma Nyl.	Cr.in
Arthonia radiata (Pers.) Ach.	Cr.in
Arthonia sp.	Cr.in
Arthopyrenia cerasi (Schrad.) A. Massal.	Cr.in
Arthopyrenia cinereopruinosa (Schaer.) A.Massal.	Cr.in
Arthothelium spectabile A. Massal.	Cr.in
Athallia alnetorum (Giralt, Nimis & Poelt) Arup, Frödén & Søchting	Cr.in
Athallia cerinella (Nyl.) Arup, Frödén Søchting	Cr.in
Athallia cerinelloides (Erichsen) Arup, Frödén Søchting	Cr.in
Athallia pyracea (Ach.) Arup, Frödén Søchting	Cr.in
Bacidia biatorina (Körb.) Vain.	Cr.in
Bacidia laurocerasi (Duby) Zahlbr.	Cr.in
Bacidia rosella (Pers.) De Not.	Cr.in
Bacidia rubella (Hoffm.) A.Massal.	Cr.in
Bacidia sp.	Cr.in
Bellicidia incompta (Borrer) Kistenich, Timdal, Bendiksby & S.Ekman	Cr.in
Biatora beckhausii (Körb.) Tuck.	Cr.in
Biatoridium monasteriense J. Lahm ex Körb.	Cr.in
Blastenia ferruginea (Huds.) A. Massal.	Cr.in
Blastenia herbidella (Hue) Servít	Cr.in
Blastenia hungarica (H. Magn.) Arup, Søchting Frödén	Cr.in
Bryoria fuscescens (Gyeln.) Brodo D.Hawksw.	Frut
Buellia hyperbolica Bagl.	Cr.in

Calicium abietinum Pers.	Cr.in
Calicium notarisii (Tul.) M. Prieto Wedin	Cr.in
Calicium sp.	Cr.in
Calicium viride Pers.	Cr.in
Caloplaca cerina (Hedw.) Th. Fr. s.lat.	Cr.in
Caloplaca obscurella (J. Lahm ex Körb.) Th. Fr.	Cr.in
Candelaria concolor (Dicks.) Stein	Fol.n
Candelariella reflexa (Nyl.) Lettau	Cr.in
Candelariella xanthostigma (Ach.) Lettau	Cr.in
Catillaria nigroclavata (Nyl.) J. Steiner	Cr.in
Catillaria subviridis (Nyl.) Zahlbr.	Cr.in
Catinaria atropurpurea (Schaer.) Vezda Poelt	Cr.in
Chaenotheca ferruginea (Sm.) Mig.	Cr.in
Chaenotheca furfuracea (L.) Tibell	Cr.in
Chaenotheca sp.	Cr.in
Chrysothrix candelaris (L.) J.R.Laundon	Cr.in
Cladonia fimbriata (L.) Fr.	Frut
Cladonia macilenta Hoffm. subsp. *macilenta*	Frut
Cladonia parasitica (Hoffm.) Hoffm.	Frut
Cladonia pyxidata (L.) Hoffm.	Frut
Cliostomum griffithii (Sm.) Coppins	Cr.in
Coenogonium pineti (Ach.) Lücking Lumbsch	Cr.in
Collema flaccidum (Ach.) Ach.	Fol.gel.swo
Collema furfuraceum (Arnold) Du Rietz	Fol.gel.swo
Collema nigrescens (Huds.) DC.	Fol.gel.swo
Collema subflaccidum Degel.	Fol.gel.swo
Collema subnigrescens Degel.	Fol.gel.swo
Dactylospora parasitica (Flörke ex Sprengel) Zopf	Cr.in
Dichoporis ziziphi (A. Massal.) S.H. Jiang, Lücking Sérus.	Cr.in
Diploicia canescens (Dicks.) A.Massal.	Cr.in
Diplotomma alboatrum (Hoffm.) Flot.	Cr.in
Enchylium conglomeratum (Hoffm.) Otálora, P.M. Jørg. & Wedin	Fol.gel.swo
Evernia prunastri (L.) Ach.	Frut
Flavoparmelia caperata (L.) Hale	Fol.b
Flavoparmelia soredians (Nyl.) Hale	Fol.b
Fuscopannaria mediterranea (Tav.) M.Jørg.	Sq
Gyalecta carneola (Ach.) Hellb.	Cr.in
Gyalecta truncigena (Ach.) Hepp	Cr.in
Gyalolechia flavorubescens (Huds.) Søchting, Frödén & Arup var. flavorubescens	Cr.in
Haematomma ochroleucum var. *porphyrium* (Pers.) J.R. Laundon	Cr.in

