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Rehydration and photosynthetic reactivation of old forest cephalolichen members of *Lobaria* in humid air

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Abstract

Most lichens tolerate desiccation, yet sufficient hydration remains a general prerequisite to photosynthesis and growth. Species-specific morphologies likely reflect important hydration traits and may therefore provide insight to niche preferences and species ranges. This study quantifies and compares kinetics of rehydration in humid air and concurrent PSII reactivation in the co-occurring yet morphologically and functionally distinct cephalolichens *Lobaria amplissima*, *L. pulmonaria* and *L. virens*. High-temporal resolution monitoring of rehydrating thalli by automatic weighing combined with chlorophyll fluorescence imaging of maximal PSII efficiency (F_v/F_m) was applied to determine species-specific rates of water vapor uptake and photosynthetic activation. The thinner thalli of *L. pulmonaria* and *L. virens* rehydrate and reactivate faster in humid air than the thicker thalli of *L. amplissima*. The thin and loosely attached lobes of *L. pulmonaria* are especially well-designed for water vapor harvest, providing a flexible hydration strategy consistent with its broader geographical distribution, which stretches from rainy coastal forests to humid continental forests. The thick, resupinate *L. amplissima* reactivates more slowly in humid air, but can store greater amounts of water when provided in abundance, prolonging active periods after saturating hydration events. This water conserving strategy could represent an advantage where abundant rain or stem flow alternates intense drying. Understanding the links between morphological traits, functional responses and their ecological implications for species at risk is crucial to conservation planning and has the potential for modelling populations under various climate scenarios.

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1. Introduction

Lichens arise from intimate associations between fungal hyphae and green algae, cyanobacteria or both. The resulting symbiotic organism lacks specialized structures for water uptake, transport and release, and thus has no active mechanisms to prevent desiccation. Such organisms, defined as poikilohydric, exchange water directly through their outer surfaces, tending towards a hydrological equilibrium with the ever-changing ambient conditions (Green et al., 2011). Lichens may therefore experience great fluctuations in water content, depending on the hydration dynamics of their habitat.

The poikilohydric strategy allows lichens to pursue an opportunistic life style, with metabolic activation during favorable moisture conditions in the form of rain, dew or humid air, alternated by dormancy and low metabolic rate during dry periods (Kershaw, 1985). Most lichens tolerate drought to some degree (Kranner et al., 2008), yet sufficient hydration remains a general prerequisite to sustain photosynthesis and growth (Bidussi et al., 2013; Lange et al., 1986). The frequency and intensity of hydration events differ among habitats (Gauslaa, 2014), and constitute an important ecological constraint to species' ranges (Lidén et al., 2010). Thus, the spatial distribution of lichen species often reflects hydrological patterns shaped by climate (Ellis, 2016; Giordani & Incerti, 2008).

Lichens have evolved morphological strategies to reach and prolong the hydrated state (Gauslaa & Solhaug, 1998; Larson & Kershaw, 1976), maximizing photosynthetic output under specific hydration regimes (Palmqvist, 2000). An important morphological trait varying within (Gauslaa & Coxson, 2011) and among (Esseen et al., 2015) species shaping their capacity to store water, is specific thallus mass (STM), a proxy of thallus thickness. A thick thallus can store more water within its tissues than a thinner thallus, exhibiting higher internal water-holding capacity ($WHC_{internal}$). High $WHC_{internal}$ enables lichens to harvest water when occasionally abundant, and to some extent retain the hydrated state beyond such events (Gauslaa et al., 2017). Thick lichens therefore thrive in habitats where periodic rainfall constitutes an important hydration source, such as in the top canopy of a forest stand (Merinero et al., 2014), in temporal drainage channels on rocks (Phinney et al., 2018), on hill tops in the general landscape, or in oceanic climates on a regional scale (as reviewed by Gauslaa, 2014).

A thin thallus offers less room for water storage, yet has a higher surface area–to-mass ratio compared to a thicker one, and thus has greater exposure to ambient air. A high surface area-

to-mass ratio assures efficient water absorption (Larson, 1981), but entails equally efficient water loss (Larson & Kershaw, 1976; Larson, 1979). When water is available only as vapor, species with thinner or more dissected growth forms rehydrate and reactivate photosynthesis more rapidly than thicker species (Phinney et al., 2018), although this is only documented for a few species so far. Lichens with thin thalli therefore seem optimized for habitats characterized by frequently high air humidity, conditions found in the shaded lower canopy of a forest stand, in ravines and north-facing slopes in the landscape, and in continental regions experiencing strong nocturnal cooling and less wind (as reviewed by Gauslaa, 2014).

Lichens' water demand for photoactivation is further influenced by their photobiont type (Schlensog et al., 2000). While green algal chlorolichens readily reactivate in humid air, cyanolichens having cyanobacteria as their only photobiont require liquid water in the form of rain or dew to resume photosynthesis (Lange et al., 1986; Lange et al., 1993). Green algal lichens with supplementary colonies of cyanobacteria, known as cephalolichens, exhibit intermediate WHC (Gauslaa & Coxson, 2011). Their water requirements for photoactivation are less known than for chloro- and cyanolichens (but see Lidén et al., 2010).

Photosynthetic activity in lichen thalli at different hydration levels can be quantified by chlorophyll fluorescence imaging. Chlorophyll fluorescence represents the energy fraction derived from absorbed light that is not utilized in photosynthesis nor dissipated as heat (Maxwell & Johnson, 2000). Thus, the yield of chlorophyll fluorescence is inversely proportional to PSII efficiency and provides valuable insight to photosynthetic performance. Chlorophyll fluorescence imaging allows non-invasive measurements of functional responses in entire lichen thalli and has become a widespread approach in eco-physiological studies of lichens. Such techniques have allowed comparisons of photosynthetic activation in green algal versus cyanobacterial lobes of tripartite lichens in humid air (Schlensog et al., 2000), quantification of activation time-lags in hydrophilic species upon hydration (Lidén et al., 2010), assessments of the duration of photoactive periods during desiccation (Gauslaa et al., 2017), and visualization of the photosynthetic resurrection of dry lichen thalli when exposed to humid air (Phinney et al., 2018). However, apart from Phinney et. al 2018, all these studies have measured PSII reactivation at low temporal resolution.

The species employed in this study, *Lobaria amplissima* (Scop.) Forssell, *L. pulmonaria* (L.) Hoffm. and *L. virens* (With.) J.R. Laundon, are conspicious, foliose cephalolichens belonging to the continuity-favored *Lobarion* community (Rose, 1976). *Lobaria amplissima* and *L. virens* were recently assigned to a separate genus and reported as *Ricosalia amplissima* (Scop.) De

Not. and *Ricosalia virens* (With.) H. H. Blom & Tønsberg (Tønsberg et al., 2016). The three species often share substrate and habitat preferences, and typically grow as epiphytes on old, broad-leaved deciduous trees, but also occur among bryophytes on rock walls (Holien & Tønsberg, 2006, p. 112). *Lobaria pulmonaria* further thrives on spruce twigs in boreal rainforests (Holien & Tønsberg, 1996). In Norway, the three species are especially abundant in coastal lowland forests from Vestfold to Nordland, even if *L. pulmonaria* has a wider distribution than the other two, including continental forests in eastern parts of Norway (Fig. 1) (Artsdatabanken, 2018; Bratli & Halvorsen, 2018). *Lobaria virens* is more exclusive to coastal lowland forests, and is presumably restricted to milder climates due to lower frost tolerance (Solhaug et al., 2018). A recent classification of British epiphytes according to their dependency on various measures of oceanity (Ellis, 2016), report all three species as indicators of oceanic temperate rainforests, with *L. virens* having the strongest association to an oceanic climate and *L. pulmonaria* the weakest. In Italy, *L. pulmonaria* is further shown to be a valid indicator of species richness and occurrence of nationally rare lichen species across forest types and bioclimatic conditions (Nascimbene et al., 2010).

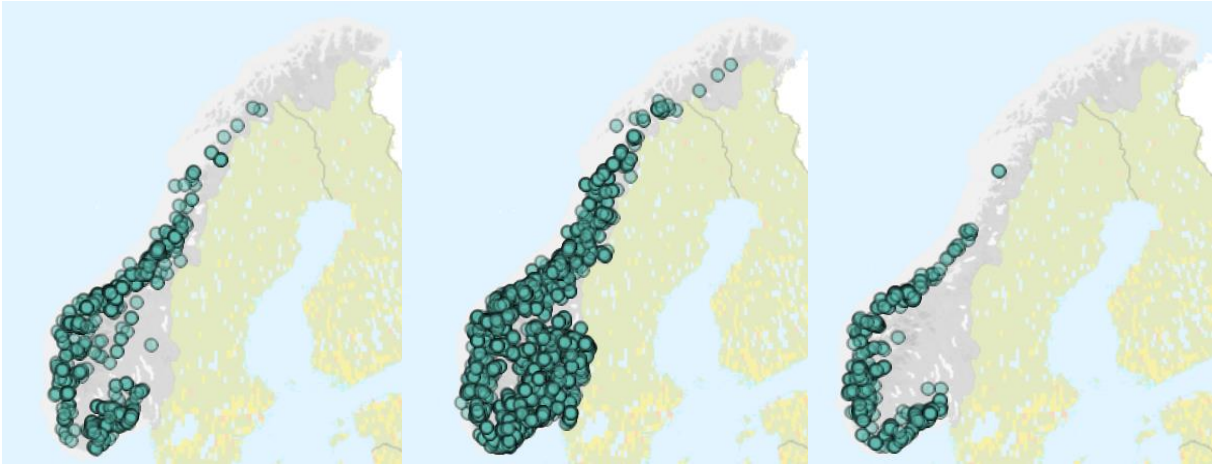

Fig. 1 Maps showing the current distribution of *Lobaria amplissima* (left), *L. pulmonaria* (middle) and *L. virens* (right) in Norway. Green points represent species observations with coordinate precisions of minimum 10m reported by citizens and professionals between 2000 and 2018 through the Norwegian Species Map Service (Artskart 2.0) provided by the Norwegian Biodiversity Information Centre (Artsdatabanken). Background maps are provided by the Norwegian Mapping Authority (Kartverket). Available at: https://artskart.artsdatabanken.no (retrieved 07.05.2018).

