

Standard Paper

Effects of organic substrates on growth rate parameters of a boreal cyanolichen

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Abstract

We investigated the influence of availability as well as type of organic substrate on the growth of the cyanolichen *Peltigera membranacea*. A total of 145 lichen lobes were grown in a plant growth chamber for 28 days. Of these, 73 were kept in permanent darkness and another 72 were exposed to a diurnal light-dark cycle. A third of the lobes from both treatments were grown on pulverized leaf litter, the second third on pulverized bryophytes, and the remainder were grown without an organic substrate to serve as a control group. Growth was quantified via relative growth rate, relative thallus area growth rate, and changes in specific thallus mass. The lobes kept in a diurnal light-dark cycle showed higher growth rates than those kept in darkness, as is expected for an organism that obtains its carbon from its photoautotrophic symbiosis partner. Furthermore, growth rates were higher in lobes growing on organic substrates. The results show that the availability of an organic substrate positively affects lichen growth in a growth cabinet. Leaf litter led to a higher biomass gain in lichen lobes, whereas area gain was unrelated to substrate type.

Keywords: growth cabinet; *Peltigera membranacea*; relative growth rate; relative thallus area growth rate; specific thallus mass

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Introduction

Measuring the growth of lichens in their natural environment has a long-standing tradition in lichenology (Denton & Karlén 1973; Fink 1917; Porter 1981; Proctor 1977) and a large number of studies have focused on transplantation experiments to determine lichen growth (Bidussi *et al.* 2013a; Dahlman & Palmqvist 2003; Gauslaa & Goward 2012; Gauslaa *et al.* 2007; Hilmo 2002; Merinero *et al.* 2015). Furthermore, there is great interest in developing protocols to grow lichens under controlled laboratory conditions, to detect the effects of abiotic factors on lichen growth and vitality (Alam *et al.* 2015; Bidussi *et al.* 2013b; Gauslaa *et al.* 2016; Gauslaa *et al.* 2021). Despite a large number of studies, the exact factors that influence lichen growth are still not fully understood. However, it is most likely that a combination of internal and external factors (Gauslaa *et al.* 2009), including temperature, precipitation, light intensity, day length, thallus moisture, the substratum, quantity of pollution, is of relevance (Armstrong 2015; Benedict 1990). Even the type and composition of the water that lichens come into contact with might affect their growth (Armstrong 1977). Thallus size also influences growth rate: in crustose lichens, small thalli have low but increasing radial growth rates whereas large thalli exhibit higher radial growth rates that remain more or less constant over time (Armstrong & Smith

1996), and relative growth rates have been shown to decline in larger thalli of foliose lichens (Larsson & Gauslaa 2011). Mass gain in lichens is so far better understood than area gain and has been demonstrated to be dependent on photosynthetic activity and hence light (Larsson *et al.* 2012).

Research regarding the influence of substrate on lichen growth is relatively scarce (e.g. Colesie *et al.* 2012; Ficko *et al.* 2023; Tolpysheva & Timofeeva 2008). Substrate has mainly been a research object in studies focusing on lichen diversity, composition and establishment (Löhmus *et al.* 2023; Pereira *et al.* 2014; Rosabal *et al.* 2013; Roturier *et al.* 2007; Spier *et al.* 2010). Some lichens show high substrate specificity while the majority have a broader substrate spectrum (Brodo 1973; Resl *et al.* 2018) and changes in substrate preference have been described for several species (Osyczka & Węgrzyn 2008; Vondrák & Liška 2010). In general, it has been proposed that various chemical (e.g. pH value, mineral content) and physical properties (e.g. surface texture, water-holding capacities) of the substrate affect lichen composition as well as growth (Armstrong & Bradwell 2011; Brodo 1973; Favero-Longo & Piervittori 2010; Henssen & Jahns 1974a; Rosabal *et al.* 2013). Furthermore, substrate seems to be important for the lichen's bacterial community composition (Cardinale *et al.* 2012; Leiva *et al.* 2021) and might possibly serve as a photobiont source (Zúñiga *et al.* 2017). To determine the effect of substrates on lichen growth, we collected bryophytes both adhered to and growing in the immediate vicinity of our study object, the foliose macrolichen *Peltigera membranacea* (Ach.) Nyl. Additionally, we collected fallen leaves from the

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same site as we noticed that many *P. membranacea* specimens were tightly attached to decaying (i.e. discoloured and brittle) leaves.

