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Non-invasive monitoring of photosynthetic activity and water content in forest lichens by spectral reflectance data and RGB colors from photographs

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ABSTRACT

There is a need for non-invasive monitoring of temporal and spatial variation in hydration and photosynthetic activity of red-listed poikilohydric autotrophs. Here, we simultaneously recorded kinetics in RGB-colors (photos), reflectance spectra, water content, maximal (F_V/F_M), and effective quantum yield of PSII (Φ_{PSII}) during desiccation in foliose lichens differing in cortical characteristics and photobionts. The spectral absorbance peaks of chlorophyll *a*, phycocyanin, and phycoerythrin were clearly displayed at high hydration levels. Brightness and total RGB colors of the lichens strongly increased during desiccation. The normalized difference vegetation index (NDVI) efficiently estimated hydration level and Φ_{PSII} – a proxy for lichen photosynthesis – in all species, including threatened old forest lichens. Color and reflectance indices based on green wavelengths gave good estimates of water content in cephalo- and chlorolichens, but not in cyanolichens with a wider range of photosynthetic pigments. Due to species-specific characteristics, species-wise calibration is essential for non-invasive assessments of lichen functioning.

1. Introduction

While most plants freely expose their chlorophylls to the sun, and thus to our eyes, the visibility of photosynthetic pigments in lichens is often reduced. The green reflectance peak is weak in many lichens (Gauslaa 1984) because lichen cortices in sun-exposed habitats are deeply colored by species-specific pigments (e.g., Brodo et al., 2001; pp 34–35). The mycobiont produces such pigments to reduce the photoinhibitory stress for its photosynthetic partner (Solhaug and Gauslaa 1996; Gauslaa and Solhaug 2001, 2004) and to optimize growth across natural light gradients (Gauslaa and Goward 2020). Light screening not only involves cortical pigments (Solhaug et al., 2010), but also reflectance from surfaces of air spaces inside dry cortices and external structures (e.g., pruina, hairs, calcium oxalate crystals). Such structures become exposed after surface water has evaporated and they contribute to the increased opaqueness of lichen cortices during drying. Thereby, light availability for the underlying photobiont is reduced, which is essential because dry and inactive lichens can experience photo-oxidative stress (Beckett et al., 2021).

Hydration is required for lichen photosynthesis (Lange et al., 1970, 1990) and growth (Palmqvist and Sundberg 2000; Gauslaa et al., 2007). While internal layers of a lichen thallus always retain air spaces, the cortex often becomes entirely filled with water during hydration (Honegger and Peter 1994; Honegger 2006). Thereby, hydration opens a window to the photobiont layer beneath the cortex and increases the exposure of lichen photobionts to solar radiation (Gauslaa and Solhaug 2001), which facilitates photosynthesis and growth in shaded habitats (Palmqvist 2000; Gauslaa et al., 2020). At the same time, increased reflectance and thus brightness during desiccation is a strategy to reduce photoinhibition and heat load in lichens during sunny weather (Sancho et al., 1994; McEvoy et al., 2007). Species-specific light-absorbing (e.g., melanin, parietin) or light-reflecting cortical pigments (e.g., atranorin, usnic acid) further adjust the solar radiation reaching the photobionts (Solhaug et al., 2010; Phinney et al., 2019). Even though the interaction of hydration and spectral properties profoundly shapes lichen functioning (Phinney et al., 2022), such relationships are not well studied, and – due to species-specific screening pigments and photobiont associations – there is a need to study these interactions across a range of species with different light requirements.

Vegetative parts of lichens vary more in color than those of plants (Rikkinen 1995). Spectral characteristics and color analyses are therefore interesting tools in lichen ecology. For example, spectral remote

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sensing in lichen-dominated landscapes allows estimation of species abundance and distribution (Nelson et al. 2013, 2022; Kuusinen et al., 2020; Macander et al., 2020), and the high albedo of dominant terricolous lichens may help counteract global warming (Beringer et al., 2005; Aartsma et al., 2020, 2021). In plants, the normalized difference vegetation index (NDVI) is correlated with the absorbed photosynthetic active radiation (Choudhury 1987). NDVI has thus been widely used to estimate plant productivity (Petorelli 2013). For lichens, however, water content and species-specific cortical pigments largely modify their spectral reflectance (Gauslaa 1984). Because lichen photosynthesis depends on light and hydration, we need to know how water content affects the reflectance before using spectral analyses to estimate lichens' productivity. Assessments of spectral characteristics or RGB colors may allow simple non-invasive estimates of lichens' water content and photosynthesis. Some of this knowledge is established for lichens in exposed polar environments (Barták et al., 2015, 2018a), but not for less pigmented and green forest lichens. Our motivation was to study old forest species because non-invasive monitoring of lichen functioning is especially needed for threatened species for which collection should be minimized.