Lobaria amplissima, *L. pulmonaria* and *L. virens* have viable populations along the Norwegian coast, and their extinction risk is considered of least concern (LC) on a national level (Artsdatabanken, 2018). However, population declines have been reported in most other European countries, such as in Sweden (ArtDatabanken, 2015). Owing to the spectacular appearance and widespread decline of these epiphytes, they have been featured as flagship species in the conservation of old growth forests, and are widely used as a model species in

ecological (Nascimbene et al., 2010) as well as physiological (Gauslaa & Solhaug, 1996) studies of lichens.

Specific thallus mass (STM) and water holding capacity (WHC) were recently determined for sympatric specimens of *L. amplissima*, *L. pulmonaria* and *L. virens* (Longinotti et al., 2017). Despite partly overlapping habitat preferences and geographical distributions, the three species significantly differ in both STM and WHC, suggesting species-specific hydration strategies. STM and WHC are important traits in determining desiccation rates, and largely explain the differences observed between these species regarding the length of photoactive periods during drying (Gauslaa et al., 2017). Differences in STM and WHC should also imply species-specific rates of water vapor uptake and photoactivation, but this remains to be quantified across *Lobaria* species.

To complement previous studies on hydration traits in these three species (Gauslaa et al., 2017; Longinotti et al., 2017), I conducted lab experiments to investigate the kinetics of rehydration and photoactivation in humid air in the co-occurring yet morphologically and functionally dissimilar *L. amplissima, L. pulmonaria* and *L. virens*. Water vapor uptake was monitored at high temporal resolution by automatic weighing of hydrating thalli, applying new protocols developed by Phinney et al. (2018). By simultaneous fluorescence imaging measurements of maximal PSII efficiency (F_v/F_m), I aimed to assess rates and patterns of photoactivation upon hydration. The overall aim was to determine and compare species-specific hydration and photoactivation traits, and to evaluate potential links between morphological and functional traits and their ecological implications.

Due to substantial differences in growth form, STM and WHC, I hypothesized that the study species would rehydrate (hypothesis 1a) and reactivate (hypothesis 1b) at different rates. I expected to observe a trade-off between high STM and rapid reactivation within and across my study species, as thinner growth forms have been shown to achieve hydration and resume photosynthesis faster than thicker ones in humid air (Phinney et al. 2018). In addition to basic water-storage parameters (e.g. STM, $WHC_{internal}$ and $WHC_{external}$), various hydration parameters (e.g. water content per dry mass (WC_{DM}), water content per area (WC_A) and relative water content (RWC)) were employed to achieve a broader view on the hydration kinetics of the study species, and to explore connections between specific hydration levels and PSII activation across species. The results could, however, underestimate the real magnitude of interspecific variation, as samples were exclusively collected from sympatric populations, where the species are acclimated to the same local conditions. Yet, species' co-occurrence is a necessary condition

for determining intraspecific differences caused by adaptation rather than acclimation, as STM and WHC also vary within species depending on realized niche conditions (Merinero et al., 2015).

A similar experimental set-up was recently applied to seven species of chlorolichens covering a range of growth forms (Phinney et al., 2018). I expected foliose cephalolichens to absorb water vapor and resume PSII activity more slowly than previously tested fruticose species, due to thicker thalli and thus higher STM (hypothesis 2a). Compared to previously tested foliose chlorolichens with similar STM, slower PSII reactivation in humid air was expected as an effect of photobiont type (hypothesis 2b). I thereby aimed to confirm the importance of both growth form and photobiont to lichens' water requirements and fill the current knowledge gap on rehydration and PSII reactivation rates in humid air for these foliose cephalolichens.

My results further contribute to the knowledge on habitat requirements of species at risk at a European level. Such knowledge is crucial to conservation planning, enabling both the identification of suitable protection areas, and the execution of successful reintroductions (Gauslaa et al., manuscript under revision).

2. Materials and methods

2.1 Sampling

Epiphytic specimens of co-occurring *Lobaria amplissima*, *L. pulmonaria* and *L. virens* were collected from broad-leaved deciduous forests in three Norwegian sites: two mesotrophic *Quercus petraea*-dominated forests in the south (Kristiansand, Vest-Agder, 58°08'N 8°07'E and 58°11'N 8°00'E, Fig. 2) and one forest dominated by *Quercus robur*, *Acer platanoides* and *Tilia cordata* in the southeast (Porsgrunn, Telemark, 59°06′N 09°50′E). Further details on the site in Porsgrunn is given by Våland Strandin (2017). A total of ten trees hosting sympatric populations of the study species were sampled. Seven were oak trunks (*Q. petraea*) in the southern sites, and three were Norway maple trunks (*A. platanoides*) in the southeastern site. The selected trees were separated by at least 100 meters to avoid pseudoreplication. The lichen thalli were randomly selected from mixed populations on the same side of each tree stem.

In the lab, the 30 collected thalli were cleaned from debris, air-dried at room temperature and stored at -18°C for 5-7 months. Freezing and storing lichens prior to physiological tests does not influence performance (Honegger, 2003).

Fig. 2 Sympatric specimens of *Lobaria amplissima* (middle) and *L. pulmonaria* (upper and lower) growing epiphytically on *Quercus petraea* in a mesotrophic old-growth forest in the south of Norway (Kristiansand, Vest-Agder). Photo: Yngvar Gauslaa.

2.2 Measurements of mass, surface area and water-storage traits

The thalli were removed from the freezer and repeatedly sprayed with deionized water until fully hydrated. Water-saturated specimens were immediately weighed to obtain wet mass (WM), first after shaking off external water droplets ($WM_{shaking}$), and again after removing surface water with dry filter paper ($WM_{blotting}$).

Individual thalli were then gently pressed under a glass plate on top of a transilluminator (Model TW-43 White light, UPV, Upland, CA, USA) and photographed from above with a Pentax K-5 II DSLR fitted with a Sigma 70mm F2.8 DG macro lens. The surface area (A_{wet}) of each hydrated thallus was determined using ImageJ 1.50i (Beteshda, Maryland, USA).

Afterwards, all thalli were left to air-dry at room temperature (20°C) before air dry mass was measured. A modest pressure was applied to the drying thalli to avoid excessive curling. Three additional thalli from each species were oven-dried at 70°C to measure oven dry mass (DM), and thus compute correction factors needed to adjust the air-dried mass of experimental thalli to DM. Based on mass and area measurements, specific thallus mass was calculated as STM = DM / A_{wet}, and water-holding capacity as WHC = (WM - DM) / A_{wet}, using $WM_{blotting}$ and $WM_{shaking}$ for $WHC_{internal}$ and $WHC_{shaking}$, respectively. External water-holding capacity was then calculated as $WHC_{external} = WHC_{internal} - WHC_{shaking}$.

2.3 Experimental set-up

The experiment was carried out in a temperature-controlled room (16°C) to minimize the possibility of cooling-induced condensation. One randomly selected thallus among the studied species was measured each day. Each thallus was taken from the freezer and kept air dry before it was glued to a sheet of aluminum foil (9 x 11 cm). The thallus on the foil was then placed in a desiccator for 48 h before measurements. The aluminum foil served to mimic the lichens' natural bark substrate, ensuring unilateral vapor uptake during the subsequent hydration treatment. No condensation occurred on aluminum foils without attached thalli during control trials. Thus, the aluminum foil did not contribute to water vapor uptake. All thalli were dark-adapted for at least 20 min prior to trials.

Desiccated and dark-adapted thalli were suspended into a glass tank (5 x 22 x 8 cm) by a thin wire connected to a scale (Sartorius CP Gemplus Series balance, Bradford, MA, USA) placed on a platform above (Fig. 3). The inside walls of the tank were covered with wet filter paper, apart from a transparent window allowing chlorophyll fluorescence measurements, and the tank floor was covered by a 1 cm layer of water. This set-up has been shown to ensure persistently high relative humidity (RH ≈ 100%) inside the chamber, without causing condensation over time (Phinney et al., 2018). The wire passed through a 13 mm wide hole in the tank lid into the tank, where the thallus was tied in a fixed vertical hanging position for the entire measurement cycle of 20 h in humid air.

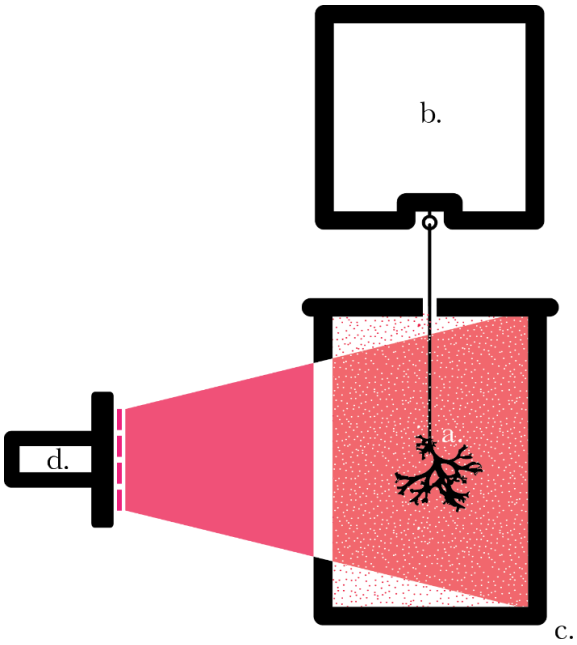

Fig. 3 Schematic representation of the experimental setup. A lichen thallus (a) glued to a tin foil (not shown) was hung from a scale (b) and into a high humidity glass tank (c) to measure weight increase from water vapor uptake. A chlorophyll fluorometer (d) was aimed through the tank wall to record changes in maximal PSII efficiency (F_v/F_m) during rehydration. Illustration: Phinney et al. 2018.

