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ORIGINAL ARTICLE

Photobiont-dependent humidity threshold for chlorolichen photosystem II activation

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Abstract

Main conclusion **Photobiont type influences the relative humidity threshold at which photosystem II activates in green algal lichens.**

Abstract Water vapor uptake alone can activate photosynthesis in lichens with green algal photobionts. However, the minimum relative humidity needed for activation is insufficiently known. The objective of this study was to quantify the humidity threshold for photosystem II (PSII) activation in a range of chlorolichen species associated with photobionts from Trebouxiaceae, Coccomyxaceae and Trentepohliaceae. These lichens exhibit distribution, habitat and substrate patterns that are likely coupled to their efficiency in utilizing water vapor at lower levels of relative humidity (RH) for photosynthesis. Using chlorophyll fluorescence imaging during water uptake from humid air of 25 species of chlorolichens representing the above photobiont groups, we monitored PSII activation within controlled chambers with constant RH at five levels ranging from 75.6 to 95.4%. The results demonstrate clear photobiont-specific activation patterns: the trentepohlioid lichens activated PSII at significantly lower RH (75.6%) than trebouxioid (81.7%) and coccomyxoid (92.0%) lichens. These responses are consistent with a preference for warm and sheltered habitats for trentepohlioid lichens, with cool and moist habitats for the coccomyxoid lichens, and with a more widespread occurrence of the trebouxioid lichens. Within each photobiont group, lichen species exposed to marine aerosols in their source habitats seemed to be activated at lower RH than lichens sampled from inland sites. High osmolyte concentration may therefore play a role in lowering a photobiont's activation threshold. We conclude that photobiont type influences water vapor-driven photosynthetic activation of lichens, thereby shaping the ecological niches in which they occur.

Keywords Chlorophyll fluorescence · Ecophysiology · Green algae · Osmolytes

Abbreviations

F_v/F_m	Maximal quantum yield of PSII
RH	Relative humidity

Introduction

The planetwide colonization of lichenized fungi is in part a reflection of their high diversity of symbiotic lifestyle combinations (Spribille 2018), which allows them to fill an extensive array of niches. While lichen systematics so far has been based on the unique fungal partner (mycobiont) that normally shapes overall thallus morphology, the symbiotic autotroph (photobiont)—either an alga, cyanobacterium, or both—plays fundamental roles in providing the composite organism with photosynthates, and sometimes even shapes the lichen phenotype (e.g. Ertz et al. 2018). Ninety percent of all lichen species have green algal photobionts (chlorolichens), and the roughly 100 algal photobionts commonly reported probably represent only a portion of all algal species present in lichens (Tschermak-Woess 1988).

Nearly 60% of chlorolichens associate with algae in the Trebouxiophyceae (Blaha et al. 2006), which are ubiquitous lichenized photoautotrophic partners in e.g. the hyperdiverse Parmeliaceae (Lecanoromycetes) (see Miadlikowska et al. 2014). Many species in the Parmeliaceae dominate open forest and well-lit upper canopies exposed to frequent desiccation. *Trebouxia* (Trebouxiaceae, Trebouxiophyceae)

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is a widespread and highly studied green algal photobiont genus, represented in the majority of lichen growth forms (Muggia et al. 2018). Lichenized *Trebouxia* can be highly tolerant to desiccation (Kosugi et al. 2009, 2013) and can withstand UV-radiation and oxidative stress—even in a hostile outer space environment (Onofri et al. 2012)—although the combination of prolonged desiccation and light can be detrimental (Gauslaa et al. 2012). Free-living *Trebouxia* also tolerates extended periods without hydration (Carniel et al. 2015), but with less efficiency than those in a lichen symbiosis (Kosugi et al. 2009).

Coccomyxa (Coccomyxaceae, Trebouxiophyceae) is a less common lichen photobiont that has been identified in just 68 lichen species, mainly in polar, boreal-arctic alpine or cooler temperate zones (Gustavs et al. 2017). As free-living algae, *Coccomyxa* is globally distributed, and was even recently found as an epiphyte on trebouxioid lichens in Antarctica (Cao et al. 2018).