We conducted a 28-day growth experiment with *P. membranacea* lobes to test the effect of organic substrate availability and organic substrate type on lichen growth. In order to identify lichen growth, the area (A), dry mass (DM), relative growth rate (RGR), relative thallus area growth rate (RT_AGR), and changes in specific thallus mass (ASTM) of the lichen lobes were determined. The substrates were ground before the experiment to standardize conditions. Half of the samples were grown in darkness to investigate the possibility of saprotrophic nutrition, since we saw hyphae of *P. membranacea* growing into leaves while handling the lobes. Also, several studies have reported potential saprotrophic activities in lichens as well as the presence of enzymes that may allow some lichenized fungi to obtain carbon via saprotrophic nutrition (Beckett *et al.* 2013; Resl *et al.* 2022; Wedin *et al.* 2004).

Organic substrates are expected to promote lichen growth not only by facilitative physical properties (e.g. increased water-holding capacities) but also by potentially providing nutrients. Therefore, we hypothesize that 1) lichen lobes placed on organic substrates exhibit increased growth rates, relative to lobes growing without substrate. As leaves and bryophytes differ in chemical composition (e.g. Carnol & Bazgir 2013; Melick & Seppelt 1992) and physical properties (e.g. Kim *et al.* 2023; Michel *et al.* 2013), we also hypothesize that 2) lichen growth rates differ between these substrate types. Finally, we tried to determine whether there is any evidence in the growth rate parameters that would suggest that lichen lobes might switch to saprotrophic nutrition when growing in the dark. This should be reflected as 3) growth, or at least a less severe loss, in biomass of dark-treated lobes placed on organic substrates compared to those without a substrate.

Materials and Methods

Study species and biological material

The lichen *Peltigera membranacea* (Supplementary Material Fig. S1, available online) was used as the study species; it is a foliose lichen which grows on damp mineral soil, humus, among bryophytes, on damp rocks, stumps and rotten wood in mesic to humid forests (Galloway 2000; Goffinet & Hastings 1994; Goward *et al.* 1995). This species occurs in temperate and boreal regions of the Northern Hemisphere where it is usually found on the western side of continents. The photobiont of this lichen symbiosis is the cyanobacterium *Nostoc* sp. (Miao *et al.* 1997; Werth *et al.* 2021). Species of the suborder *Peltigerineae* are relatively fast growing (Beckett *et al.* 2003); thus *P. membranacea* should be a suitable candidate for growth experiments.

The lichen specimens were collected on 20 March 2015 at Öskjuhlíð, a hill located in Reykjavík, Iceland, by Sophie S. Steinhäuser and Ólafur S. Andrésson. Collections took place in a forest consisting of *Betula pubescens* Ehrh. with some *Sorbus aucuparia* L., where the lichens grew on the ground among bryophytes (*Rhytidiadelphus* sp. and *Hylocomiadelphus* sp.). The thalli were air-dried at room temperature and shipped to Graz, Austria, where they were immediately prepared for the experiment upon arrival (27 March 2015). For the experiment, 145 lobes were selected, based on their vitality and size. Damaged lobes were excluded from the experiment; the same is true for lobes that were too small or too large, since we tried to use similarly sized specimens as they should have similar growth rates. The selected lobes were inspected for any substrate adhering

to them under a stereomicroscope (WILD Heerbrugg M3B), and any substrate detected was removed using fine forceps as, 1) it would influence the weight of the lichen lobes; 2) every lobe was grown on a defined amount of substrate (or on none) and any already present on the lobes would change the total amount of substrate. Cleaned lobes were temporarily stored in a fridge until further processing (i.e. weighing and photographing).

The pH values of the substrates were determined with a pH meter by dissolving 2 g of each substrate in 10 ml of distilled water. After the experiment, substrate adhering to the lobes was removed carefully so it would not affect the weight measurements.