A chlorophyll fluorometer (red-LED IMAGING-PAM M-series, Walz Effeltrich, Germany) was aimed through the transparent window in the tank wall and focused on the lichen thallus. Maximal quantum yield of PSII (F_v/F_m) was recorded in response to a saturating light pulse at 2800 μmol m^{-2} s^{-1}, activated at five-minute intervals throughout the 20 h cycle. Pulse intensity, frequency and number of repetitions were predefined in a software (ImagingWin v2.46i, Walz,

Effeltrich, Germany) coupled to the fluorometer, and kept constant among trials. The mass of each hydrating thallus was recorded automatically at 1 min intervals throughout the measurement cycle, using WinWedge v4.0.4 Professional (TAL Technologies, Philadelphia, PA, USA) to run the scale. The scale and fluorometer programs were started simultaneously to obtain matching time series of weight and F_v/F_m data, respectively.

Based on the time series of wet mass data obtained in the experiments, corresponding time series of water content per thallus area (WC_A, mg H_2O cm^{-2}), and per thallus dry mass (WC_{DM}, %), were computed as WC_A = (WM - DM) / A_{wet} and WC_{DM} = (WM - DM) / DM * 100, employing values of WM from every measurement time in the hydration cycle. Time series of relative water content (RWC, %) were computed as WC_A / WHC * 100, using the calculated values of WC_A for each measurement time.

2.4 Statistical analyses

One-way ANOVAs and Tukey HSD tests with species as factor were performed to detect interspecific similarities and differences within the measured traits. Data were log-transformed when required to meet test assumptions. Simple linear regression models were built to investigate relationships between morphological and functional traits. Multiple linear regressions models were run to explore the relative importance of the continuous variable STM and the categorical variable species in explaining the variation in all other measured traits.

Time series of WC_A, WC_{DM}, RWC and F_v/F_m data were compared by a mean *t*-statistic permutation test with species as factor, using the function compareGrowthCurves from the package Statmod v1.4.30 (Elso, C. M. et al., 2004). Models to describe uptake curves were found using Curve Expert 1.4 (Hyams Development, Hixton, TN, USA).

The statistical analyses were conducted in R v3.4.1 (R Core Team, Vienna, Austria), and the results were plotted in SigmaPlot v14.0 (Systat Software, San Jose, CA).

One specimen of *L. amplissima* was excluded from parts of the analysis due to a particularly thick growth form and deviant functional responses causing a disproportional impact on the species' means. Although this specimen likely reflects the large natural variation observed within *L. amplissima* (Gauslaa et al., 2017), it was sometimes necessary to exclude it from the small dataset employed in this study to meet test assumptions. The exclusion of this specimen did not affect measured trends.

3. Results

Individual thallus areas ranged from 24 to 99 cm^2, with an overall mean of approximately 50 cm^2 (Table 1). Mean thallus area did not differ significantly among the studied species, facilitating comparisons of all other traits between species. Interspecific differences were detected within most inspected traits, despite the similar thallus areas across species.

3.1 Water-storage traits

Lobaria amplissima had 2.4 and 1.9 times higher mean STM (40.4±4.1 mg cm^{-2}) than *L. pulmonaria* and *L. virens*, respectively. The two latter species did not differ in STM (Table 1). By contrast, all three species differed in $WHC_{internal}$. *Lobaria amplissima*, with a mean $WHC_{internal}$ of 75.6±4.1 mg H_2O cm^{-2}, held 2.8 times more internal water than *L. pulmonaria*, and 1.8 times more than *L. virens*. Similarly, $WHC_{external}$ was significantly higher in *L. amplissima* (26.0±4.7 mg H_2O cm^{-2}) than in *L. pulmonaria* (15.3±0.7 mg H_2O cm^{-2}), while the $WHC_{external}$ of *L. virens* (22.8±2.1 mg H_2O cm^{-2}) was not different from either of the previous. However, $WHC_{external}$ constituted only 34% of $WHC_{internal}$ in *L. amplissima*, compared to 56 and 54% in *L. pulmonaria* and *L. virens*, respectively. The WC_{DM} measured after wetting and blotting (i.e. saturated WC_{DM}) was similar in *L. amplissima and L. virens*, but significantly lower in *L. pulmonaria*. There were strong, linear relationships between STM and $WHC_{internal}$ in log-log plots across and within species (Fig. 4). Both STM and species were significant predictors of $WHC_{internal}$ in a multiple linear regression model (Table 2), with STM having the highest impact on R^2_{adj} and thus explaining most of the variation in $WHC_{internal}$.

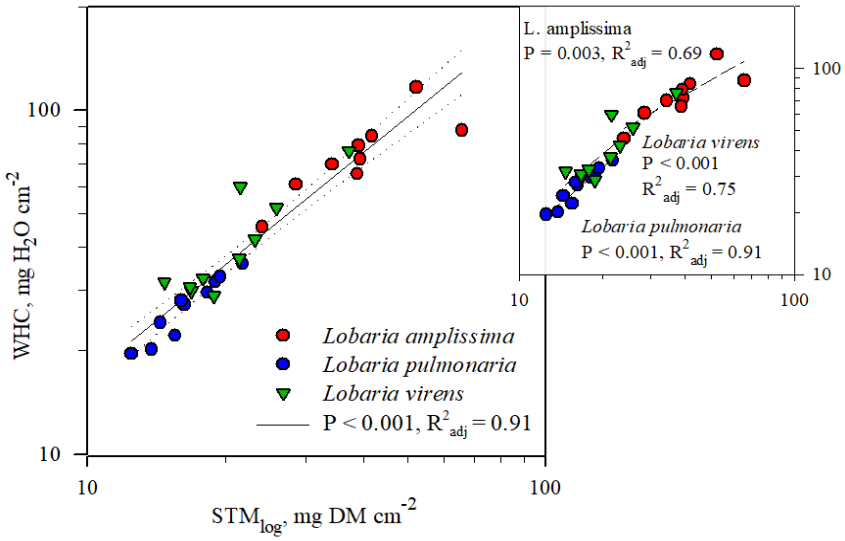

Fig. 4 Simple linear regressions showing the relationship between specific thallus mass (STM_{log}) and water-holding capacity (WHC_{log}) across (left) and within (right) species (*Lobaria amplissima* n = 9; *L. pulmonaria* n = 10; *L. virens* n = 10). P-values and R^2adj-values are provided for each regression line (α=0.05). Dotted lines in the left figure delineate 95% confidence intervals.

Table 1 Species means ±SE (*Lobaria amplissima* n = 9; *L. pulmonaria* n = 10; *L. virens* n = 10) of lichen traits, including thallus area, specific thallus mass (STM), water holding capacity (WHC), water content per thallus dry mass after wetting and blotting (saturated WC_{DM}), time employed to reach 10 and 95% of maximum F_v/F_m in humid air (time to 10 and 95% F_v/F_m), as well as relative water content (RWC), water content per thallus area (WC_A) and water content per thallus dry mass (WC_{DM}), each at 10 and 95% of maximum F_v/F_m and after 20 h in humid air (denoted by $_{end}$ subscript). Similar superscript letters among species indicate no significant difference ($\alpha = 0.05$) in one-way ANOVA and Tukey post-hoc tests. Variables are marked with $_{log}$ subscripts where log-transformation was required to meet test assumptions. R^2_{adj}-values were obtained from linear models. Ns: Not significant.

Species	*Lobaria amplissima*	*Lobaria pulmonaria*	*Lobaria virens*		
	Mean ± *SE*	Mean ± *SE*	Mean ± *SE*	R^2_{adj}	P_{adj}
Basic traits					
Thallus area, cm^2	53.8 ± 8.5^a	54.9 ± 5.4^a	42.8 ± 3.1^a	0.28	Ns
STM_{log}, mg DM cm^{-2}	40.4 ± 4.1^a	16.7 ± 0.9^b	21.4 ± 2.1^b	0.67	**<0.001**
$WHC_{internal\ log}$, mg H_2O cm^{-2}	75.6 ± 6.6^a	27.1 ± 1.7^b	42.1 ± 5.0^c	0.69	**<0.001**
$WHC_{external\ log}$, mg H_2O cm^{-2}	26.0 ± 4.7^a	15.3 ± 0.7^b	22.8 ± 2.1^{ab}	0.18	**0.0287**
Saturated $WC_{DM\ log}$, %	190.9 ± 9.0^a	161.4 ± 3.2^b	194.8 ± 10.7^a	0.23	**0.0119**
PSII Activation traits					
Time to 10% F_v/F_m, min	48.3 ± 3.3^a	18.5 ± 4.2^b	22.5 ± 3.6^b	0.55	**<0.001**
Time to 95% F_v/F_m, h	8.8 ± 0.4^a	7.1 ± 0.3^b	6.6 ± 0.4^b	0.38	**<0.001**
Water content at specific times					
RWC at 10% $F_v/F_{m\ log}$, %	7.2 ± 0.6^{ab}	8.7 ± 0.8^a	6.0 ± 0.6^b	0.21	**0.0168**
WC_A at 10% F_v/F_m, mg H_2O cm^{-2}	5.1 ± 0.4^a	2.4 ± 0.2^b	2.5 ± 0.3^b	0.63	**<0.001**
WC_{DM} at 10% $F_v/F_{m\ log}$, %	12.9 ± 0.5^a	14.1 ± 1.3^a	11.6 ± 0.6^a	0.05	Ns
RWC at 95% $F_v/F_{m\ log}$, %	18.9 ± 1.8^a	30.0 ± 1.3^b	21.0 ± 1.3^a	0.50	**<0.001**
WC_A at 95% F_v/F_m, mg H_2O cm^{-2}	13.2 ± 0.9^a	7.9 ± 0.3^b	8.5 ± 0.7^b	0.56	**<0.001**
WC_{DM} at 95% F_v/F_m, %	33.8 ± 1.7^a	48.1 ± 1.5^b	40.9 ± 1.5^c	0.59	**<0.001**
RWC_{end}, %	25.6 ± 2.3^a	42.0 ± 1.4^b	31.7 ± 1.9^a	0.57	**<0.001**
$WC_{A,\ end}$, mg H_2O cm^{-2}	17.9 ± 1.0^a	11.2 ± 0.4^b	12.8 ± 0.9^b	0.56	**<0.001**
$WC_{DM,\ end}$, %	44.6 ± 2.3^a	67.6 ± 1.7^b	61.8 ± 2.3^b	0.66	**<0.001**