This study aims to quantify relationships between water content, spectral reflectance, and RGB colors versus maximal (F_V/F_M) and effective quantum yield of photosystem II (Φ_{PSII}) in forest lichens with different photobionts and cortical pigments. For comparison, we include one species from sun-exposed rocks. Our specific objectives are as follows: (1) Evaluate the potential of spectral reflectance as a proxy for water content in forest lichens. (2) Quantify relationships between their reflectance indices and Φ_{PSII}. (3) Compare spectral reflectance indices and color indices estimated from RGB photos of forest lichens as predictors of F_V/F_M that may function as an indicator of a physiological active state and of Φ_{PSII} – a proxy for lichen photosynthesis (as documented by Solhaug et al., 2021).

2. Material and methods

2.1. Lichen material

We used foliose lichens differing in photobionts and cortical properties, including chlorolichens (with green algae as the only photobiont), cyanolichens (with cyanobacteria only) and cephalolichens hosting both algae and cyanobacteria. The cephalolichen *Peltigera leucophlebia* and the cyanolichen *P. praetextata* were collected in rocky, East-facing open forests in Ås, SE Norway (59°41′48″N, 10°44′18″E, 50 m a.s.l.). The cephalolichens *Lobaria pulmonaria*, *L. virens*, and *L. amplissima* were sampled from large mixed populations on *Quercus robur* trunks in open old-growth broadleaved deciduous forests in Porsgrunn (Longinotti et al., 2017), SE Norway (59°06′43″ N, 9°50′05″ E, 150 m.a.s.l.). The two latter species are now often referred to as *Ricasolia amplissima* and *R. virens*. Collected cephalo- and cyanolichens were partly shade-adapted. By contrast, the chlorolichen *Umbilicaria spodochroa* was sampled on sun-exposed seashore rocks in Bohuslän, W Sweden (58°50′23''N, 11°09′15''E). Ten separate specimens of each species were sampled in September 2019, air-dried at 20 °C, and stored 1–4 weeks in the fridge (4 °C) until start of experiments.

The upper cortex of *L. pulmonaria* and *L. virens* in open habitats contains the UV- and PAR-absorbing pigment melanin, but lacks pigments in shade-adapted thalli (Gauslaa and Solhaug 2001). Our specimens had some melanin. *Lobaria amplissima* lacks melanin, but has white atranorin crystals in the upper cortex (Jørgensen and Tønsberg 2007), likely contributing to its whitish green color. Traces of atranorin were reported in *L. virens* (Asplund et al., 2010), but could not visibly be detected in our specimens. No cortical compounds were reported in *Umbilicaria spodochroa* (Hitch and Purvis 2009) and *Peltigera praetextata* (Vitikainen 2007).

2.2. Pretreatment

All specimens were sprayed with de-ionized water and acclimated for 24 h at 5 μmol photons m^{-2} s^{-1} and 20 °C. For each species, 3–4 hydrated thalli were shaken to remove external water and then immediately weighed before the hydrated thallus area (A) was measured with a leaf area meter (LI-6100, LiCor, Lincoln, NE, USA).

2.3. Desiccation cycles

Before each desiccation cycle, we hydrated 3–4 randomly selected specimens of each species and pre-adapted them to light (185 μmol photons m^{-2} s^{-1}) at 20 °C for 10 min. The species did not differ in size ($P = 0.405$; one-way ANOVA; mean A = 15.3 cm^2; total range = 5–25 cm^2. Their mean specific thallus mass (DM/A in mg cm^{-2} ± 1 SE) was: *L. amplissima* = 22.8 ± 2.5, *U. spodochroa* = 18.6 ± 1.8, *L. virens* = 15.0 ± 2.2, *L. pulmonaria* = 14.0 ± 0.3, *P. praetextata* = 11.2 ± 0.6, *P. leucophlebia* = 9.7 ± 0.9. After shaking the excess water, effective quantum yield of PSII (Φ_{PSII}) was measured by a red light Imaging PAM M-series fluorometer (Heinz Walz GmbH, Effeltrich, Germany) using actinic light of 200 μmol red photons m^{-2} s^{-1}. Immediately after the Φ_{PSII} measurement, thalli were weighed and photographed. This was repeated every 15 min until the samples were dry.

Afterwards, lichens were again rehydrated at 20 °C and kept moist for 24 h. Then nine discs (25 mm diameter) were cut with a cork borer from 3 to 4 specimens of each species to secure repeated measurements of spectral reflectance and maximum quantum yield of PSII (F_V/F_M) on the same spot during drying. Spectral reflectance was measured with PolyPen RP 410 (Photon Systems Instruments, Czech Republic). F_V/F_M was measured in 15 min intervals using a PEA fluorimeter (Hansatech Instruments, United Kingdom) until the lichens were dry. Samples were reweighed immediately after every recording of spectra and F_V/F_M. To reduce the between-disc variation noise in spectral signals, the spectra for all nine discs of each species were first sorted after decreasing water contents from fully hydrated to dry. In this sorted set of data, the water contents and the reflectance spectra were averaged for 10 by 10 measurements.