Heterodermia speciosa (Wulfen) Trevis.	Fol.n
Huneckia pollinii (A. Massal.) S.Y. Kondr., Elix, Kärnefelt, A. Thell, J. Kim, A.S. Kondratiuk & J.-S. Hur	Cr.in
Hyperphyscia adglutinata (Flörke) H.Mayrhofer Poelt	Fol.n
Hypogymnia physodes (L.) Nyl.	Fol.n
Hypogymnia tubulosa (Schaer.) Hav.	Fol.n
Lecania cyrtella (Ach.) Th.Fr.	Cr.in
Lecania koerberiana J.Lahm	Cr.in
Lecanographa amylacea (Pers.) Egea Torrente	Cr.in
Lecanora albella (Pers.) Ach.	Cr.in
Lecanora allophana (Ach.) Nyl. f. allophana	Cr.co
Lecanora argentata (Ach.) Malme	Cr.co
Lecanora carpinea (L.) Vain.	Cr.co
Lecanora chlarotera Nyl.	Cr.co
Lecanora chlarotera subsp. *meridionalis* (H. Magn.) Clauzade & Cl. Roux	Cr.co
Lecanora expallens Ach.	Cr.in
Lecanora horiza (Ach.) Linds.	Cr.co
Lecanora leptyrodes (Nyl.) Degel.	Cr.in
Lecanora pulicaris (Pers.) Ach.	Cr.co
Lecanora saligna (Schrad.) Zahlbr.	Cr.in
Lecanora strobilina (Spreng.) Kieff.	Cr.in
Lecanora symmicta (Ach.) Ach.	Cr.in
Lecidella elaeochroma (Ach.) M.Choisy	Cr.co
Lecidella elaeochroma var. *elaeochroma* f. *soralifera* (Erichsen) D. Hawksw.	Cr.co
Lepra albescens (Huds.) Hafellner	Cr.co
Lepra amara (Ach.) Hafellner	Cr.co
Lepra multipuncta (Turner) Hafellner	Cr.co
Lepraria sp.	Cr.in
Leprocaulon quisquiliare (Leers) M. Choisy	Frut
Leucodermia leucomelos (L.) Kalb	Fol.n
Lobaria pulmonaria (L.) Hoffm.	Fol.large
Lobarina scrobiculata (Scop.) Cromb.	Fol.large
Megalaria grossa (Nyl.) Hafellner	Cr.in
Melanelixia glabratula (Lamy) Sandler Arup	Fol.b
Melanelixia subaurifera (Nyl.) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch	Fol.b
Melanohalea elegantula (Zahlbr.) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch	Fol.b
Melanohalea exasperata (De Not.) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch	Fol.b

Melanohalea exasperatula (Nyl.) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch	Fol.b
Melanohalea laciniatula (H. Olivier) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch	Fol.b
Melaspilea ochrothalamia Nyl.	Cr.in
Micarea lignaria (Ach.) Hedl. var. lignaria	Cr.in
Mycomicrothelia confusa D. Hawksw.	Cr.in
Mycoporum antecellens (Nyl.) R.C. Harris	Cr.in
Myriolecis hagenii (Ach.) Śliwa, Zhao Xin Lumbsch	Cr.in
Myriolecis sambuci (Pers.) Clem.	Cr.in
Naetrocymbe punctiformis (Pers.) R.C. Harris	Cr.in
Naevia punctiformis (Ach.) A. Massal.	Cr.in
Nephroma laevigatum Ach.	Fol.b
Normandina pulchella (Borrer) Nyl.	Sq
Ochrolechia alboflavescens (Wulfen) Zahlbr.	Cr.in
Ochrolechia androgyna (Hoffm.) Arnold	Cr.in
Ochrolechia arborea (Kreyer) Almb.	Cr.in
Ochrolechia balcanica Verseghy	Cr.in
Ochrolechia pallescens (L.) A.Massal.	Cr.in
Ochrolechia subviridis (Høeg) Erichsen	Cr.in
Opegrapha niveoatra (Borrer) J.R. Laundon	Cr.in
Opegrapha sp.	Cr.in
Pannaria rubiginosa (Ach.) Bory	Fol.n
Parmelia saxatilis (L.) Ach.	Fol.b
Parmelia submontana Hale	Fol.b
Parmelia sulcata Taylor	Fol.b
Parmelina pastillifera (Harm.) Hale	Fol.b
Parmelina quercina (Willd.) Hale	Fol.b
Parmelina tiliacea (Hoffm.) Hale	Fol.b
Parmotrema perlatum (Huds.) M. Choisy	Fol.b
Parmotrema reticulatum (Taylor) M.Choisy	Fol.b
Parmotrema stuppeum (Taylor) Hale	Fol.b
Pectenia atlantica(Degel.) P.M. Jørg., L. Lindblom, Wedin & S. Ekman	Fol.n
Pectenia plumbea (Lightf.) P.M. Jørg., L. Lindblom, Wedin & S. Ekman	Fol.n
Peltigera praetextata (Sommerf.) Zopf	Fol.large
Pertusaria coccodes (Ach.) Nyl.	Cr.co
Pertusaria coronata (Ach.) Th.Fr.	Cr.co
Pertusaria flavida (DC.) J.R.Laundon	Cr.co
Pertusaria heterochroa (Müll.Arg.) Erichsen	Cr.co
Pertusaria hymenea (Ach.) Schaer.	Cr.co
Pertusaria leioplaca DC.	Cr.co