3.2 PSII activation

All specimens readily began PSII reactivation when exposed to humid air (RH ≈100%), shown by a rapid increase in F_v/F_m in the first part of the measurement cycle (Fig. 5). All the species exceeded an F_v/F_m-value of 0.6 within 4.25-10.75 h, before stabilizing at slightly higher levels (*L. amplissima* 0.634±0,01; *L. pulmonaria* 0.632±0.01; *L. virens* 0.656±0.01, Table 1). *Lobaria virens* displayed significantly higher F_v/F_m than *L. amplissima* throughout the cycle. *Lobaria pulmonaria* initially activated at a similar pace as did *L. virens*, but later settled at a slightly lower F_v/F_m level, reaching final F_v/F_m values similar to those of *L. amplissima.*

L. pulmonaria and *L. virens* passed 10 and 95% of their maximum F_v/F_m level at similar rates in humid air, exceeding 10% F_v/F_m after 18.5±4.2 min and 22.5±3.6 min, and 95% within 7.1±0.3 h and 6.6±0.4 h, respectively (Table 1). *Lobaria amplissima* required a significantly longer time to reach corresponding percentages of maximal F_v/F_m (48.3±3.3 min and 8.8±0.4 h). The time required to reach 10 and 95% of maximum F_v/F_m in humid air increased with STM across species and within *L. virens* (Fig. 6A and B). In a multiple linear regression model with STM and species as independent variables (Table 2), species was the only

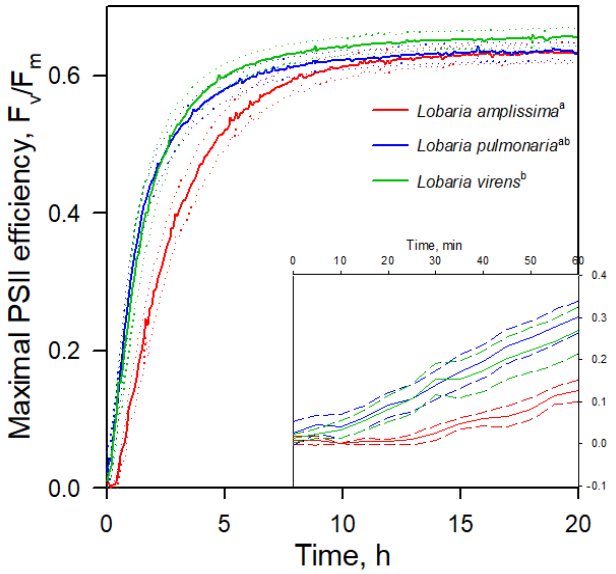

Fig. 5 PSII activation measured as change in F_v/F_m over time during a 20h trial in humid air (RH ≈100%), with the first 60 min enlarged in an insert plot. Solid lines represent species means (*Lobaria amplissima* n = 9; *L. pulmonaria* n =10; *L. virens* n =10), while dotted lines indicate 95% confidence intervals of the means. Different superscript letters mark significant difference at α =0.05 in a mean t-statistic permutation test.

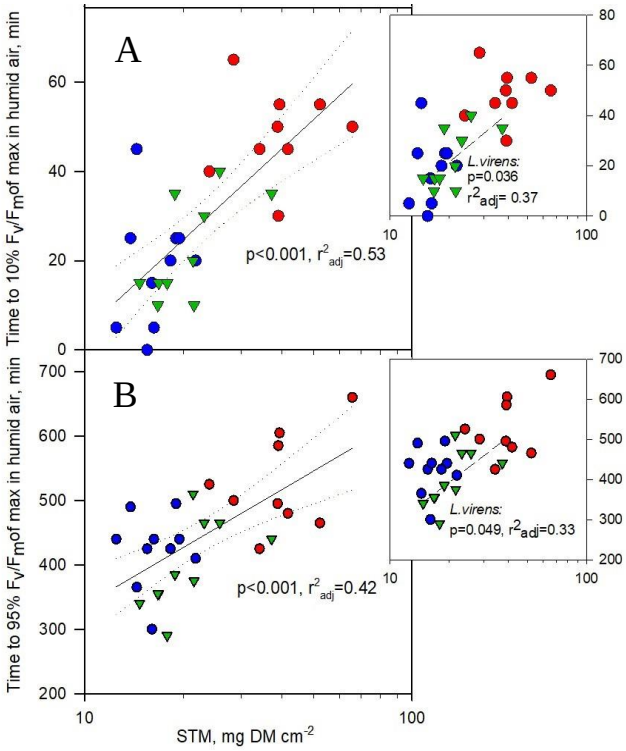

Fig. 6 The relationship between specific thallus mass (STM_{log}) and time employed to reach 10 and 95% of maximum Fv/Fm in humid air (6A and B, resp.). Main figures show the relationships across species, while species-wise regressions are shown in insert plots. Regression lines with P- and R^2_{adj}-values are provided where significant (α = 0.05). Dotted lines indicate 95% confidence intervals.

significant predictor for the time to reach 10% PSII activation, while species as well as STM were significant predictors for the time required to reach 95%, with STM explaining most of the variation at this time point.

The PSII activation started in exposed lobe ends, and later spread towards central parts of the thallus (Fig. 7). Photoactivation in humid air did not occur in the external cephalodia that were present on some specimens of *L. amplissima* (Fig. 8).

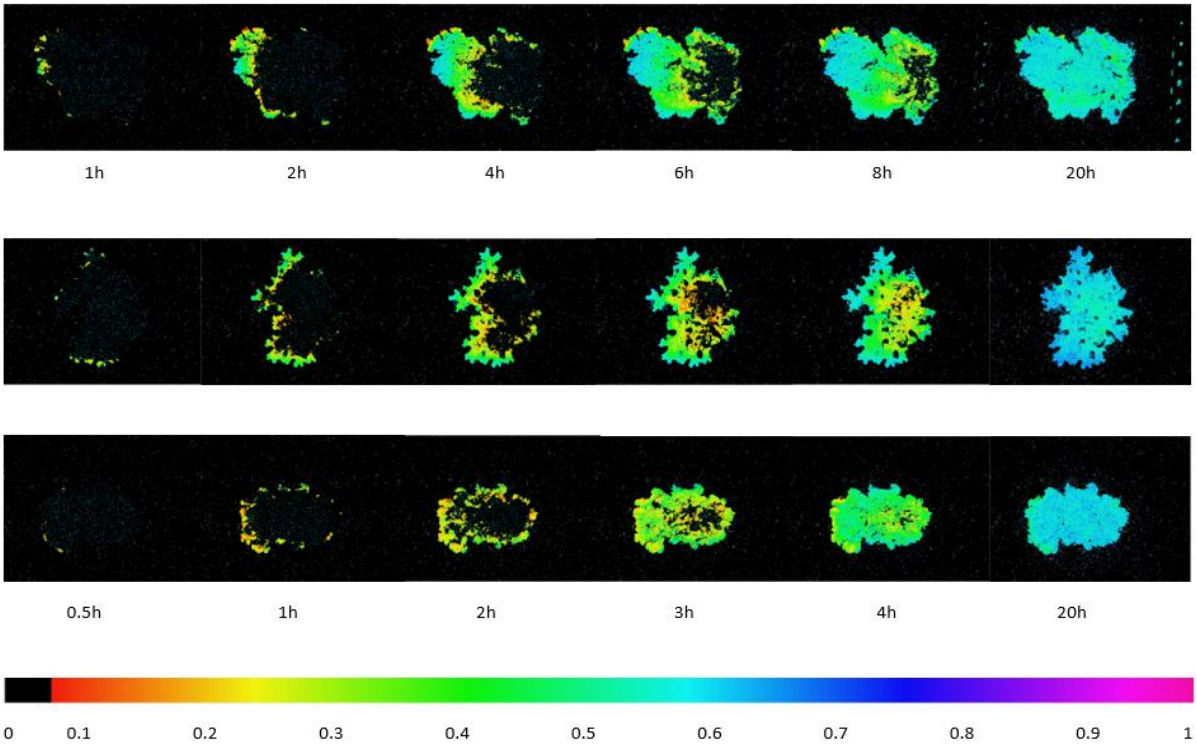

Fig. 7 Patterns of photoactivation upon rehydration in humid air in representative specimens of *Lobaria amplissima* (top), *L. pulmonaria* (middle) and *L. virens* (lower), measured as change in maximal PSII efficiency (F_v/F_m) during rehydration in humid air by chlorophyll fluorescence imaging. Note the different time scales in the top (*L. amplissima*) and lower (*L. pulmonaria* and *L. virens*) image series.

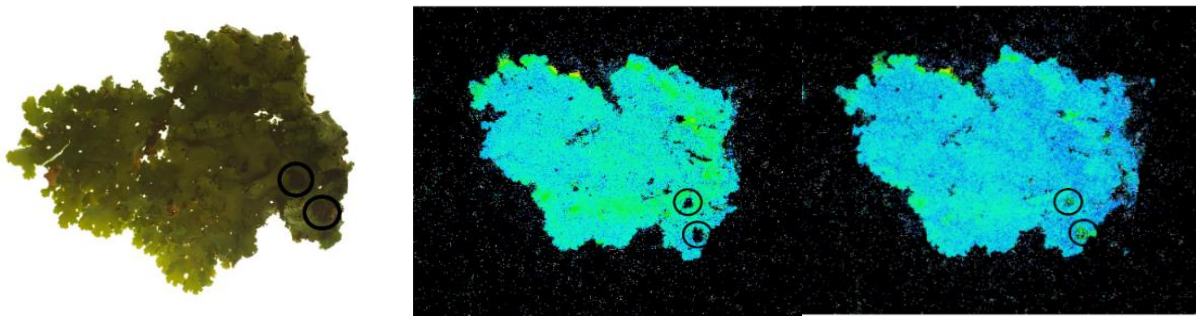

Fig. 8 Picture showing a thallus of *Lobaria amplissima* bearing external cephalodia (left) and chlorophyll fluorescence images of the same specimen after 20 h of rehydration in humid air (middle) and after subsequent wetting with liquid water (right). The coloring indicates level of maximal PSII efficiency (F_v/F_m), with black coloring translating to zero F_v/F_m. Two cephalodia in the lower right part of the thallus are marked with black circles in each picture. No PSII activity was observed in the cephalodia after 20 h of exposure to humid air (middle), as the cephalodia remained black in contrast to the otherwise increasingly colored thallus. PSII activation only occurred in the cephalodia after subsequent wetting with liquid water, shown by the coloring of the cephalodia in the right image.