Experimental set-up

Lichen lobes were divided into two major groups: one group was exposed to a 12-h light/12-h dark cycle using the energy-saving lamp Elektrox 250W Grow 6500K (100 $\mu\text{mol m}^{-2} \text{s}^{-1}$); the other group was kept in permanent darkness inside a box in a plant growth chamber (Vötsch VB 0714, 1998). The light intensity setting of 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ was chosen as it comes close to the light saturation of *Peltigera neopolydactyla* (Gyeln.) Gyeln. (c. 120 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and *Peltigera malacea* (Ach.) Funck (< 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$) (Sundberg *et al.* 1997). The temperature of the growth chamber was set to 12 °C during the light periods and to 10 °C during darkness, to simulate conditions of a summer day-night rhythm in Iceland (Bjornsson *et al.* 2007). The relative humidity in the growth chamber was c. 85% for the light periods and 70% for the dark periods. Temperature [°C], dew point [°C] and relative humidity [%] were measured throughout the experiment (EL-USB-2 data logger; Supplementary Material Fig. S2, available online).

A third of the specimens of both groups were grown in Petri dishes (92 × 16 mm) on 2 g of powdered leaf litter (Supplementary Material Fig. S3, available online) (24 in light, 25 in darkness), another third on 2 g of powdered bryophytes (Supplementary Material Fig. S3) (24 in light, 23 in darkness), and the last third was grown in empty Petri dishes as control (24 in light, 25 in darkness). Leaves and bryophytes were collected from the same site as *P. membranacea* and therefore represent substrates that the lichen specimens encountered in their natural environment. Additional organic and inorganic materials such as grass, wood and plastic were removed from the leaves and bryophytes; in addition, bryophytes were sorted out from leaves and vice versa. Leaves and bryophytes were air-dried and ground with a kitchen mill. The leaf and bryophyte substrates were powdered in order to standardize conditions, so that each sample in the respective experimental groups receiving organic material was exposed to an identical substrate. The substrate was placed in Petri dishes and moistened with distilled water; the lichen lobes were softly pressed onto the substrate to ensure contact of the rhizines with the substrate. The lobes were sprayed daily with approximately equal amounts of distilled water dispensed via a spray bottle (pumping five times per lobe, per day). As the Petri dishes were closed throughout the growth experiment, the relative humidity inside the dishes was most likely higher than that stated for the growth chamber. Once a week the lichens were left to dry out to prevent the lobes and the substrate from getting mouldy (Y. Gauslaa, personal communication).

Measurements

The cleaned and air-dried lobes were weighed using an analytical balance (A&D Instruments Ltd) with a precision of 0.1 mg. Five

additional lobes were first air-dried and weighed, then oven-dried for 48 h at 65 °C and immediately weighed to account for differences in air humidity. Dry masses were adjusted proportionally to account for the reduced weight of oven-dried to air-dried specimens. In order to determine the area of the lichen lobes, they were moistened, placed between two glass sheets to flatten them, and then photographed with a Canon EOS 7D SLR digital camera (maximum resolution 5184 × 3456 pixels) equipped with a Canon Ef-S 60 mm/2.8 macro lens. Each lobe was photographed four times, and was rotated for every photograph. Afterwards, the lobes were placed onto the moistened substrates or into empty Petri dishes and all specimens were sprayed with distilled water. The area of the lobes was calculated with ImageJ v. 1.48v (Schneider *et al.* 2012); for each lobe the area was determined four times, based on the four photographs, and then averaged to obtain the mean area of every lobe. ImageJ recognizes only green structures, so parts of the lobes that were not green (as the glass sheets reflected light) were coloured to that of the lichen in GIMP 2 (available from <http://www.gimp.org/>) prior to determining the thallus area. After the growth period, the lobes were weighed, photographed and analyzed as before. Ten of the samples were oven-dried to account for ambient air humidity.

Dry mass (DM) and area (A) values were measured before and after the experiment. Mass difference [mg] was calculated as $DM_{\text{end}} - DM_{\text{start}}$ and area difference [mm^2] as $A_{\text{end}} - A_{\text{start}}$. DM was used to compute the relative growth rate (RGR) following Bidussi *et al.* (2013a), with $RGR = (\ln(DM_{\text{end}}/DM_{\text{start}})) * 1000 / \Delta t$ ($\text{mg g}^{-1} \text{day}^{-1}$), where t is the number of days the lichens were cultivated (28 days). The relative thallus area growth rate (RT_AGR) was also used to determine lichen growth, with $RT_AGR = (\ln(A_{\text{end}}/A_{\text{start}})) * 100 / \Delta t$ ($\text{mm}^2 \text{cm}^{-2} \text{day}^{-1}$) (Bidussi *et al.* 2013b). Specific thallus mass (STM) was calculated with the values determined before and after the experiment, with $STM = DM/A$. Changes in STM were calculated with the following formula: $\Delta STM = 100 * (STM_{\text{end}} - STM_{\text{start}}) / STM_{\text{start}}$ (mg mm^{-2}) (Bidussi *et al.* 2013b).