The following reflectance indices were computed for each disc and each hydration level: Normalized Difference Vegetation Index (NDVI) = $(R_{740} - R_{660})/(R_{740} + R_{660})$, Greenness index (G) = R_{554}/R_{677}, Average Visible Reflectance (AVR) = Average (R_{380}; R_{740}) and Green/Blue reflectance index (G550/B450) = R_{550}/R_{450}. Finally, all samples were dried for at least 12 h at 80 °C to record DM of all thalli and discs. Percent water content (WC) was calculated and attributed to each measurement (Φ_{PSII}, analysis of colors in photos, and spectral reflectance).

2.4. Photography analyses

Photographs of all thalli were taken immediately after each measurement with a Pentax K–5II SLR camera (Sigma 70 mm macro lens) attached to a stand with a fluorescent tube positioned at 45° angle on each side in a room without windows to standardize the exposures. Shutter time, aperture and color balance were fixed. The pictures were saved in high resolution JPG-format and analyzed with the ImageJ 1.52R software (National Institutes of Health, USA). The whole thallus was selected as the area of interest for each analysis. The mean unweighted intensity (Tot) and the intensity of the blue (B), green, (G) and red (R) intensity were measured with the histogram function. The same parameters (B_{ref}, G_{ref}, R_{ref} and Tot_{ref}) were measured for the white paper background as a reference. The relative percent total reflectance was calculated = (Tot x 100)/(Tot_{ref}). This was done separately for blue, green, and red light by inserting B, G, and R values, respectively, in the formula.

2.5. Statistical analyses

Plots showing trend lines with ±1 standard error bands for each species were made. Linear regression models for some dependent variables for sets of data fulfilling the requirements of the analysis were run; the full models are given in Supplementary Material. Values of r^2_{adj} were given in the text for these models. Independent variables were water content (numerical parameter; log-transformed when required) and species (categorical parameter). The variation inflation factors in linear regression models were less than 1.4, consistent with low levels of multicollinearity. Statistical analyses were done in in Jamovi 1.8.1 (https://www.jamovi.org/).

3. Results

3.1. Color changes during desiccation

The lichen colors changed in species-specific ways during desiccation (Fig. 1). *Lobaria pulmonaria* and *L. virens* changed from green with a slight brownish tinge when hydrated to pale brownish grey (slightly melanic) when dry. While hydrated, *Lobaria amplissima* was greyish green and *P. leucophlebia* pure green, they both became bright grey without any brownish tinge during desiccation. The chlorolichen *U. spodochroa* showed minor color changes with the disappearance of a rather subtle greenish tinge of grey to slightly brownish grey when