3.3. Water vapor uptake

All specimens immediately begun to absorb water when exposed to humid air (Fig. 9A-C), with initial uptake rates varying from 0.13 (*L. amplissima*) to 0.24 (*L. pulmonaria*) mg H_2O DM^{-1} min^{-1} during the first 10 min. The uptake rates gradually slowed down over time, decreasing to 0.02 (*L. amplissima*) and 0.03 (*L. pulmonaria* and *L. virens*) mg H_2O $DM^{-1}min^{-1}$ after 10 h of exposure. However, none of the species reached saturation in humid air within the 20 h duration of the trial, even if the uptake rates had decreased to 0.01 mgH_2O $DM^{-1}min^{-1}$ for all species towards the end of the hydration cycle. All the water uptake curves (Fig. 9A-C) matched the MMF model $y = (ab + cx^d) / (b + x^d)$, with x = time and $r \geq 0.99$ (coefficients for all graphs given in supplementary material).

3.3.1 Change in water content per dry mass (WC_{DM})

For WC_{DM}, the increase over time significantly differed between all the study species (Fig. 9A). The WC_{DM} in *L. pulmonaria* increased most rapidly, reaching 67.6±1.7% at the end of the measurement cycle (Table 1). *Lobaria amplissima* had the slowest increase in WC_{DM}, reaching a WC_{DM} of only 44.6±2.3% after 20 h in humid air. By comparison, *L. pulmonaria* had attained the same WC_{DM} as the final WC_{DM} of *L. amplissima* within 6 h. *Lobaria virens* showed an intermediate response, although more similar to *L. pulmonaria* than to *L. amplissima*. All species reached 10% PSII activation, measured as per cent of their

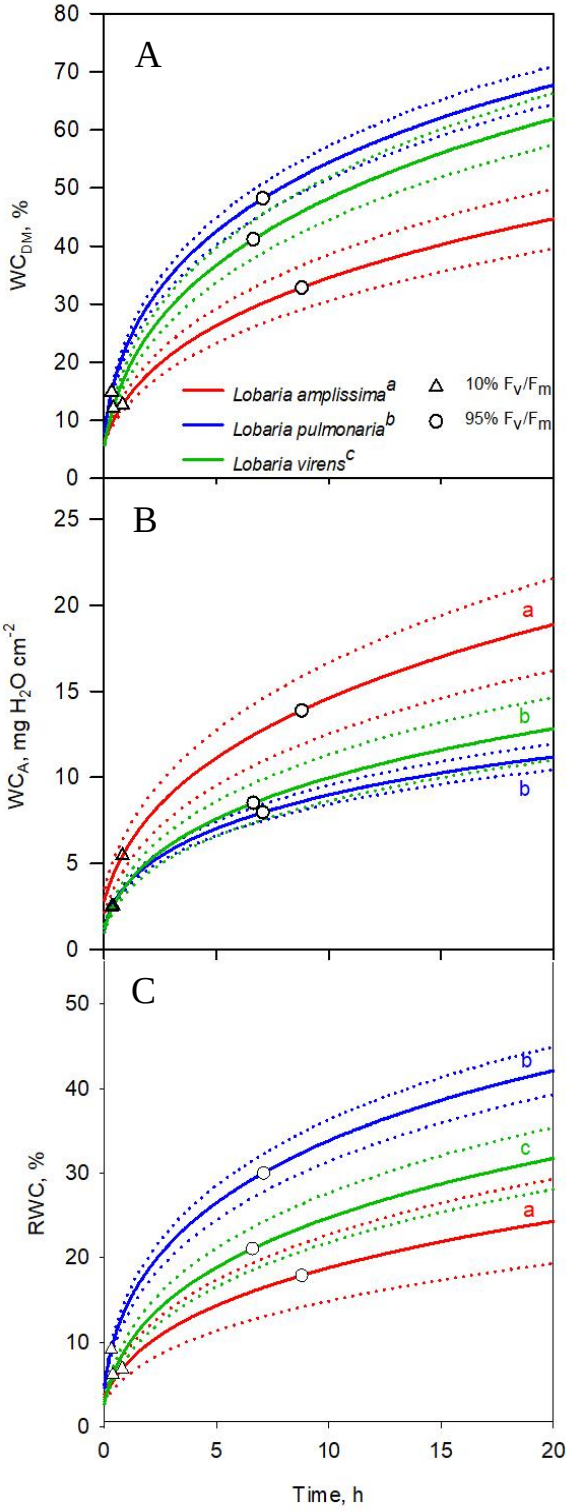

Fig. 9 Water vapor uptake measured as change in water content per dry mass (A, WC_{DM}), water content per area (B, WC_A) and relative water content (C, RWC) during a 20 h trial in humid air (RH ≈100%). Solid lines represent species means (n =10), while dotted lines indicate 95% confidence intervals of the means. White triangles and circles sign 10 and 95% PSII activation, respectively. Different superscript letters (in A) and colored letters (in B and C) mark significant difference at α = 0.05 in a mean t-statistic permutation test.

respective maximum F_v/F_m in humid air, at the same WC_{DM} (Fig. 9A, Table 1). However, the WC_{DM} required for an activation of 95% of maximal F_v/F_m varied substantially between species (Fig. 9A, Table 1). Yet, 95% PSII activation occurred after a similar exposure time for *L. pulmonaria* and *L. virens* (7.1±0.3 and 6.6±0.4 h), and shortly after for *L. amplissima* (8.8±0.4 h) (Fig. 9A, Table 1). Final WC_{DM} after 20 h in humid air correlated negatively with STM across species, and intraspecifically for *L. pulmonaria* and *L. amplissima* (Fig. 10B). Both STM and species were significant predictors of WC_{DM} at 95% F_v/F_m and after 20 h in humid air in a multiple linear regression model (Table 2), with each predictor explaining considerable variation in WC_{DM} at 95% F_v/F_m, and STM explaining most of the variation in WC_{DM} after 20h.

3.3.2 Change in water content per area (WC_A)

With respect to WC_A, *L. amplissima* exhibited the most rapid increase (Fig 9B), measuring higher WC_A than the two other study species throughout the hydration cycle, and reaching a mean after 20 h of 17.9±1.0 mg H_2O cm^{-2} (Table 1). *Lobaria pulmonaria* and *L. virens* developed similar WC_A curves, reaching maximums of respectively 11.2±0.4 and 12.8±0.9 mg H_2O cm^{-2} after 20 h. By comparison, *L. amplissima* had gained the equivalent WC_A within only 5 and 7 h. *Lobaria pulmonaria* and *L. virens* had attained the same WC_A when reaching 10 and 95% PSII activation (Fig. 9B, Table 1), while *L. amplissima* measured significantly higher WC_A at corresponding points of photoactivation. After 20 h in humid air, there was a strong, positive linear correlation between STM and WC_A across and within species (Fig. 10A). Both STM and species were significant predictors of WC_A at 10% and 95% PSII activation as well as after 20 h

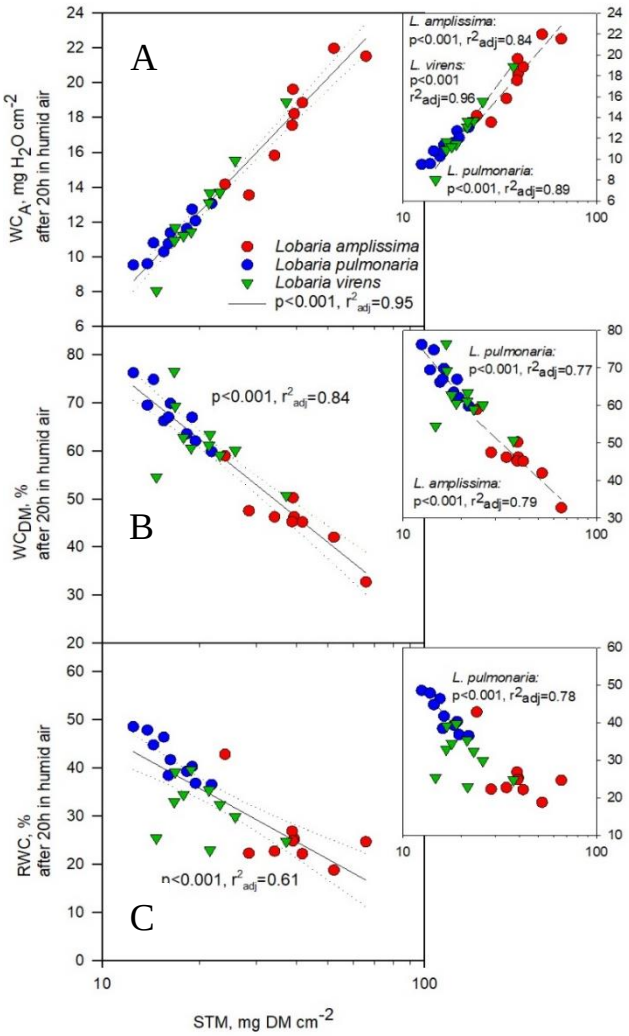

Fig. 10 The relationship between specific thallus mass (STM_{log}) and water content per area (A, WC_A), water content per dry mass (B, WC_{DM}) and relative water content (B, RWC) after 20h in humid air (RH≈100%). Main figures show the relationships across species, while species-wise regressions are shown in insert plots. Regression lines with P- and R^2_{adj}-values are provided where significant (α=0.05). Dotted lines indicate 95% confidence intervals.

in a multiple linear regression model (Table 2). STM had the strongest influence on the model fit, explaining most of the variation in WC_A at all three measurement points.