Statistical analyses

Statistical analyses were performed with R v. 4.2.1. The R package *stats* was used to conduct paired-samples *t*-tests to assess whether the area and biomass differences before and after the growth experiment were significant. Furthermore, two-way ANOVAs were run to determine the statistical significances of the parameters 'RGR', ' RT_AGR ' and 'STM' (Table 1). Tukey's HSD post-hoc tests were conducted in R (using the 'TukeyHSD' function) for significant results (P -values ≤ 0.05) (R Core Team 2022). The effect size was computed using the package *rstatix* (function 'cohens_d') (Kassambara 2023). Data was visualized in R using the package *ggplot2* (Wickham 2016) and *multcompView* was used for compact letter displays to indicate significant differences (Graves *et al.* 2023).

Results

The leaf substrate had a pH value of 6.89 and the moss substrate of 7.22. The pH value of the distilled water was 6.96. During the course of the growth experiment, it was noticeable that light-treated lobes dried faster than dark-treated ones within a day. Also, lobes growing in empty Petri dishes stayed hydrated for longer, whereas the added water was absorbed by the organic substrates in the treatment groups. While the bryophytes absorbed

most of the water, the leaves were more hydrophobic (probably due to cuticular waxes), causing small drops of water to remain on the surface. The lobes did not desiccate completely after one day, but stayed damp even when growing on organic substrates and in light. Once a week, the lichen lobes were left to dry out; the substrates however stayed moist, especially the ground bryophytes. In the leaf litter treatments, the water settled at the bottom of the Petri dishes, and in the control groups, water did not evaporate either but collected at the edge of the dishes.

RGRs were negative for lobes that were kept in permanent darkness, whereas lobes grown in a diurnal light-dark cycle exhibited positive RGRs (Fig. 1; Supplementary Material Table S1, available online). These differences were highly significant (difference in means = $8.7 \text{ mg g}^{-1} \text{day}^{-1}$, adjusted $P < 0.001$) (Table 2). RGRs were significantly higher (i.e. positive) for light-treated samples grown on organic substrates compared to light-treated samples without substrates (difference in means = $5.7 \text{ mg g}^{-1} \text{day}^{-1}$, adjusted $P < 0.001$), whereas there were no significant differences in RGRs between substrate type (difference in means = $1.1 \text{ mg g}^{-1} \text{day}^{-1}$, adjusted $P = 0.478$). In general, lobes growing without a substrate showed either low (light-dark treatments) or negative (dark treatments) values. RGRs were similar for all dark treatments (difference in means $\leq 1.4 \text{ mg g}^{-1} \text{day}^{-1}$, adjusted $P \geq 0.193$). Overall, light had the largest effect on RGR and substrate availability a moderate effect, while the effect of substrate type was negligible (Table 3; Supplementary Material Table S2, available online).

On average, RT_AGR s were smaller for specimens kept in permanent darkness than for specimens exposed to a diurnal light-dark cycle; the values were positive for most treatments, with the exception of the treatment 'dark_empty' (Fig. 1, Supplementary Material Table S1). All comparisons between lobes grown with light and those grown in darkness were significantly different (difference in means = $0.51 \text{ mm}^2 \text{cm}^{-2} \text{day}^{-1}$, adjusted $P \leq 0.001$) (Table 2). The availability as well as type of substrate did not affect RT_AGR s; there were neither significant differences within the three dark-treated groups (difference in means $\leq 0.16 \text{ mm}^2 \text{cm}^{-2} \text{day}^{-1}$, adjusted $P \geq 0.327$) nor within the three light-treated groups (difference in means $\leq 0.19 \text{ mm}^2 \text{cm}^{-2} \text{day}^{-1}$, adjusted $P \geq 0.150$). Light had the largest effect on RT_AGR , while substrate availability had a small to moderate effect. The effect of substrate type on RT_AGR was small (Table 3, Supplementary Material Table S2).