Pertusaria pertusa (L.) Tuck. var. pertusa	Cr.co
Pertusaria pustulata (Ach.) Duby	Cr.co
Phaeophyscia ciliata (Hoffm.) Moberg	Fol.n
Phaeophyscia hirsuta (Mereschk.) Essl.	Fol.n
Phaeophyscia nigricans (Flörke) Moberg	Fol.n
Phaeophyscia orbicularis (Neck.) Moberg	Fol.n
Phaeophyscia pusilloides (Zahlbr.) Essl.	Fol.n
Phlyctis agelaea (Ach.) Flot.	Cr.in
Phlyctis argena (Spreng.) Flot.	Cr.in
Physcia adscendens (Fr.) H.Olivier	Fol.n
Physcia aipolia (Humb.) Fürnrh.	Fol.n
Physcia biziana (A.Massal.) Zahlbr. var. *biziana*	Fol.n
Physcia biziana var. *leptophylla* Vězda	Fol.n
Physcia dimidiata (Arnold) Nyl.	Fol.n
Physcia dubia(Hoffm.) Lettau	Fol.n
Physcia leptalea (Ach.) DC.	Fol.n
Physcia tenella (Scop.) DC.	Fol.n
Physcia vitii Nádv.	Fol.n
Physciella chloantha (Ach.) Essl.	Fol.n
Physconia detersa (Nyl.) Poelt	Fol.n
Physconia distorta (With.) J.R.Laundon	Fol.n
Physconia enteroxantha (Nyl.) Poelt	Fol.n
Physconia grisea (Lam.) Poelt subsp. grisea	Fol.n
Physconia grisea subsp. *algeriensis* (Flagey) Poelt	Fol.n
Physconia muscigena (Ach.) Poelt var. *muscigena*	Fol.n
Physconia perisidiosa (Erichsen) Moberg	Fol.n
Physconia servitii (Nádv.) Poelt	Fol.n
Physconia subpulverulenta (Szatala) Poelt var. *subpulverulenta*	Fol.n
Physconia venusta (Ach.) Poelt	Fol.n
Piccolia ochrophora (Nyl.) Hafellner	Cr.in
Pleurosticta acetabulum (Neck.) Elix Lumbsch	Fol.b
Porina aenea (Wallr.) Zahlbr	Cr.in
Punctelia subrudecta (Nyl.) Krog	Fol.b
Pyrenula chlorospila Arnold	Cr.in
Pyrrhospora quernea (Dicks.) Körb.	Cr.in
Ramalina canariensis J.Steiner	Frut
Ramalina farinacea (L.) Ach.	Frut
Ramalina fastigiata (Pers.) Ach.	Frut
Ramalina fraxinea (L.) Ach.	Frut
Ramalina lacera (With.) J.R.Laundon	Frut
Ramalina subgeniculata Nyl.	Frut
Ramonia chrysophaea (Pers.) Vezda	Frut