Trentepohliaceae (Ulvophyceae) species (most notably *Trentepohlia* spp.) make up the photobionts of approximately 20–23% of lichenized fungi (Nelsen et al. 2011; Grube et al. 2017). They are most abundant in warmer climates (Marini et al. 2011) and are ubiquitous in tropical rainforests as photobionts in foliicolous lichens (Nelsen et al. 2011). As photobionts, they often grow in sheltered positions inside old forests (Stofer et al. 2006; Jüriado and Paal 2019). Some lichens depart from the typical mycobiont-dominated thallus definition of a lichenized fungus, in which *Trentepohlia* dictates the general thallus morphology (e.g. *Coenogonium*; Meier and Chapman 1983) or displays unique modes of lichenization (e.g. *Eremithallus costaricensis*; Lücking et al. 2008). Trentepohlioid algae form free-living colonies on various substrata (see e.g. Rindi and Guiry 2002)—even on sloth fur (Suutari et al. 2010).

High air humidity alone can support high photosynthetic rates in chlorolichens (Lange and Redon 1983; Lange et al. 1986; Pintado and Sancho 2002) and can fill a substantial proportion of chlorolichen hydration requirements (Phinney et al. 2018). Lichens inhabit some of the Earth's driest regions by exploiting non-rainfall water sources (see e.g. Palmer and Friedmann 1990). Humid air is not only a key water source in dry regions (see e.g. Maphangwa et al. 2012), but also in microhabitats sheltered from rain, such as under overhanging rocks and on tree trunks in tropical rainforests (Lakatos et al. 2012).

Nevertheless, we need to better understand how various green-algal photobionts respond to water vapor-driven activation. In addition, because diurnal air humidity oscillations strongly contribute to thallus hydration status, it is important to document threshold RH-levels for photosynthetic activation. This study uses the optimal yield of photosystem II (PSII) (F_v/F_m) as a proxy for the activation of photosynthesis, since F_v/F_m can easily be measured without influencing thallus hydration status—measurement of photosynthetic CO_2 uptake or PSII yield in light will inevitably alter the RH surrounding a thallus. We have unpublished data showing that F_v/F_m is activated at approximately the same RH as photosynthetic CO_2 uptake or PSII yield. Therefore, F_v/F_m is a good proxy for the potential for photosynthesis, although it does not tell much about the level of CO_2 uptake. In this study, we aim to quantify PSII activation in 25 lichenized fungi associated with four green-algal photobiont families, Trebouxiaceae, Coccomyxaceae, Trentepohliaceae and Prasiolaceae at constant RH levels ranging from 75 to 95%. Although Lange et al. (1986) found that RH above 96% yielded negligible differences in photosynthetic responses across lichen species from these three photobiont types, measurements across such groups have not yet been recorded at RHs below 96%. We expect that photobiont type influences the reactivation of PSII depending on RH and hypothesize that the *Coccomyxa* photobiont, being associated with cool climates needs higher RH for photosynthetic activation than *Trentepohlia* associated with sheltered and warm climates. Finally, we hypothesize that the ubiquitous group of lichens with trebouxioid photobionts shows a high variation in RH-dependent activation pattern, depending on the habitat requirements of the associated lichenized fungus.

Materials and methods

Lichen material

Twenty-five species of lichens were collected from Norway, Madeira (Portugal), South Africa, and British Columbia (Canada) in September–October 2017 (Table 1). All lichens were air-dried at room temperature and kept frozen for < 6 months at − 18 °C until the experiments started. From the thalli collected of each species, a batch of thirty 1 cm^2 discs, including the dead tree bark underneath the crustose lichens, were taken randomly using a cork borer. Hair lichens were cut with scissors to approximately the same dimensions. Discs were sprayed with deionized water, and kept hydrated under low light (5 µmol photons m^{-2} s^{-1}) for 24 h, to reduce the effects of possible photoinhibition experienced before collection. At the end of the hydration period, initial maximal PSII efficiency (F_v/F_m) for each disc was recorded using a red-LED IMAGING-PAM M-series chlorophyll fluorometer (Walz, Effeltrich, Germany) and ImagingWin v2.46i (Walz) after 15 min dark adaptation.