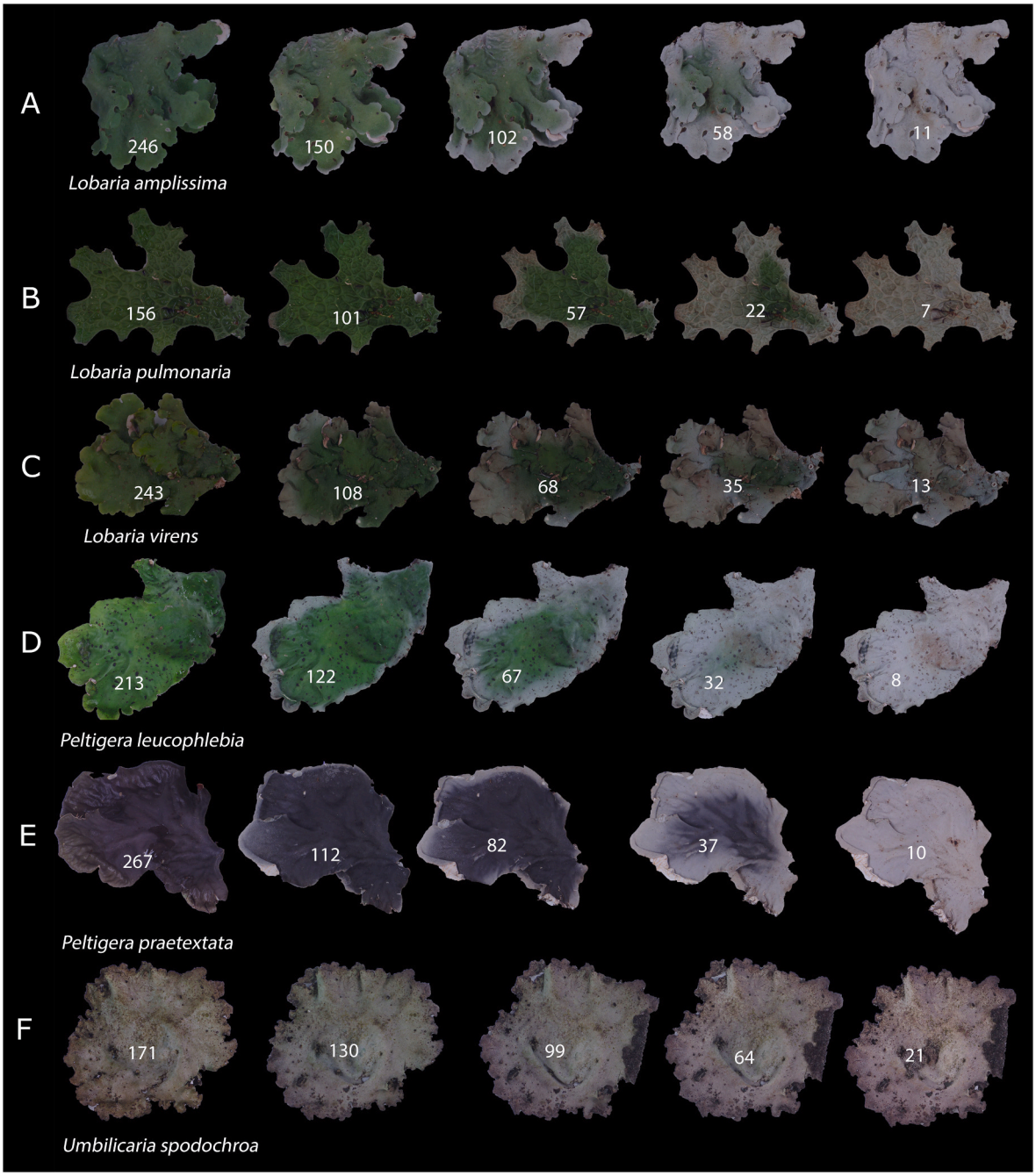

Fig. 1. Color changes in six foliose lichens during desiccation cycles from the fully hydrated (left side) to the air-dry state (right side). The lichens were (starting from top): the cephalolichens (A) *Lobaria amplissima,* (B) *L. pulmonaria,* (C) *L. virens* and (D): *Peltigera leucophlebia*; (E) the cyanolichen *P. praetextata*; and (F) the chlorolichen *Umbilicaria spodochroa*. The white number in each photo refers to the water content in percent when the photograph was taken. The time to become air-dry was 465 min for *Lobaria* species; 405 min for *U. spodochroa* and *Peltigera* species.

desiccated. By contrast, the cyanolichen *P. praetextata* changed from dark brownish black in the hydrated state to pale grey with a faint reddish tinge when dry (Fig. 1).

The reflectance spectra (Fig. 2) sharply peaked at green wavelengths (550 nm) for all fully hydrated cephalolichens. However, the overall visible reflectance (400–700 nm) was highest in *L. amplissima* (Fig. 2), manifested by a whitish appearance visible even at full hydration (Fig. 1). In the chlorolichen *U. spodochroa*, the green reflectance peak at 550 nm was less distinct and faintly detectable at high water contents only (Fig. 2). In all species, the reflectance at visible wavelengths increased with decreasing water content but stayed at a high constant level in the near infrared (700–800 nm) during desiccation. The drying-induced increase in visible reflectance was most rapid at more advanced drying stages (water content ≤50%) and most strong in lichens that became bright grey when dry (Fig. 1; *L. amplissima, P. leucophlebia, P. praetextata*).

For all well hydrated cephalo- and cyanolichens, the absorbance peak of chlorophyll (Chl) *a* at 665 nm was visible as a sharp reflectance minimum. This minimum was also visible, but less sharp, for the sun-adapted chlorolichen *U. spodochroa*. For the cyanolichen, *P. praetextata*, the absorbance peak of phycocyanin at 620 nm was visible as a second sharp reflectance minimum to the left of that of Chl *a*, whereas phycoerythrin with maximal absorbance at 560 nm was visible as a third less sharp reflectance minimum.

3.2. Reflectance indices during desiccation

The darkening in color at increasing water contents was clear both from the average visible reflectance data (Fig. 3A) and the total RGB color analysis (Fig. 3B). Total RGB colors linearly declined with water contents, whereas the average visible reflectance showed a linear response with log-transformed values. A linear regression analysis for visible reflectance in Fig. 3A versus the factors log-transformed water content and species showed that these parameters were significant ($P < 0.001$; $r^2_{adj} = 0.659$; Supplementary Material A1). For the RGB-colors shown in Fig. 3B this regression model (but with non-transformed water content) was even stronger ($r^2_{adj} = 0.727$; Supplementary Material A2). RGB colors were associated with visible reflectance in a regression analysis using species as a categorical factor ($r^2_{adj} = 0.556$; $P < 0.001$; data not shown). The cephalolichen *P. leucophlebia* and the cyanolichen *P. praetextata* were highest in RGB colors throughout the desiccation cycle (Fig. 3B) and these two *Peltigera* species showed the