3.3.3 Change in relative water content (RWC)

The RWC increased over time in clearly species-specific ways (Fig. 9C). *Lobaria pulmonaria* increased in RWC most rapidly, followed by *L. virens*, and ultimately *L. amplissima.* After 20h in humid air, *L. pulmonaria* had filled 42.0±1.4% of its $WHC_{internal}$, while *L. virens* and *L. amplissima* finally reached 31.7±1.9% and 25.6±2.3%, respectively (Table 1). *Lobaria amplissima* and *L. virens* reached 95% PSII activation at the same RWC, and at a significantly lower RWC than *L. pulmonaria* (Fig. 9C, Table 1). The RWC after 20 h in humid air decreased with increasing STM across species, but at an intraspecific level only within *L. pulmonaria* (Fig 10C). In a multiple linear regression model with STM and species as independent variables, species was the only significant predictor of RWC at 10 and 95% F_v/F_m, while both species and STM were significant predictors of RWC after 20 h (Table 2).

Table 2 Summary of multiple linear regressions models testing the effect of the continuous variable STM and the categorical variable species (*Lobaria amplissima* n=9; *L. pulmonaria* n=10; *L. virens* n=10) on thallus area, internal and external water holding capacity ($WHC_{internal\ log}$ and $WHC_{external\ log}$), water content per dry mass after wetting and blotting (saturated WC_{DM}), time employed to reach 10 and 95% of maximum Fv/Fm in humid air, and relative water content (RWC), water content per area (WC_A) and water content per dry mass (WC_{DM}) at 10 and 95% Fv/Fm, as well as after 20h in humid air. Log-transformed response variables are denoted by $_{log}$ subscripts in the left column, while log-transformed independent variables (STM) are marked with $_{log}$ subscripts after corresponding F-value. Significant P-values are shown in bold. Interaction factors were excluded from the models as they were not found significant (α=0.05).

Independent variables		STM (df=1)		Species (df=2)	
	R^2_{adj}	*F*	*P*	*F*	*P*
Dependent variables					
Basic traits					
Thallus area	0.16	5.3_{log}	**0.029**	1.5	0.233
$WHC_{internal\ log}$	0.93	83.2_{log}	**<0.001**	136.5	**<0.001**
$WHC_{external\ log}$	0.52	7.0_{log}	**0.004**	19.3	**<0.001**
Saturated WC_{DM}	0.21	0.8	0.383	4.9	**0.016**
PSII activation traits					
Time to 10% F_v/F_m	0.56	1.4	0.244	18.4	**<0.001**
Time to 95% F_v/F_m	0.47	5.1	**0.032**	11.2	**<0.001**
WC at specific times					
RWC at 10% $F_v/F_{m\ log}$	0.19	0.1_{log}	0.730	4.6	**0.019**
WCA at 10% F_v/F_m	0.88	58.3	**<0.001**	79.7	**<0.001**
WC_{DM} at 10% F_v/F_m	0.06	0.6	0.434	2.1	0.143
RWC at 95% F_v/F_m	0.56	3.6	0.071	17.6	**<0.001**
WCA at 95% F_v/F_m	0.93	140.8_{log}	**<0.001**	120.3	**<0.001**
WCDM at 95% F_v/F_m	0.71	11.7	**0.002**	29.3	**<0.001**
RWC_{end}	0.70	12.0_{log}	**0.002**	28.1	**<0.001**
$WC_{A\ end}$	0.95	206.7_{log}	**<0.001**	165.0	**<0.001**
$WC_{DM\ end}$	0.83	27.6	**<0.001**	56.1	**<0.001**

4. Discussion

Photoactivation in humid air is well documented for a range of chlorolichens (Lange et al., 1986; Phinney et al., 2018). This study demonstrates that also cephalolichen members of *Lobaria* succeed in utilizing water vapor as a hydration source to resurrect from the dormant dry-state and resume photosynthesis within ecologically relevant time spans. My results confirm that thinner species with low STM fill their thalli with water more rapidly than thicker species, allowing photosynthesis to occur after shorter exposure to humid air, as shown for other lichen growth forms (Phinney et al., 2018). Furthermore, my analyses reveal that the important morphological trait STM not only drives functional traits such as WHC (Longinotti et al., 2017), but also predicts achieved cephalolichen hydration levels after 20 h in humid air, and influences the time and hydration level required to initiate and fully reactivate photosynthesis in humid air.

4.1 Water storage traits

The high correlation observed between STM and $WHC_{internal}$ both within and between the study species affirms that lichens have to invest in thallus thickness to enhance their ability to store water within their tissues (Gauslaa & Coxson, 2011). The studied cephalolichens had STM-$WHC_{internal}$ ratios ranging from 1:1.6 to 1:2, implying substantially larger water storage per thallus area than inferred from the 1:1 ratio observed in a wide spectrum of chlorolichens (Esseen et al., 2015). In fact, these cephalolichen STM-$WHC_{internal}$ ratios are closer to the 1:2 ratio detected in cyanolichens (Gauslaa & Coxson, 2011). The species-specific STM-$WHC_{internal}$ ratios found in this study are somewhat lower than previously recorded ratios for the same species (Longinotti et al., 2017), as a result of slightly higher values of $WHC_{internal}$ compared to STM. Such differences could imply inconsistent measuring protocols, which is not unlikely as $WHC_{internal}$ measurements entail several steps (e.g. spraying, shaking and blotting) that are prone to subjectivity and difficult to replicate consistently. For example, it seems that some species or genera take up liquid water during spraying more slowly than other species, presumably due to more hydrophobic surfaces (Erikson et al., manuscript under revision). Assuming accurate measurements, however, one could suggest that the higher values of $WHC_{internal}$ found across species in the present study is a result of the use of larger thalli, as $WHC_{internal}$ is known to increase with thallus size (Gauslaa & Solhaug, 1998). Yet, both STM and $WHC_{internal}$ increase with thallus area at a similar slope in log-log plots (Merinero et al.,

2014), and the STM-$WHC_{internal}$ ratios should therefore remain unaffected by thallus size. Alternatively, higher species-specific $WHC_{internal}$ could be a result of a higher frequency of thalli with particularly well-developed cephalodia, as $WHC_{internal}$ also depends on photobiont (Gauslaa & Coxson, 2011). Cyanobacterial cells are coated with a gelatinous sheath that can swell and store great amounts of water (Honegger et al., 1996), increasing extensively the $WHC_{internal}$ of cyanolichens. Therefore, the $WHC_{internal}$ of cephalolichens should increase considerably with their relative concentration of cyanobacterial cells. The $WHC_{internal}$ of *L. amplissima* is substantially higher in specimens bearing external cephalodia than in specimens with only internal cephalodia (Y. Gauslaa, pers. comm.). Yet, cephalodiate specimens also have higher STM than specimens without (Y. Gauslaa, pers. comm.), confounding the effect of external cephalodia vs. thallus thickness on water storage traits in this species. The amount of cephalodia per thallus varies between *Lobaria* species depending on thallus size, shape and growth form (Jordan, 1970), but whether the frequency of internal cephalodia varies within cephalolichen species depending on thallus area is not known. Nitrogen fixation activity is however highly variable among replicates of *L. pulmonaria* (Millbank, J. & Kershaw, K., 1970), suggesting intraspecific variation in cephalodia development, which could partly contribute to the variation in $WHC_{internal}$ in cephalolichens.

Among the study species, *L. amplissima* exhibited the highest STM and the highest $WHC_{internal}$. Growth forms favoring water storage are often observed in species growing in open, sun-exposed microhabitats, where abundant rain is alternated by high solar irradiance (Phinney et al., 2018). Here, all lichen species were collected from mixed population, but *L. amplissima* often grows higher on tree stems than the two other *Lobaria* species (Asplund et al., 2010). In such positions, it receives generous amounts of liquid water at rainfall, but also experiences higher sun-exposure and more intense drying than species growing in the more continuously humid, shaded canopy below (Gauslaa, 2014). *Lobaria amplissima* also occurs on old trees in open and sparsely wooded cultural landscapes (Bratli & Halvorsen, 2018), without a closed rain- and sun-shielding canopy. The upper canopy and open cultural landscape undergo stronger nocturnal cooling than the lower canopy of a closed forest stand, and thus also provide hydration in the form of abundant morning dew (Gauslaa, 2014). With its thick, resupinate rosettes *L. amplissima* seem optimized for harvesting much water when available and retaining the hydrated state over time, which could represent a competitive advantage under the shifting moisture conditions of the upper canopy and open cultural landscape. This water conserving strategy, in combination with higher light resistance compared to other *Lobaria* species

(Gauslaa & Solhaug, 1996), allows *L. amplissima* to stretch active periods beyond occasional hydration events, prolonging photosynthesis during subsequent drying (Gauslaa et al., 2017). The distribution of epiphytes along the complex vertical gradients of forest canopies is however not determined by climate alone, and may as much be a result of biotic factors such as the intensity of gastropod grazing, which increases towards the lower parts of the tree stem (Asplund et al., 2010). *Lobaria amplissima* is highly palatable to gastropods (Gauslaa 2018, manuscript), and could be restricted to higher parts of tree trunks due to low grazing resistance (Asplund et al., 2010). Favorable functional traits such as high $WHC_{internal}$ and higher light resistance than other *Lobaria* species would nevertheless give *L. amplissima* an advantage within its realized niche in the upper canopy, as well as in the open cultural landscape.