Activation experiment

After pretreatment, the lichen discs were dried at room temperature, and when air-dry, were randomly attached with a small drop of glue with acetone solvent that does not harm

Table 1 Twenty-five lichen species associated with four photobiont families, collected from Norway, Madeira (Portugal), South Africa and Canada

Lichen species	Photobiont class	Photobiont family	Photobiont taxa	Collection location
Baeomyces rufus	Trebouxiophyceae	Coccomyxaceae	*Elliptochloris* sp.	Ås, Akershus, Norway
Nephroma arcticum	Trebouxiophyceae	Coccomyxaceae	*Coccomyxa* sp.	Ski, Akershus Norway
Peltigera aphthosa	Trebouxiophyceae	Coccomyxaceae	*Coccomyxa* sp.	Ski, Akershus, Norway
Peltigera britannica	Trebouxiophyceae	Coccomyxaceae	*Coccomyxa* sp.	Lyngdal, Agder, Norway
Solorina saccata	Trebouxiophyceae	Coccomyxaceae	*Coccomyxa* sp.	Frogn, Akershus, Norway
Crocodia aurata[a]	Trebouxiophyceae	Trebouxiaceae	*Symbiochloris* sp.	uMlalazi, South Africa
Lobaria pulmonaria	Trebouxiophyceae	Trebouxiaceae	*S. reticulata*	Kristiansand, Agder, Norway
Lobaria virens	Trebouxiophyceae	Trebouxiaceae	*S. reticulata*	Kristiansand, Agder, Norway
Phlyctis argena	Trebouxiophyceae	Trebouxiaceae	*Dictyochloropsis splendida*	Ås, Akershus, Norway
Rhizocarpon geographicum[a]	Trebouxiophyceae	Trebouxiaceae	*Trebouxia jamesii*	Jeløya, Østfold, Norway
Evernia prunastri	Trebouxiophyceae	Trebouxiaceae	*Trebouxia* sp.	Ås, Akershus, Norway
Lecanora allophana	Trebouxiophyceae	Trebouxiaceae	*Trebouxia* sp.	Ås, Akershus, Norway
Lecidella elaeochroma	Trebouxiophyceae	Trebouxiaceae	*T. arboricola*[b]	Ås, Akershus, Norway
Xanthoria parietina	Trebouxiophyceae	Trebouxiaceae	*T. arboricola*[b]	Ås, Akershus, Norway
Bryoria capillaris	Trebouxiophyceae	Trebouxiaceae	Trebouxioid	Ås, Akershus, Norway
Dermatocarpon miniatum	Trebouxiophyceae	Prasiolaceae	*Diplosphaera* sp.	Ås, Akershus, Norway
Parmotrema austrosinense[a]	Trebouxiophyceae	Trebouxiaceae	Trebouxioid	uMlalazi, South Africa
Parmotrema dilitatum[a]	Trebouxiophyceae	Trebouxiaceae	Trebouxioid	uMlalazi, South Africa
Ramalina thrausta	Trebouxiophyceae	Trebouxiaceae	Trebouxioid	Clearwater valley, BC, Canada
Usnea dasopoga	Trebouxiophyceae	Trebouxiaceae	Trebouxioid	Ås, Akershus, Norway
Graphis scripta[a]	Ulvophyceae	Trentepohliaceae	*Trentepohlia* sp.	Frogn, Akershus, Norway
Lecanactis abietina	Ulvophyceae	Trentepohliaceae	*Trentepohlia* sp.	Ås, Akershus, Norway
Opegrapha rufescens	Ulvophyceae	Trentepohliaceae	*Trentepohlia* sp.	Ås, Akershus, Norway
Roccella montagnei[a]	Ulvophyceae	Trentepohliaceae	*Trentepohlia* sp.	uMlalazi, South Africa
Roccella tuberculata[a]	Ulvophyceae	Trentepohliaceae	*Trentepohlia* sp.	Madeira