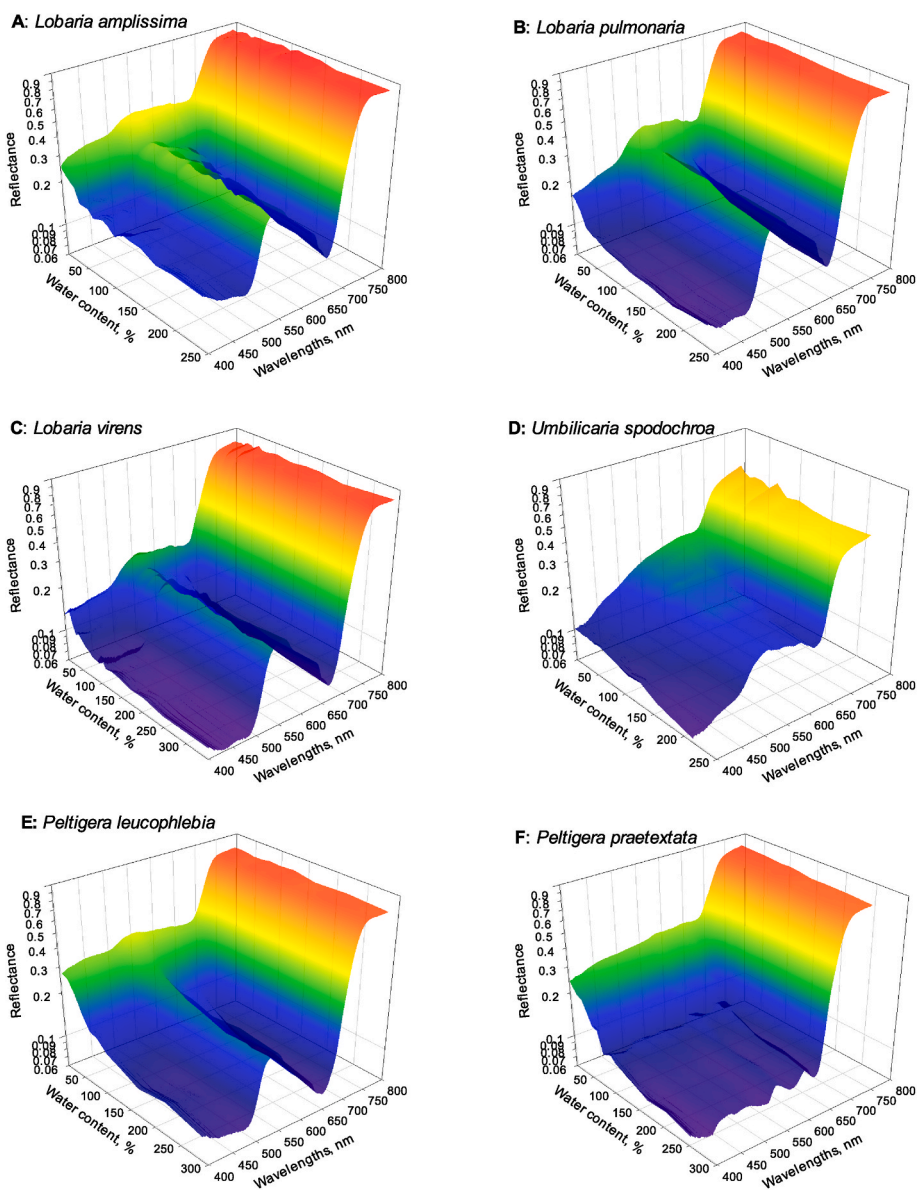

Fig. 2. Spectral reflectance (400–800 nm) as a function of percent water content measured from the upper side of six foliose lichen species. The species were as follows. Four cephalolichens: (A) *Lobaria amplissima,* (B) *L. pulmonaria,* (C) *L. virens* and (E) *Peltigera leucophlebia*. One chlorolichen: (D) *Umbilicaria spodochroa*, and one cyanolichen: (F) *P. praetextata*. The artificial colors (shown in the online version only) illustrate the level of reflectance. The reflectance (y-axis) is log-transformed to emphasize details at visible wavelengths.