Ricasolia amplissima (Scop.) De Not. - chloromorph	Fol.large
Ricasolia amplissima (Scop.) De Not. - cyanomorph	Frut
Ricasolia virens (With.) H.H. Blom. Tønsberg	Fol.large
Rinodina colobina (Ach.) Th.Fr.	Cr.in
Rinodina exigua (Ach.) Gray	Cr.in
Rinodina pyrina (Ach.) Arnold	Cr.in
Rinodina roboris (Nyl.) Arnold	Cr.in
Rinodina sophodes (Ach.) A.Massal.	Cr.in
Rinodina sp.	Cr.in
Sanguineodiscus haematites (Chaub. ex St.-Amans) I.V. Frolov & Vondrák	Cr.in
Scoliciosporum chlorococcum (Stenh.) Vezda	Cr.in
Scoliciosporum umbrinum (Ach.) Arnold	Cr.in
Scytinium lichenoides (L.) Otálora, P.M. Jørg. Wedin	Fol.n
Scytinium teretiusculum (Wallr.) Otálora, P.M. Jørg. Wedin	Sq
Sphinctrina tubiformis A.Massal.	Cr.in
Sphinctrina turbinata (Pers.) De Not.	Cr.in
Strangospora microhaema (Norman) R.A. Anderson	Cr.in
Tephromela atra var. *torulosa* (Flot.) Hafellner	Cr.co
Thelenella sp.	Cr.in
Usnea ceratina Ach.	Frut
Varicellaria hemisphaerica (Flörke) I. Schmitt Lumbsch	Cr.co
Waynea stoechadiana (Abbassi Maaf & Cl. Roux) Cl. Roux & P. Clerc	Sq
Xanthomendoza fallax (Hepp) Søchting, Kärnefelt & S.Y. Kondr.	Fol.n
Xanthoria parietina (L.) Th. Fr.	Fol.b

A.8 Bibliography

Abatzoglou, J. T., Dobrowski, S. Z., Parks, S. A., & Hegewisch, K. C. (2018). TerraClimate, a high-resolution global dataset of monthly climate and climatic water balance from 1958–2015. Scientific data, 5(1), 1-12.

Bellocchi, G., Acutis, M., Fila, G., & Donatelli, M. (2002). An indicator of solar radiation model performance based on a fuzzy expert system. Agronomy Journal, 94(6), 1222-1233.

Frazer, G. W., Canham, C. D., & Lertzman, K. P. (1999). Gap Light Analyzer (GLA), Version 2.0: Imaging software to extract canopy structure and gap light transmission indices from true-colour fisheye photographs, users manual and program documentation. Simon Fraser University, Burnaby, British Columbia, and the Institute of Ecosystem Studies, Millbrook, New York, 36.

Hauck, M., Jung, R., & Runge, M. (2000). Does water-holding capacity of bark have an influence on lichen performance in dieback-affected spruce forests?. The Lichenologist, 32(4), 407-409.

Iqbal, M. (2012). An introduction to solar radiation. Elsevier.

Johnsen, I., & Søchting, U. (1973). Influence of air pollution on the epiphytic lichen vegetation and bark properties of deciduous trees in the Copenhagen area. Oikos, 344-351.

Limousin, J. M., Rambal, S., Ourcival, J. M., & Joffre, R. (2008). Modelling rainfall interception in a Mediterranean Quercus ilex ecosystem: lesson from a throughfall exclusion experiment. Journal of Hydrology, 357(1-2), 57-66.

Pinho P., Branquinho C., Cruz C., Tang S., Dias T.R., Rosa A.P., Máguas C., Martins-Loução M. & Sutton M. (2009). “Assessment of critical levels of atmospherically ammonia for lichen diversity in cork-oak woodland, Portugal” in “Atmospheric Ammonia - Detecting emission changes and environmental impacts - Results of an Expert Workshop under the Convention on Long-range Transboundary Air Pollution” (Sutton M, Reis S & Baker S eds.). Springer, pp. 109-119.

Ribeiro, M. C., Pinho, P., Llop, E., Branquinho, C., Sousa, A. J., & Pereira, M. J. (2013). Multivariate geostatistical methods for analysis of relationships between ecological indicators and environmental factors at multiple spatial scales. Ecological indicators, 29, 339-347.

Sadeghi, S. M. M., Gordon, D. A., & Van Stan II, J. T. (2020). A global synthesis of throughfall and stemflow hydrometeorology. In Precipitation partitioning by vegetation (pp. 49-70). Springer, Cham.

Turner, B., Hill, D. J., Carlyle-Moses, D. E., & Rahman, M. (2019). Low-cost, high-resolution stemflow sensing. Journal of Hydrology, 570, 62-68.

Valente, F., David, J. S., & Gash, J. H. C. (1997). Modelling interception loss for two sparse eucalypt and pine forests in central Portugal using reformulated Rutter and Gash analytical models. Journal of Hydrology, 190(1-2), 141-162.

Wang, D., & Wang, L. (2019). Canopy interception of apple orchards should not be ignored when assessing evapotranspiration partitioning on the Loess Plateau in C hina. Hydrological Processes, 33(3), 372-382.

Xiao, Q., McPherson, E. G., Ustin, S. L., Grismer, M. E., & Simpson, J. R. (2000). Winter rainfall interception by two mature open-grown trees in Davis, California. Hydrological processes, 14(4), 763-784.