[a]From sea shore habitats with marine aerosols

[b]Beck et al. (1998)

dry lichens (Solhaug and Gauslaa 2001) onto plastic mesh within a plastic box (10.5 × 7.5 × 6 cm). One disc from each species was included per box. Five replicate boxes were used for each of five RH levels. Saturated salt solutions (after Rockland 1960 and Greenspan 1977) producing five RHs (75.6, 81.7, 85.9, 92.0, and 95.4%), equivalent to a water potential (Ψ) ranking from − 37.2 to − 6.3 (Table 2) were placed in the bottom of the boxes below the specimens; the RHs used are hereafter referred to as 76, 82, 86, 92 and 95%. The saturated salt solutions were used to create stable microenvironments to study the effects of RH on PSII activation. The salts themselves were not airborne (i.e. there was no wind producing aerosols inside the boxes), and as such they were not intended to mimic marine aerosols. The boxes were sealed with cling film and rubber bands and were placed in a temperature-regulated room at 15 °C. Because lichens equilibrate in humid air at different rates (Phinney et al. 2018), F_v/F_m was repeatedly recorded using the imaging fluorometer through the transparent cling film in the sealed boxes after 18, 22, 40, 45, and 64 h to allow for equilibrium to be reached. Finally, the boxes were unsealed, the discs fully hydrated by spraying with deionized water, and F_v/F_m was again recorded. All fluorescence measurements were performed in darkness.

Table 2 Salts in saturated solutions used to create specific relative humidities (RH) at 15 °C, as measured by Greenspan (1977), and Rockland (1960)

Salt	RH, % at 15 °C	Water potential (Ψ), MPa
Sodium chloride (NaCl)	75.6	− 37.2
Ammonium sulfate ($(NH_4)_2SO_4$)	81.7	− 26.9
Potassium chloride (KCl)	85.9	− 20.2
Barium chloride ($BaCl_2$)	92	− 11.1
Potassium nitrate (KNO_3)	95.4	− 6.3

Corresponding water potentials (Ψ) in MPa were calculated by $\Psi = 10^{-6} \times (R \times T/V_w^o)$*ln(RH), where R is the universal gas constant (8.3143 J mol^{-1} K^{-1}), V_w^o is the molar volume of pure water (18.02 × 10^{-6} m^3 mol^{-1}), and T is Kelvin temperature

Statistical analyses

A generalized mixed linear model for maximal PSII efficiency, F_v/F_m, computed as percent of start values, was run using Minitab v18 (Minitab Inc., State College, PA, USA). Fixed factors were relative humidity level (five RHs) and photobiont group (Coccomyxaceae, Trebouxiaceae Trentepohliaceae). The photobiont group Prasiolaceae was excluded because it only occurred in one lichen species (*Dermatocarpon miniatum*). The random factor was lichen species ($n = 24$) nested in the three photobiont groups. Two of the 625 lichen discs were excluded as extreme outliers (one *E. prunastri* and one *L. pulmonaria* thallus with unusually low start values) to satisfy homoscedasticity requirements of the analyses. One-way ANOVAs were run to test for differences in F_v/F_m between photobiont groups and between F_v/F_m at the start and after the experiment. Means ± 1 standard error are given in the text and Figs.

Results

Hydration by deionized water before the start of the experiment gave the same F_v/F_m in all three photobiont families (one-way ANOVA; $P = 0.060$). Due to a common start level, as indicated by the horizontal line in Fig. 1, the trends in Fig. 1 were also valid for F_v/F_m expressed as percent of start values. When the air-dried lichens had reached an equilibrium at each of the respective five RH levels, the F_v/F_m of the trentepohlioid, trebouxioid and coccomyxoid lichens exhibited significantly different RH-dependent activation profiles (Fig. 1). While the trentepohlioid lichens already activated their PSII at the lowest RH used (76%), trebouxioid and coccomyxoid lichens did not start to activate until 82 and 92%, respectively (Fig. 1). However, none of the photobiont groups fully reached the activity level achieved after hydration by liquid water before the start of the experiment (Fig. 1). The fixed factors RH, photobiont type (P), and the RH × P interaction, were all highly significant factors in a generalized mixed linear model accounting for 78.3% of the RH-dependent change in F_v/F_m during the 64 h period (Table 3). The lichen species, treated as a random factor nested in photobiont type, also contributed significantly to the variation in humidity-induced activation.