Changes in STM were on average higher for treatments exposed to a light-dark cycle than for dark treatments (difference in means = 9.8 mg mm^{-2} , adjusted $P < 0.001$) (Fig. 1, Supplementary Material Table S1). Comparing the treatments and experimental groups in more detail regarding ΔSTM showed highly variable results (Table 2, Supplementary Material Table S3). While there were no significant differences within the dark treatments (difference in means $\leq 3.8 \text{ mg mm}^{-2}$, adjusted $P \geq 0.259$), all ΔSTM s within the light-treated groups were significantly different (difference in means $\leq 14.4 \text{ mg mm}^{-2}$, adjusted $P \leq 0.026$), highlighting an influence of substrate availability as well as substrate type on ΔSTM . Changes in STM were highest (i.e. positive) for lichen lobes growing on leaf litter and second highest for those growing on bryophytes, whereas the ΔSTM s were on average negative for the treatment 'light_empty' as well as for the dark-treated groups. The treatment 'light_empty' did not differ significantly from any of the dark treatments (difference in means $\leq 4.1 \text{ mg mm}^{-2}$, adjusted $P \geq 0.199$). Both light and substrate availability had a large effect on ΔSTM , with lobes growing on leaves exhibiting increased ΔSTM when compared to lobes growing in empty

Table 1. ANOVA (two-way) table for relative growth rate (RGR), relative thallus area growth rate (RT_AGR), and changes in specific thallus mass (Δ STM) of *Peltigera membranacea* grown under different conditions. Results are given for the categories light (L, light vs dark), substrate (S, bryophytes vs leaves vs empty), and the interaction of light and substrate (L:S). Significant results are indicated with *

Parameter	df	RGR		RT _A GR		Δ STM	
		F	P	F	P	F	P
light (L)	1	596.3	<2e-16*	127.8	<2e-16*	95.3	<2e-16*
substrate (S)	2	41.6	6.91e-15*	5.0	0.008*	21.0	1.11e-08*
L:S	2	18.9	5.73e-08*	0.05	0.952	16.1	5.25e-07*

Note: 'L:S' is the combination of light and substrate and is made up of six groups: 'light_leaves', 'light_bryophytes', 'light_empty', 'dark_leaves', 'dark_bryophytes' and 'dark_empty'.