Lobaria virens exhibited significantly higher $WHC_{internal}$ than *L. pulmonaria*, despite the two species displaying similar STM. Thus, although STM shapes the lichens' capacity to store water, other species-specific traits clearly influence this ability. In heteromerous lichens, $WHC_{internal}$ depends to a large extent on the thickness of the cortex and the photobiont layer (Gauslaa & Coxson, 2011), while the thickness of the underlying medulla contributes less to water storage due to its hydrophobic nature (Honegger, 2006). The higher $WHC_{internal}$ measured in *L. virens* could therefore reflect a thicker upper cortex or photobiont layer in this species compared to *L. pulmonaria*. Chlorophyll measurements from sympatric populations showed 1.6 times higher chlorophyll content in *L. virens* than in *L. pulmonaria* (Gauslaa & Solhaug, 1996), indicating the presence of a thicker photobiont layer in *L. virens*. A thicker photobiont layer in *L. virens* may explain the significantly higher $WHC_{internal}$ measured in *L. virens* compared to *L. pulmonaria* despite their similar STM.

4.2 Water vapor uptake

In accordance with hypothesis 1a, interspecific differences in growth form, STM and WHC were reflected in species-specific hydration rates. *Lobaria pulmonaria* and *L. virens*, having larger surface areas relative to their masses and thus lower STM, fill their thalli with water from vapor more rapidly than the thicker thalli of *L. amplissima*. Interestingly, *Lobaria pulmonaria* was more efficient in raising its WC_{DM} and RWC than *L. virens*, in contrast to what would be expected by species sharing STM (Phinney et al., 2018). This suggests that the ability to attain rapid hydration from humid air does not exclusively depend on surface area-to-mass ratio. The difference in water vapor uptake observed between *L. pulmonaria* and *L. virens* could be influenced by growth form, as the more loosely attached lobes of *L. pulmonaria* are more

exposed to the ambient air, and thus are better designed for vapor harvest than the resupinate rosettes of *L. virens* that likely benefit from stem flow during rain. Although the specimens in this study were glued to aluminum foil to ensure unilateral water vapor uptake, the projecting lobes of *L. pulmonaria* often resulted at least partially exposed to humid air from both sides, as they do under natural field conditions. In nature, this loosely attached growth form could potentially double the thallus area involved in water vapor uptake, as water vapor in contrast to liquid water can pass unimpededly through hydrophobic parts of the thallus also from the lower thallus surface (Honegger, 1991). By contrast, water vapor uptake in the more closely attached *L. virens* is likely restricted to the upper thallus surface and could be further hampered by a thicker boundary layer, as this species typically forms more compact thalli than *L. pulmonaria* (Larson, 1979).

A disproportional underestimation of the projected surface area of *L. pulmonaria* could provide an alternative or additional explanation for the similar STM yet different water vapor uptake rates observed in *L. pulmonaria* and *L. virens*. *Lobaria pulmonaria* develops ridges and pits on its upper surface extending the thallus area exposed to the ambient air (Gauslaa et al., 2009). The thallus surface area would be further amplified in highly isidiate specimens (Larson 1981), yet isidia were uncommon among the specimens employed in this study. Two-dimensional area measurements of three-dimensional lichen thalli inevitably entail underestimations, and such inaccuracies are especially large in highly three-dimensional species, such as *L. pulmonaria*. Comparisons of two- and three-dimensional area measurements of *L. pulmonaria* suggest an underestimation of 10-30% in this species, with the highest inaccuracy in larger and thicker thalli (N. H. Phinney, pers. comm.). The three-dimensional surface area of *L. virens* is unknown, which complicates species-wise comparisons of real surface areas. If *L. virens* truly has lower surface area-to-mass ratio and thus higher STM than *L. pulmonaria*, this would explain both the slower hydration rates and the higher $WHC_{internal}$ measured in *L. virens* compared to *L. pulmonaria*. The different efficiency by which the two species attain hydration from humid air could further elucidate their divergent geographical distributions. In fact, *L. pulmonaria* frequently occurs in continental forests where hydration is mainly provided in the form of humid air, whereas *L. virens* is restricted to more rainy habitats along the coast (Bratli & Halvorsen, 2018; Ellis, 2016).

Although the thick *L. amplissima* had the slowest water uptake per thallus dry mass, this species was the fastest in raising its WC_A, and thus had the fastest water uptake per area unit among the studied species. Water vapor uptake in lichens is driven by the difference in water potential

between the lichen thallus and its surrounding air, as water always moves towards lower water potentials in the attempt to reach a hydrological equilibrium. The water potential gradient is particularly strong between a dry lichen thallus and humid air, causing initially rapid water uptake by dry thalli when exposed to air at 100% RH. As lichens hydrate, their thallus water potential inevitably increases, resulting in progressively weaker water attraction force and thus lower water uptake rate over time. However, the thick thalli of *L. amplissima* remain relatively dry for a longer time in humid air compared to species with thinner thalli, conserving a lower water potential that can sustain higher water uptake per area for longer time. Lichens' water potential is further influenced by their intracellular solute concentration, caused by accumulation of ions or organic molecules reducing the symplastic osmotic potential. Interspecific variation in intracellular carbohydrate concentrations may explain observed differences between species in intracellular water potentials (Beckett, 1995). Previous studies indicate lower intracellular water potentials and higher cell wall elasticity in species from xeric habitats compared to species from mesic habitats, assuring higher turgor at lower water contents in species subject to frequent drought stress (Beckett, 1995). Variation in intracellular water potential should also affect water vapor uptake by influencing the ability of a thallus to attract water. Measurements of species-specific water potentials could therefore provide deeper insight to the differences in water vapor uptake observed in *L. amplissima, L. pulmonaria* and *L. virens*, although this is probably not the main explanation for differences observed in species sampled from sympatric populations. Lower intracellular water potential in *L. amplissima* would nevertheless contribute to explaining the higher water uptake per area observed in this species, and could further represent an advantage under the shifting moisture conditions that often characterize its habitat by assuring higher cell turgidity at lower water contents.

4.3 PSII activation

In line with hypothesis 1b, morphological differences between the species causing interspecific variation in hydration rates were further reflected in the species-specific kinetics of photoactivation. In fact, *L. amplissima* and *L. virens*, exhibiting different STM and hydrating at different rates, also developed significantly different photoactivation curves. *Lobaria virens* and *L. pulmonaria* on the other hand, having similarly low STM and hydrating more rapidly than the thicker *L. amplissima* in terms of WC_{DM} and RWC, also showed a similarly rapid PSII activation. Moreover, the foliose cephalolichens employed in this study filled their thalli with water vapor and reactivated photosynthesis at a slower pace than previously tested fruticose

chlorolichens with lower STM (Fig. 11), as expected by hypothesis 2a. However, compared to previously tested foliose chlorolichens with similar STM (i.e. *Lasallia pustulata* and *Dermatocarpon miniatum*), the studied cephalolichens reactivated somewhat faster, initiating and reaching full photoactivation at lower water contents, although methodological differences in F_v/F_m calculations could confound such comparisons. As the hydration level required for maximum PSII activation in humid air was not higher in cephalolichens compared to chlorolichens with similar STM, it seems that additional colonies of cyanobacteria do not increase cephalolichens' water demand for photoactivation, contrary to what was expected by hypothesis 2b. Cyanolichens, having cyanobacteria as their only photobiont, are known to require liquid water to resume photosynthesis (Lange et al., 1986, but see Schlensog et al. 2000). Cephalolichens per definition incorporate colonies of cyanobacteria, yet these play a secondary role to their photosynthesis and carbon budget, and more importantly constitute a source of nitrogenous compounds and alternative carbohydrates (Millbank, J. W. & Kershaw, K. A., 1970). As green algae remain the dominant photobiont in cephalolichens, it is reasonable that cephalolichens' hydration requirements for photoactivation resemble more those of chlorolichens than those of cyanolichens.

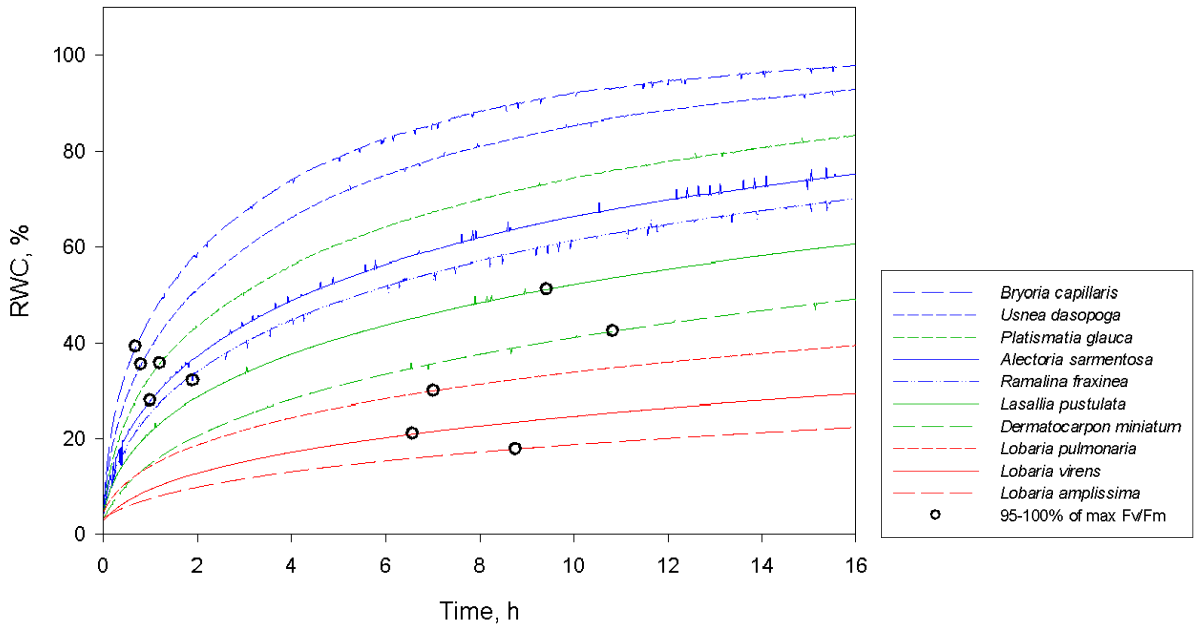

Fig. 11 Rehydration from the dry-state shown as change in relative water content (RWC) during 16 h in humid air (RH ≈ 100%) in foliose cephalolichens (red) compared to foliose (green) and fruticose (blue) chlorolichens. Lines represent species means (cephalolichens n=10; chlorolichens n=5). Circles mark the point of 95% (cephalolichens) and 100% (chlorolichens) of maximum F_v/F_m in humid air. The cephalolichens rehydrated and reactivated slower than fruticose chlorolichens with lower STM. Compared to foliose chlorolichens with similar STM, the cephalolichens increased their RWC more slowly and reactivated PSII after shorter time at lower RWC. Chlorolichen data retrieved from Phinney et al. 2018.