After spraying with water, post-experiment F_v/F_m values were lower than pre-start values (ANOVA; $P < 0.001$). Due

Table 3 Generalized mixed linear models for maximal photosystem II efficiency, F_v/F_m, as percent of start values of twenty-four lichen species nested in the three lichen photobiont groups Coccomyxaceae, Trebouxiaceae and Trentepohliaceae

Source	*df*	*F*	*P*
Relative humidity, fixed factor (RH)	4	155.84	0.000
Photobiont class, fixed factor (P)	2	13.16	0.000[a]
RH × P	8	24.44	0.000
Species (random factor) nested in P	23	15.98	0.000
Error	560		
Lack-of-Fit	90	5.23	0.000
Pure error	470		
Total	597		

$R^2_{adj} = 0.783$. Mean values and standard errors for the analyzed data are shown in Fig. 3

[a]Not an exact *F* test

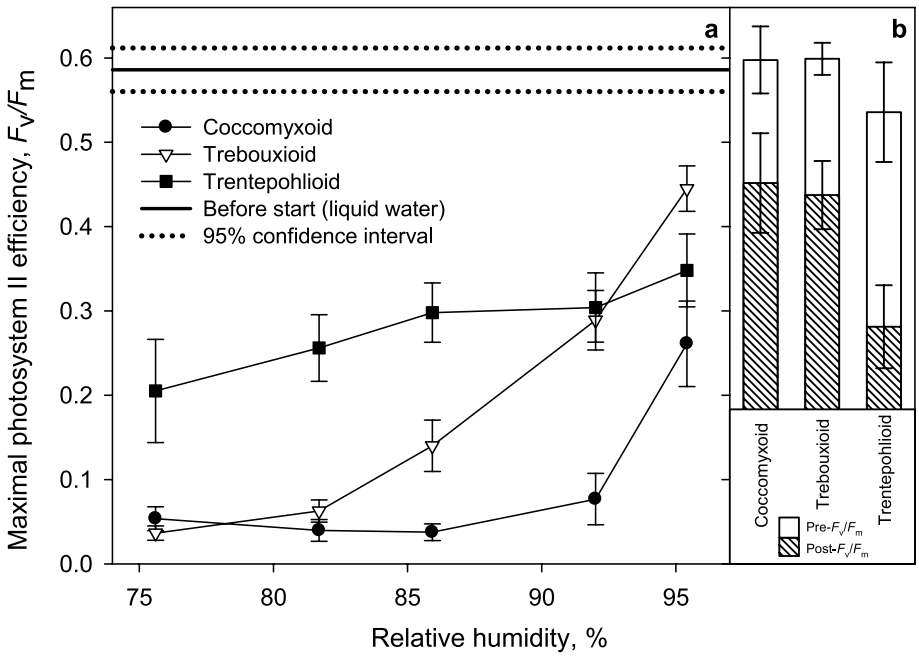

Fig. 1 a The effect of relative humidity (RH) on maximal chlorophyll fluorescence (F_v/F_m) in dried discs of lichens from three photobiont groups (Coccomyxaceae ($n = 5$ spp.), Trebouxiaceae ($n = 14$ spp.), and Trentepohliaceae ($n = 5$ spp.) placed in sealed boxes with constant humidity (see Table 1), after allowing the discs to reach equilibrium within each RH. Symbols and error bars indicate means and standard errors, respectively, of the maximum F_v/F_m recorded within the sealed boxes after a 64 h period. **b** F_v/F_m was also recorded prior to and following being sealed in the boxes after spraying with water; F_v/F_m means and SE within photobiont type are given in the bar graph

to this apparent decline in viability during the experiment, we used the highest F_v/F_m readings for each disc in statistical analyses (normally already at 18 h). This decline in F_v/F_m was significantly greater in the trentepohlioid lichen group (down to 0.294 ± 0.0391) than in the two other photobiont groups (trebouxioid: 0.445 ± 0.0311; coccomyxoid: 0.469 ± 0.0320). For each particular lichen species, the F_v/F_m levels recorded after liquid hydration are given as short, bold ticks on the left y-axis in Fig. 2 before (upper ticks) and after the experiment (lower ticks).