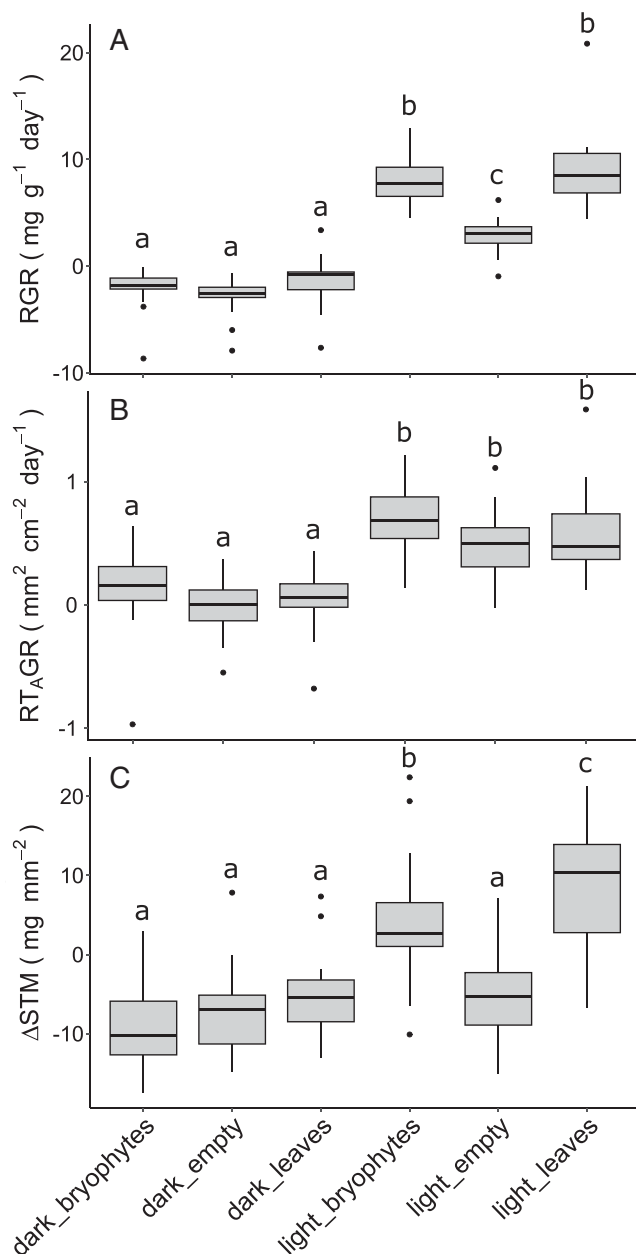


Figure 1. Boxplots showing the effects of light availability (dark vs light), substrate availability (bryophytes/leaves vs empty) and organic substrate type (bryophytes vs leaves) on the different growth parameters of the lichen *Peltigera membranacea*. A, relative growth rate (RGR) ($\text{mg g}^{-1} \text{day}^{-1}$). B, relative thallus area growth rate (RT_AGR) ($\text{mm}^2 \text{cm}^{-2} \text{day}^{-1}$). C, changes in specific thallus mass (Δ STM) (mg mm^{-2}). Different letters above the bars indicate statistically significant differences (adjusted $P \leq 0.05$) (see also Table 2).

Petri dishes. The effect size of bryophytes on Δ STM was small, as was that of substrate type (Table 3, Supplementary Material Table S2).

Discussion

In general, the rate of growth of lichens under experimental conditions depends on the species (Dahlman & Palmqvist 2003; Gauslaa & Goward 2012; Hilmo 2002). The species we used for this experiment, *P. membranacea*, belongs to a genus of rather fast-growing lichen-forming fungi (Henssen & Jahns 1974b) and we were able to measure significant growth differences after a 28-day growth period (Supplementary Material Table S4, available online). The results of our study show that growth of *P. membranacea* lobes depends on light as well as substrate availability and, to a certain extent, on the type of substrate. The measured growth parameters were positive for lobes that had been exposed to a diurnal light-dark cycle, and were even higher when an organic substrate was also used.

Light influences the photosynthetic activity of the photobiont; therefore, the carbon balance of lichen lobes exposed to light for a period of time is likely to be positive, which is reflected in an increase in biomass and hence RGR (Larsson *et al.* 2012; Palmqvist 2000). The mycobiont, rather than the photobiont, seems to contribute to the area growth of lichens, as fungal hyphae expand when hydrated (Larsson *et al.* 2012). Hydration also initiates a lichen's physiological activity, and lichens with long active periods have been shown to have higher area growth rates (Raggio *et al.* 2018; Sancho *et al.* 2011). Hence humidity along with light are the main factors influencing RT_AGR (Larsson *et al.* 2012). In fast-growing lichens of the genus *Lobaria*, comparable growth rates (RGR, RT_AGR) were observed when lichens were hydrated not only during the daytime but also at night (Bidussi *et al.* 2013b). We did not measure the effect of hydration and desiccation on lichen growth in our study, but during the course of the experiment we sprayed the lichen lobes regularly to make sure they remained hydrated. We observed that lobes grown in a diurnal light-dark cycle in empty Petri dishes, as well as dark-treated lobes, stayed hydrated for longer during the course of the day, yet they showed only minor increases and sometimes even decreases in RT_AGRs.

Changes in STM were positive only in lichen lobes grown in a light-dark cycle with organic substrate. Thus, both light and substrate seem to be essential for Δ STM. The organic substrate partly absorbed the water added, so it might serve as a water reservoir with lobes taking up water from the substrate when needed, which could reduce water stress and extend periods of photosynthetic activity (Colesie *et al.* 2012). In empty Petri dishes, liquid water partially covered the bottom of the dish right after spraying,

Table 2. Results of the post-hoc tests (TukeyHSD) which were performed to determine potential effects of light availability, substrate availability, and substrate type on the growth of the lichen *Peltigera membranacea*. Differences in means (diff.) and adjusted *P*-values (*P* adj.) are shown for the growth parameters relative growth rate (RGR), relative thallus area growth rate (RT_AGR), and changes in specific thallus mass (ΔSTM). Significant results are indicated with *