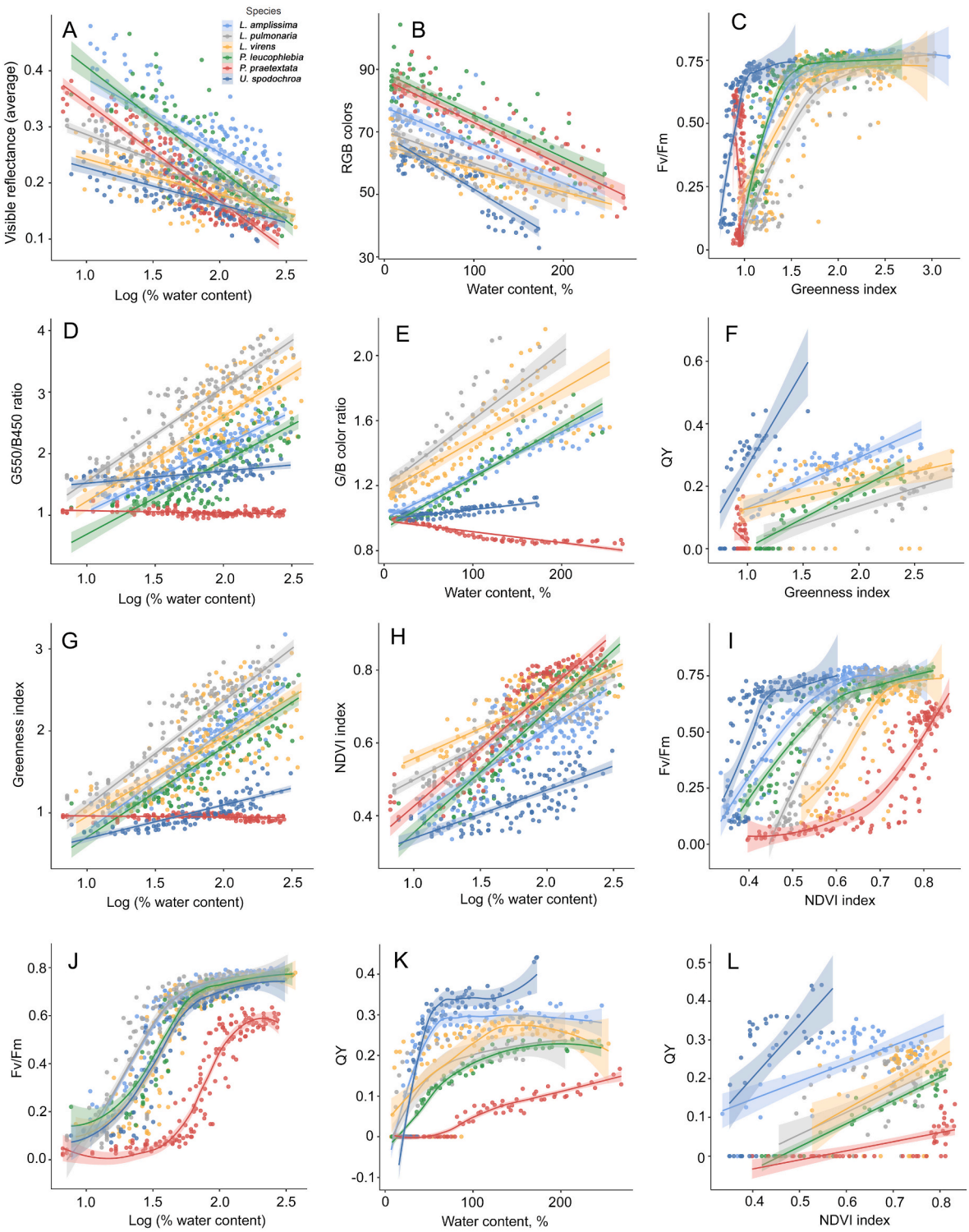

Fig. 3. Measured reflectance (A, D, G, H) and RGB-color indices (B, E) – as well as maximal (F_V/F_M; J) and effective quantum yield of PSII (Φ_{PSII}; K) – versus water content (in percent). In addition, F_V/F_M (C, I) and Φ_{PSII} (F, L) are plotted versus the greenness index (C, F) and versus the normalized difference vegetation index (NDVI; F, L). The water content was log-transformed when this resulted in linear responses of the respective parameter. The shaded band around each line shows ±1 standard error. Lines and symbols are colored in the online version.

steepest hydration-dependent decline in average visible reflectance (Fig. 3A). The cephalolichen L. *amplissima* had the highest average visible reflectance at high water contents (Fig. 3A), whereas the chlorolichen *U. spodochroa* had the lowest scores of both RGB colors and visible reflectance.

The greenest species (*L. pulmonaria, L. virens*) had the highest G550/B450 index and G/B-ratio. Overall, the G/B ratio taken from the photographs (Fig. 3E; $r^2_{adj} = 0.754$; $P < 0.001$; Supplementary Material A4) and the G550/B450 ratio (Fig. 3D; $r^2_{adj} = 0.767$; $P < 0.001$; Supplementary Material A3) strongly depended on hydration and species. A simple linear regression analysis showed that the G/B ratio and G550/B450 were strongly coupled ($r^2_{adj} = 0.741$; $P < 0.001$). The greenness

index (Fig. 3G; $r^2_{adj} = 0.751$; $P < 0.001$; Supplementary Material A5) increased strongly with the G550/B450-ratio, as shown by a linear regression analysis ($r^2_{adj} = 0.718$, increasing to $r^2_{adj} = 0.834$; $P < 0.001$) when species was added (data not shown). The cyanolichen had a low G550/B450-ratio (Fig. 3D), G/B-ratio (Fig. 3E), and greenness index (Fig. 3G), which were weakly affected by water content in this species.

Among all indices, the strongest regression ($r^2_{adj} = 0.800$; $P < 0.001$; Supplementary Material A6) was found for NDVI versus water content (log-transformed) and lichen species. NDVI increased with water content in all species (Fig. 3H). The NDVI index increased most steeply with water content in the two *Peltigera* species and most slowly in the chlorolichen *U. spodochroa* with low NDVI scores. The cyanolichen had higher NDVI than other lichen species when well hydrated.