For the trentepohlioid and trebouxioid lichen groups, F_v/F_m tended to be highest at the first measurement (18 h)

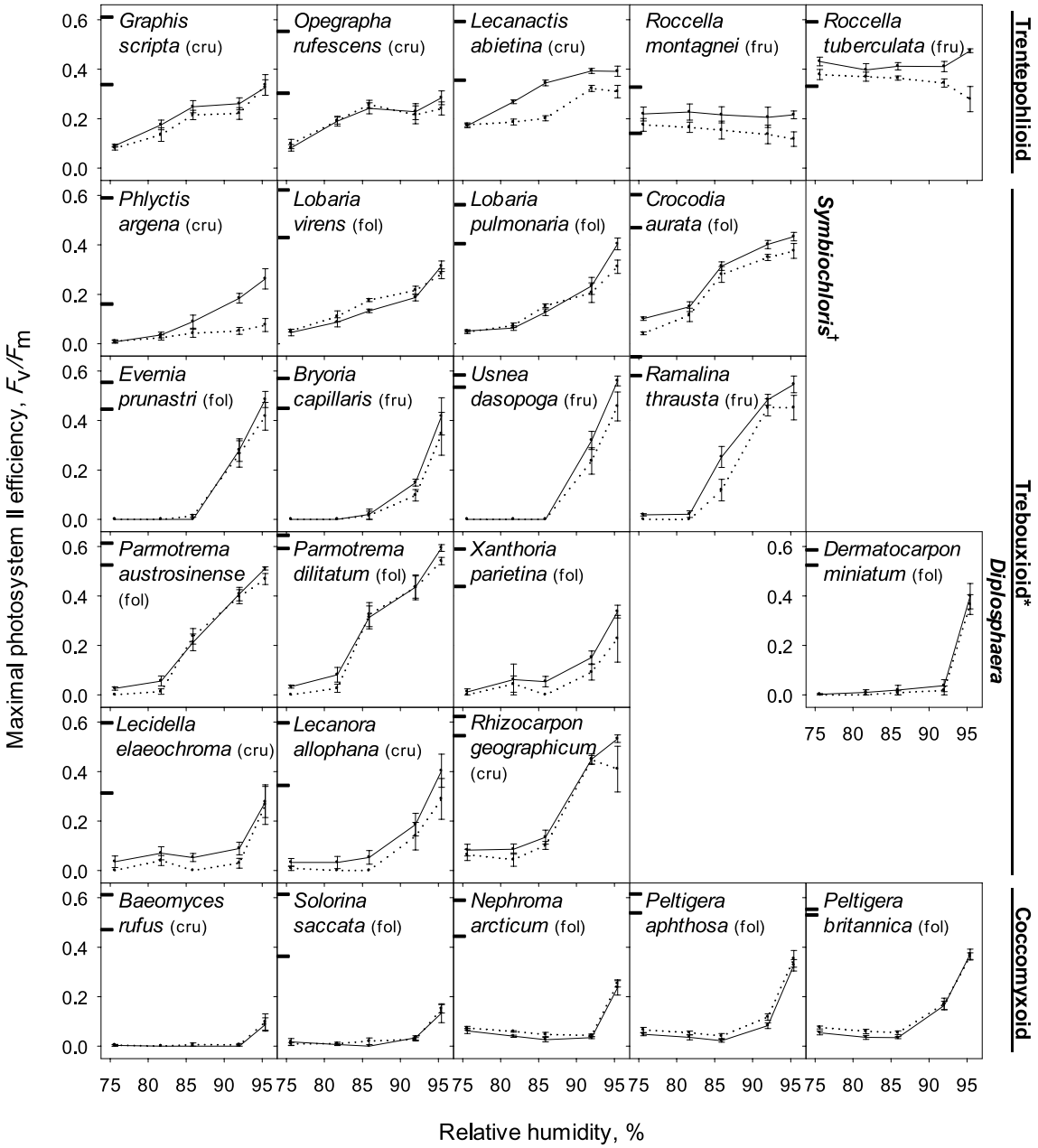

Fig. 2 The effect of relative humidity (RH) on maximal chlorophyll fluorescence (F_v/F_m) in 25 lichen species representing three photobiont groups (top row: Trentepohliaceae; middle four rows: Trebouxiaceae (including Prasiolaceae); Coccomyxaceae: bottom row. RHs were controlled using five saturated salt solutions in sealed boxes at 15 °C (see Table 1). Solid and dotted lines indicate mean F_v/F_m values measured in the sealed boxes after 18 h and 64 h, respectively. Symbols and error bars show the species means and standard errors ($n = 5$) at each RH. Thick dashes on the left hand y axes indicate the mean values after spraying, both prior to (upper dashes) and following (lower dashes) the experiment. Growth form is indicated by cru (crustose), fol (foliose) and fru (fruticose) following the species names. **Dermatocarpon miniatum* has *Diplosphaera* sp. (Prasiolaceae) as the photobiont. †*Phlyctis argena* has *Dictyochloropsis splendida* as the photobiont