Light	RGR [mg g ⁻¹ day ⁻¹]		RT _A GR [mm ² cm ⁻² day ⁻¹]		ΔSTM [mg mm ⁻²]	
	diff.	<i>P</i> adj.	diff.	<i>P</i> adj.	diff.	<i>P</i> adj.
light vs dark	8.7	< 0.001*	0.51	< 0.001*	9.8	< 0.001*
Substrate						
leaves vs bryophytes	0.8	0.131	-0.08	0.287	4.5	0.001*
empty vs bryophytes	-2.9	< 0.001*	-0.18	0.005*	-3.3	0.021*
leaves vs empty	3.8	< 0.001*	0.09	0.222	7.9	< 0.001*
Light:substrate						
dark_leaves vs dark_bryophytes	0.7	0.895	-0.08	0.900	3.8	0.259
dark_leaves vs dark_empty	1.4	0.193	0.08	0.915	1.6	0.933
dark_bryophytes vs dark_empty	0.7	0.832	0.16	0.327	-2.2	0.816
light_leaves vs light_bryophytes	1.1	0.478	-0.09	0.886	5.5	0.026*
light_leaves vs light_empty	6.2	< 0.001*	0.11	0.756	14.4	< 0.001*
light_bryophytes vs light_empty	5.1	< 0.001*	0.19	0.150	8.9	< 0.001*
light_leaves vs dark_leaves	10.4	< 0.001*	0.52	< 0.001*	14.7	< 0.001*
light_bryophytes vs dark_bryophytes	10.0	< 0.001*	0.52	< 0.001*	13.0	< 0.001*
light_empty vs dark_empty	5.6	< 0.001*	0.49	< 0.001*	1.9	0.881

Table 3. Results of the Cohen's *d* test for effect size of different growth parameters for the lichen *Peltigera membranacea*. The effect sizes (effsize) and magnitude of the effect sizes are shown for the growth parameters of relative growth rate (RGR), relative thallus area growth rate (RT_AGR), and changes in specific thallus mass (ΔSTM). Results are given for the categories light vs dark, empty vs bryophytes, empty vs leaves, and leaves vs bryophytes

Parameter	RGR		RT _A GR		ΔSTM	
	effsize	magnitude	effsize	magnitude	effsize	magnitude
light_dark	3.00	large	1.84	large	1.33	large
empty_bryophytes	0.70	moderate	0.50	moderate	0.47	small
empty_leaves	0.78	moderate	0.25	small	1.01	large
leaves_bryophytes	0.12	negligible	0.25	small	0.46	small

which could negatively affect lichen growth and vitality, especially in *Peltigera* species which lack a lower cortex (Büdel & Scheidegger 2008). Supersaturation of lichen thalli impedes gas exchange and subsequently photosynthesis (Gauslaa *et al.* 2016; Lange *et al.* 1996). This phenomenon also occurs in nature and has been observed for epilithic and soil crust lichens after heavy rainfall (Colesie *et al.* 2016; Lange 2003; Lange *et al.* 1997; Lange & Green 1996). *Peltigera membranacea* might also be affected by supersaturation under natural conditions, since this species is known to grow on rocks, soil and wood which are substrate types where water can accumulate after rainfall (Galloway 2000; Goffinet & Hastings 1994). Colesie *et al.* (2012) have shown that moss-associated *Peltigera rufescens* specimens had increased growth rates compared to those associated with soil and they propose facilitative interactions between lichens and bryophytes based on eco-physiological mechanisms including water retention. Hydration leads to prolonged physiological activity, which in turn increases lichen growth rates (Raggio *et al.* 2018;

Sancho *et al.* 2011). Additionally, net photosynthesis rates in lichens are highest in thalli during drying, that is, when the lichens are still hydrated but no longer supersaturated (Colesie *et al.* 2016; Lange 2003). The organic substrates used in our study could have retained and slowly released water, optimizing the thallus water content of the lichen lobes and extending their physiological activity. This might explain the higher growth rates in these treatments. In the empty Petri dishes, liquid water could have caused supersaturation, at least within a period of time after spraying. Since all lichen lobes were left to dry out once a week, potential supersaturation effects were most likely short in duration. Nonetheless, the control group used in this study was not ideal and we propose that the control might be improved in future experiments by growing lichens on nets (Gauslaa *et al.* 2016) or by using inorganic, water-retaining substrates to examine the effects of hydration; moreover, Petri dishes could be tilted to allow water run-off. However, growing lichens in empty Petri dishes as a control is informative as it represents an

unfavourable substrate type, which can occur in nature (e.g. a rock surface).