The cephalodia of the studied species consist in internal aggregations of *Nostoc* allocated in the medulla (Jordan, 1970; Millbank, J. W. & Kershaw, K. A., 1970), and thus below the green algal layer situated right beneath the upper cortex. Therefore, fluorescence measurements would mainly reflect changes in the photosynthetic activity of the green algae in response to humid air even if the cyanobacteria in the cephalodia below had been activated. However, *L. amplissima* bears additional cephalodia on its upper surface that mask underlying green algae. In the fluorescence images, in which change in F_v/F_m during rehydration in humid air is translated to change in color, these superficial cephalodia appeared as black and thus inactive spots in contrast to the otherwise increasingly colored and thus activating thalli (Fig. 8). Photoactivation in the external cephalodia only occurred after subsequent wetting with liquid water, applied after the 20 h trial in humid air. This observation illustrates the contrasting ability between green algal and cyanobacterial photobionts in exploiting water vapor to rehydrate and reactivate photosynthesis (Lange et al., 1986).

The time series of fluorescence images further revealed that photoactivation started in lobe tips and outer lobes, and only later spread towards the middle thallus parts (Fig. 7). This activation pattern was observed in all specimens regardless of species. Marginal thallus parts are more exposed to humid air than the more protected central and basal laminal parts of the thallus and therefore attain sufficient hydration for photoactivation after shorter time of exposure. Moreover, lower STM in thinner apices could ensure more rapid activation of peripheral thallus parts, as the thickness of the thallus cortex appears to decrease towards thallus margins (N. H. Phinney, pers. comm.), although this needs further documentation across *Lobaria* species. Different functional traits across individual thalli could provide ecological flexibility, as thinner external lobe tips may rapidly hydrate and activate photosynthesis in response to humid air, while the thicker central lobes may secure water supply over time after saturating hydration events.

The results further indicate that photoactivation does not depend exclusively on specific hydration levels across species. Water contents measured as WC_{DM}, WC_A and RWC at initiating (10% F_v/F_m) and reaching full (95% F_v/F_m) photoactivation mostly varied among the study species, with the exception of WC_{DM} at 10% F_v/F_m that was similar across all the species. The lack of consistent water contents at specific points of photoactivation across species suggests interspecific differences in the hydration level required for initiating and completing photoactivation. Although *L. amplissima* initiated and fully activated photosynthesis somewhat later than the other two other species, as expected by its thicker growth form and slower water

vapor uptake per dry mass, 95% F_v/F_m occurred at significantly lower WC_{DM} in *L. amplissima* than in *L. virens*. *Lobaria virens* and *L. pulmonaria* also differed in WC_{DM} when approaching full photosynthesis. Water content expressed per total thallus dry mass (WC_{DM}) could, however, result an inappropriate parameter for the hydration level required for photoactivation, as only the photobiont requires hydration to resume photosynthesis. Regardless of the thickness of the thallus, the photobiont layer in heteromerous lichens is located directly beneath the upper cortex, and superficial hydration could therefore be sufficient to induce photosynthetic responses. A possible interpretation is that more massive specimens, such as those of *L. amplissima*, activate at lower WC_{DM} because the mycobiont gives priority to its photobiont with respect to internal allocation of absorbed water. Such an interpretation implies that photoactivation across species occurs after a fairly fixed exposure time in humid air despite significant species-specific differences in water contents.

Water content given as percent of $WHC_{internal}$ (RWC) could represent a better predictor for photoactivation across species than WC_{DM}. Although *L. amplissima* reached full activation after longer time, *L. amplissima* and *L. virens* attained 95% F_v/F_m at the same RWC. This could indicate the presence of a threshold value of RWC for photoactivation, as observed in previously studied chlorolichens (Phinney et al., 2018). Area-based hydration traits should also be better predictors of activation times than mass-based hydration traits, as a certain amount of water per area unit would be necessary to hydrate the photobiont layer in species with similar photobiont layer thickness. In fact, WC_A was consistent between *L. pulmonaria* and *L. virens* at initiating and complete photoactivation. These two species could have comparable photobiont layer thickness when exhibiting similar STM (Gauslaa & Coxson, 2011), and thus similar hydration requirements in terms of WC_A for photoactivation, even though previous chlorophyll measurements discussed above suggest the opposite (Gauslaa & Solhaug, 1996).

Although sufficient hydration is a prerequisite for photosynthesis (Lange et al., 1986), other factors than hydration level could influence photoactivation times. Even if metabolic activation can occur almost instantaneously in dry lichens when given liquid water (Lange et al., 1989), species-specific activation time-lags have been reported for some species (Lidén et al., 2010), and such time-lags could possibly be stronger during hydration in humid air alone. Dry and dormant lichens easily accumulate damage under certain environmental conditions (e.g. Färber et al., 2014; Gauslaa & Solhaug, 1996). As lichens rehydrate, they initially release CO_2 (Smith & Molesworth, 1973). This initial "respiratory burst" has led several authors to suggest that lichens need to repair accumulated damage before positive net photosynthesis is restored

(Brown et al., 1983; Smith & Molesworth, 1973). The efficiency of such reparatory mechanisms may differ between species, causing interspecific differences in activation time-lags (Lidén et al., 2010). As a result, photoactivation may not occur in response to the sufficient hydration level alone, but rather after the sum of the time required to attain sufficient hydration and the species-specific time required for reparation and preparation for photosynthesis. Differences in activation time-lags between *L. amplissima*, *L. pulmonaria* and *L. virens* could therefore contribute to explaining the observed inconsistency in hydration level required for photoactivation in these species.

Overall, the reported results indicate a clear trade-off between water storage capacity and the ability to rapidly hydrate and reactivate photosynthesis in humid air. The thick thalli of *L. amplissima* hold higher amounts of water when provided in abundance but are relatively inefficient in hydrating and resuming photosynthesis when exposed to humid air alone compared to the thinner thalli of *L. pulmonaria* and *L. virens*. However, *L. amplissima* reached similarly high F_v/F_m to *L. pulmonaria* when given sufficient time in humid air, suggesting that conditions providing persistently high air humidity could sustain photosynthesis also in this species. The high area-to-mass ratio and projecting growth form of *L. pulmonaria* likely explain the highly efficient water vapor uptake observed in this species. The more flexible hydration strategy of *L. pulmonaria* compared to *L. amplissima* and *L. virens* may further explain its broader distribution pattern, stretching from rainy coastal forests to humid continental forests. *Lobaria virens* hydrated more slowly than *L. pulmonaria* yet was equally efficient in resuming photosynthesis in humid air. Thus, other factors than hydration requirements likely restrict this species to coastal habitats (Solhaug et al., 2018). The ability to store water after saturating hydration events provided by high $WHC_{internal}$ would nevertheless represent an advantage for both *L. virens* and *L. amplissima* in rainy habitats along the Norwegian coast.

Concluding remarks

Cephalolichen members of *Lobaria* succeed in utilizing water vapor to resurrect from the dormant dry-state and resume photosynthesis within the time frames of chlorolichens with similar STM. Morphological variation across cephalolichen species generate species-specific kinetics of water vapor uptake and concurrent photoactivation. Thallus thickness is not only an important morphological trait in shaping lichens' water relations, but also influences the time and hydration level required to initiate and fully reactivate photosynthesis upon rehydration in humid air. Divergent hydration strategies reflect the inevitable trade-off between a thick thallus providing high water holding capacity after saturating hydration events and a thin thallus assuring rapid rehydration and photoactivation when exposed to humid air alone. These contrasting abilities embedded in morphological adaptations to different hydrations regimes further elucidate species' niche preferences and geographical distributions. High temporal resolution monitoring of hydrating thalli in humid air by automatic weighing combined with chlorophyll fluorescence imaging provides valuable insight to the coupled kinetics of water vapor uptake and photoactivation in lichens. Information on species-specific hydration requirements is crucial to conservation planning, enabling both the identification of suitable protection areas, and the execution of successful reintroductions of species at risk.

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Supplementary material

Table 1 Coefficients for the species mean (n=10) water uptake curves (Fig. 9A-C) as fitted to the MMF model: $y= (ab + cx^d)/(b + x^d)$**.** R ≈ 1 indicates a particularly good fit.

MMF model: $y= (ab + cx^d)/(b + x^d)$	*Lobaria amplissima*	*Lobaria pulmonaria*	*Lobaria virens*
WC_{DM} – Time (Fig. 9A)	S = 0.141; R = 0.999	S = 0.135; R = 0.999	S = 0.115; R = 0.999
a	3.857	3.480	2.127
b	1.166	7.239	9.120
c	1.305	1.635	1.670
d	5.718	5.280	5.492
WC_A – Time (Fig. 9B)	S = 0.064; R = 0.999	S = 0.023; R = 0.999	S = 0.025; R = 0.999
a	1.800	5.868	4.638
b	1.205	7.372	9.420
c	5.570	2.728	3.522
d	5.742	5.285	5.501
RWC- Time (Fig. 9C)	S = 0.076; R = 0.999	S = 0.084; R = 0.999	S = 0.059; R = 0.999
a	2.066	2.170	1.105
b	1.149	7.199	9.135
c	7.049	1.013	8.568
d	5.707	5.282	5.493

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