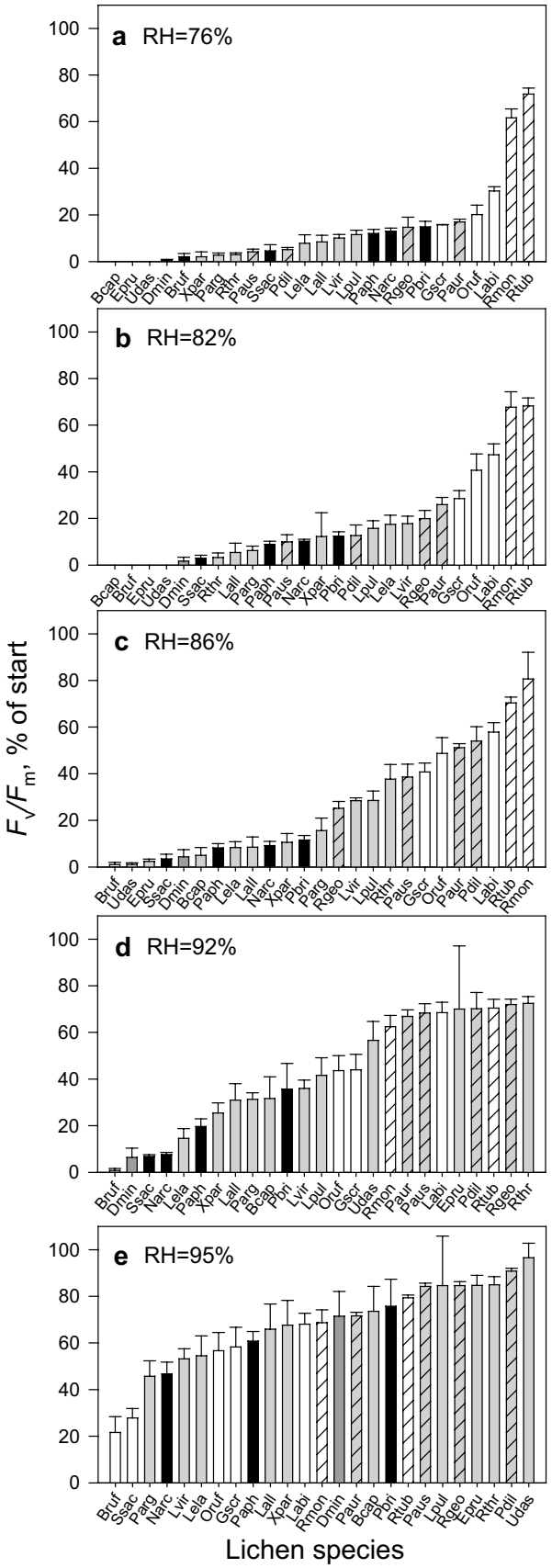

Fig. 3 Species-ranked photosystem II (PSII) activation given as percent of start values (i.e. after spraying with water) of maximal chlorophyll fluorescence (F_v/F_m). Photobiont types are differentiated by shading: coccomyxoid (black bars); trebouxioid (grey bars; *D. miniatum* is dark grey); trentepohlioid (white bars). Hatched bars indicate species collected from seashore sites. Mean values ($n = 24$, species nested in the three lichen photobiont groups) ± SE

in the sealed boxes, whereas the species with coccomyxoid photobionts yielded a slight increase in F_v/F_m at most RHs between 18 and 64 h (Fig. 2). In most species of all groups, F_v/F_m increased with increasing RH, though not in *Roccella montagnei,* and negligibly in *R. tuberculata.* These two fruticose trentepohlioid species activated already at the lowest RH used (Fig. 2). Their unique response at the lowest RH stands out in the RH-wise ranking of the species-specific PSII activation given as percent of the activation achieved after spraying with deionized water (Fig. 3a). The interspecific F_v/F_m-distribution was highly skewed at the highest and lowest RH levels; at the lowest RH, just few species had become activated, whereas most species were high in PSII activation at 95% RH (Fig. 3). As a group, the studied trentepohlioid lichens showed a significantly higher F_v/F_m at the three lowest RHs than did the other photobiont groups (Figs. 1, 3a–c).

The coccomyxoid lichens were the least efficient group in using humid air for PSII activation. They had the weakest response to increased RH, and only surpassed the F_v/F_m level of 0.1 above 92% RH. None of them approached the activity level corresponding to full hydration with sprayed water, even at the highest RH and after the longest exposure duration (Fig. 2). The only crustose coccomyxoid species, *B. rufus*, had the highest humid air-induced