The type of organic substrate partially affected lichen growth, with lobes growing on leaf litter having significantly higher Δ STMs (and higher gains in biomass; data not shown) than lobes growing on bryophytes. As leaves and bryophytes have similar pH values, it is unlikely that this factor directly affected the observed changes in STM. It seems more likely that the organic substrates used in this study differ in their water-holding and retention capacities, which would influence growth rates. As poikilohydric organisms, bryophytes absorbed and retained the water added, which could have made it more difficult for the lichen lobes to take it up. Furthermore, in some of the Petri dishes, the bryophytes started to grow during the experiment, indicating that some stayed vital throughout. The leaf litter also absorbed the water, but drops of water remained on the litter surface, so the leaves did not retain the water to the same extent as the bryophytes. Measuring the water content of lobes growing on different organic substrates would be necessary to assess which treatments stayed hydrated for longer and which desiccated faster. This could also help determine the length of physiological activity of the lobes. Moreover, grinding the substrates presumably changed their hydraulic properties and the observed growth effects could be a result of the experimental set-up rather than of natural conditions. Nonetheless, the main properties of both substrates used in the experiment resembled those of their natural counterparts, since leaves retained water to a lesser extent (e.g. Kim *et al.* 2023) than bryophytes, which stored the added water (e.g. Michel *et al.* 2013). Repeating the experiment with unground organic substrates could give further insight into natural lichen-substrate interactions.

Additionally, the presence of certain substances in the leaves could explain the significantly higher STM values in lichen lobes growing on leaf litter. The leaves were partly decayed, since they were collected in March, and it might be easier for lichens to obtain nutrients from partially broken-down leaf litter than from ground fresh bryophytes. Lichens absorb dilute nutrients through their entire surface, and rain and dew are major sources for nutrient uptake (Nash 2008). Additionally, nutrient capture in lichens is dependent on their water-holding capacity (Gauslaa *et al.* 2021). De Bruin & Hackenitz (1986) proposed the possibility of nutrient uptake from water running off bark, at least when high concentrations of trace elements are present in the substrate. Hence, substrates might indirectly supply nutrients, for example by substance leaching induced via wetting. Watering the lobes during the experiment could have leached nutrients from the substrates, especially from the decaying leaf litter. Nutrients released during decomposition of birch leaves include (but are not limited to) nitrogen (N) and phosphorus (P) (Berg & Staaf 1987), while bryophytes have been shown to lose N as well as carbohydrates via leaching (Liu *et al.* 2020; Melick & Seppelt 1992). Rowan leaf litter also contains N and P but has higher concentrations of magnesium (Mg), potassium (K) and calcium (Ca) than birch leaf litter (Carnol & Bazgir 2013). A different chemical composition of leached water could explain the substrate type-dependent differences in Δ STM. However, measuring nutrient availability and uptake, for example by tracing stable isotopes, was beyond the scope of our study. Also, in our experiment, lichen lobes grown in darkness showed only minor increases in RT_A GR as well as decreases in RGR and Δ STM, although they stayed vital. Therefore, it seems unlikely that lichens are able to switch to saprotrophy to obtain nutrients


from their substrates in the dark. Additional studies looking into nutrient concentrations and transfer in the thalli, substrates, and water in contact with both the thalli and the substrates are necessary to draw firm conclusions. Similarly, testing the presence and activity of certain carbohydrate-degrading enzymes (e.g. de los Ríos *et al.* 1997; Resl *et al.* 2022), especially in lichen rhizines, could provide information about potential substrate-specific effects on lichen growth and fitness.

Conclusions

In this study, we showed that growth of lichens, despite being mainly dependent on light, is also influenced by the availability and, to a certain extent, the type of organic substrate. The measured growth parameters were highest in lichen lobes growing on organic substrates in a diurnal light-dark cycle. Additionally, the results indicate that *P. membranacea* can be grown reliably under controlled laboratory conditions, and that lichen growth in the laboratory could be improved by growing lichens on the organic substrates with which they are associated in nature. While area gain was unrelated to substrate availability and type, biomass gain was significantly higher in lobes growing on organic substrates, and especially in lobes growing on leaf litter. Further studies regarding the physical and chemical properties of organic substrate types are necessary to understand why leaves are a more suitable substrate for lichen growth in the laboratory than bryophytes.

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