3.3. The kinetics of maximal (F_V/F_M) and effective quantum yield of PSII during desiccation

All well hydrated chloro- and cephalolichens reached a maximal F_V/F_M of approximately 0.75 (Fig. 3J). During the first parts of desiccation, F_V/F_M slightly declined to a water content of approximately 50% from where it abruptly and rapidly dropped to inactive levels at further drying. *Lobaria pulmonaria* and *L. amplissima* retained high F_V/F_M to lower water contents than the other species. The cyanolichen, by contrast, had lower F_V/F_M (≈0.6) at full hydration that rapidly declined already at a water content of 130% (Fig. 3J) and became inactive already at approximately 90%.

For the effective quantum yield (Φ_{PSII}), the level and kinetics differed substantially between species (Fig. 3K). Φ_{PSII} was highest in the sun-adapted chlorolichen *U. spodochroa*, followed by the cephalolichen *L. amplissima*, and then by the three other cephalolichens, whereas the cyanolichen had low Φ_{PSII} (Fig. 3K). In the two species with the highest Φ_{PSII} when wet, Φ_{PSII} did not start the faster decline before desiccation had lowered the water content to 50%. By contrast, the rapid decline in the other species started already at a water content of 120%.

F_V/F_M and Φ_{PSII} were plotted against the reflectance indices with the best predictive power. For chloro- and cephalolichens, F_V/F_M (Fig. 3C) and Φ_{PSII} (Fig. 3F) increased in species-specific ways with the greenness index. The cyanolichen was different by not displaying any visible greenness; average visible reflectance was a better predictor for this species (data not shown). F_V/F_M strongly increased with NDVI in highly species-specific ways; it started to increase at the lowest NDVI for *U. spodochroa*, followed by the other species in the sequence *L. amplissima* < *P. leucophlebia* < *L. pulmonaria* < *L. virens* < *P. praetextata* (Fig. 3I). The Φ_{PSII} responded in a similar way to the greenness index (Fig. 3G; $r^2_{adj} = 0.598$; $P < 0.001$; Supplementary Material A7) and the NDVI (Fig. 3L; $r^2_{adj} = 0.586$; $P < 0.001$; Supplementary Material A8), although the cyanolichen response differed for the two indices.

A simple correlation analysis showed that the Φ_{PSII} had the strongest relationship with F_V/F_M ($r = 0.736$), followed by log-transformed water content ($r = 0.652$), RBG colors ($r = -0.598$), and G550/B450-ratio ($r = 0.467$).

4. Discussion

4.1. Hydration and cortical characteristics regulate light for lichen photobionts

In forest lichens, the wetting-induced opening of the cortical window strongly enhances the visibility of the photobiont layer, evidenced by increased greenness of cephalolichens and darkening of the cyanolichen. It is noteworthy that the hydrated cortex of a cyanolichen is sufficiently transparent to expose the spectral signatures not only of Chl *a*, but also of phycocyanin and phycoerythrin.

The sun-adapted *U. spodochroa,* lacking specific cortical pigments, still has an opaque and rather closed cortical window even when fully hydrated (Fig. 1), implying that colored pigments are not required for efficient cortical screening. A thicker cortex in sun-exposed habitats, as measured in *Lobaria hallii* of open compared to closed forests (Gauslaa and Coxson 2011), may contribute to efficient light screening in the light-adapted *U. spodochroa*, likely due to more reflecting micro-structures and air spaces in thick cortices.

4.2. Spectral reflectance and color indices as proxies of water content and photosynthetic activity in chloro- and cephalolichens

Our study has shown that spectral reflectance and RGB-color parameters can, non-invasively and efficiently, assess the water content in many lichen species. The greenness index and other indices using green reflectance (550 nm) predict well the water content of cephalolichens, but not of cyanolichens, because their high green Chl *a* reflectance is counteracted by strong absorbance of green light by phycoerythrin and phycocyanin. Indices based on green color can predict water contents of the sun-exposed *U. spodochroa*, but not as efficiently as of the cephalolichens. By contrast, NDVI calculated as the (740 - 660)/(740 + 660)-ratio is not affected by the strong green reflectance peak in chloro- and cephalolichens but depends on the low Chl *a* reflectance at 660 nm characterizing cyanobacteria as well as green algae. Therefore, NDVI responds similarly to hydration in cyano- and cephalolichens. NDVI is known to correlate well with water potential in *Umbilicaria hirsuta* (Gloser and Gloser 2007) and with water content in *Dermatocarpon polyphyllizum* (Barták et al., 2018b). However, NDVI was apparently a less accurate predictor of the water content of the sun-adapted chlorolichen with the most closed cortical window, as was also the case for polar chlorolichens (Barták et al., 2015, 2018a). Nevertheless, because the NDVI was hydration-dependent in all studied species, it may serve as a general, non-invasive proxy of the hydration status in lichens. In any case, species-specific responses imply a need for species-specific calibration before using spectral or RGB-color indices as proxies for water content.

The species-specific coupling between hydration and spectral indices likely contributed to the strong species-specific relationships between such indices versus F_V/F_M and Φ_{PSII}. We have shown that the widely used NDVI used for prediction of plant production (Petorelli 2013) was also a good predictor for Φ_{PSII} – a proxy of photosynthesis (Solhaug et al., 2021) – during desiccation cycles in lichens. The strong hydration-dependence of the maximal (Fig. 3J) and effective quantum yield of PSII (Fig. 3K) during desiccation cycles is consistent with previously recorded hydration-dependent kinetics of CO_2 uptake (Lange 1965, 1969) and Φ_{PSII} (Barták et al., 2015, 2018a) in lichens. Because chloro- and cephalolichen photosynthesis is readily activated by humid air (Lange et al., 1993), spectral and RGB-based indices likely predict their Φ_{PSII} during hydration by humid air as well as by rain. By repeatedly recording the non-invasive NDVI for lichen specimens over time, it should be possible to assess C-gain and to identify windows in time and space where they can grow, which is particularly useful for rare and threatened species. However, because cyanolichens require liquid water (rain, fog and dewfall) to activate CO_2 uptake (Lange et al., 1993), such indices unlikely monitor the Φ_{PSII} in cyanobacteria during hydration in humid air.

An advantage of indices based on RGB colors from photoimages is that the entire thallus area can easily be monitored, showing an average response across both hydrated central parts and desiccated margins. RGB colors from photography can be conveniently monitored in remote field locations. Chlorophyll fluorescence does not measure CO_2 assimilation directly. Nonetheless, Solhaug et al. (2021) demonstrated that Φ_{PSII} measurements averaged across an entire thallus using imaging PAM chlorophyll fluorescence, including in dry inactive portions, strongly correlated with CO_2 uptake at hydration levels below the optimal water content. This relationship was weaker yet still significant in suprasaturated thalli.

Because thallus water content can be estimated efficiently by reflectance indices, such indices monitored even by remote sensing may

be good proxies also for photosynthetic CO_2 uptake in lichens. There is an increased interest in remote sensing of chlorophyll fluorescence (Mohammed et al., 2019). However, the interpretation of passive solar-induced chlorophyll fluorescence assessed by remote sensing is still immature and more complicated than traditional PAM fluorometry data. Traditional chlorophyll fluorescence techniques using active excitation light require a short distance between the measuring device and the photosynthetic organism and are thus not suited for remote sensing.

4.3. Spectral characteristics as species markers in lichens

For vascular plants – a predominantly homeohydric group – spectral remote sensing has a potential for a fine-scale resolution of detecting ecological dynamics and diversity (Nelson et al., 2022; Kamoske et al., 2022), even at an intraspecific level (Stasinski et al., 2021; Hejtmánek et al., 2022). Due to the strong hydration dependency of spectral reflectance and color indices, remote sensing should be used with caution in efforts to record the taxonomic diversity of lichen-dominated vegetation because effects of hydration may override the effects of species identity. The water holding capacity of lichens – and thus the duration of their photosynthetic periods – varies much between species (Gauslaa 2014; Phinney et al., 2022). At a given time, some species can therefore be dry whereas others still are moist. Finally, the intraspecific variation in colored lichen pigments is substantial (Gauslaa and Ustvedt 2003) due to seasonal (Gauslaa and McEvoy 2005; Bjerke et al., 2005) and spatial variation (Gauslaa and Solhaug 2001; Bjerke et al., 2004), which further complicates the use of spectral data as a marker for taxonomic identity in lichens.

4.4. Spectral characteristics influence the duration of hydration periods

By pigment-specific absorption efficiency of solar radiation, lichens regulate thallus temperature (Kershaw 1975) and thereby the desiccation rate and the duration of hydration periods (Phinney et al., 2022). In this way, hydration level and pigmentation can be confounded factors. For example, the higher visible reflectance in *L. amplissima* than in other measured *Lobaria* species (Fig. 3A) – in combination with its larger water holding capacity (Longinotti et al., 2017) – likely contribute not only to longer photosynthetic active periods (Gauslaa et al., 2017), but also to higher tolerance of excess solar radiation (Gauslaa and Solhaug 1996). This is consistent with its ability to grow in more exposed upper forest canopies (Asplund et al., 2010).

5. Conclusion

At a species-specific level, both RGB-colors and spectral reflectance indices have a high potential as simple and non-invasive proxies of both thallus water contents and effective quantum yield (a strong proxy for lichen photosynthesis) under undisturbed field conditions. NDVI is a good predictor in chloro-, cephalo-, and cyanolichens, whereas other spectral ratios and RGB-indices only work in some species groups. Such non-invasive and easily measured parameters have a great potential for the simple functional assessment of rare and threatened old forest lichens in particular.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.funeco.2023.101224.

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