activation requirements, yet only exhibited negligible activity at 95% RH (Figs. 2, 3). Among the coccomyxoid lichens, the two *Peltigera* species reached the highest PSII activation (Figs. 2, 3). *Dermatocarpon miniatum*, the only studied lichen species with a photobiont in Prasiolaceae, reactivated PSII only at 95% RH, and in this respect was thus more similar to the coccomyxoid lichens than to the trebouxioid ones.

Among studied trebouxioid lichens, species with *Symbiochloris*—or closely related *Dictyochloropsis*—photobionts (Fig. 2, second panel) had the most similar response to RH as the trentepohlioid lichens. These lichens (*C. aurata*, *L. pulmonaria*, *L. virens* and *P. argena*) exhibited slower and more gradually increasing F_v/F_m at higher RHs than the remaining trebouxioid lichens. Compared to the other trebouxioid lichens, the species with *Symbiochloris* photobionts had slightly but significantly higher F_v/F_m at the two lowest RH, and lower F_v/F_m at 95% (group data not shown, but see the species plots in Fig. 2).

Of the trebouxioid lichens, the thin hair lichen, *R. thrausta*, had the most rapid increase in F_v/F_m already between 82 and 86% RH. The other two hair lichens, *B.*

capillaris and *U. dasopoga* showed a similarly strong increase, but only beyond 86% RH. In general, hair lichens and *E. prunastri* (Fig. 2; third panel) were highly inefficient in using low RH-levels (76–82%) for PSII activation (Fig. 3a, b). By contrast, hair lichens were among those that came closest to full activation levels at high humidity (95% RH: Fig. 3e), followed by the majority of the remaining trebouxioid species.

Species from seashore sites (Table 1; mangrove forests and sea cliffs) that had undoubtedly experienced an addition of marine aerosols in their respective habitats reached a higher percent of PSII activation than the other lichens of the respective groups (Fig. 3; hatched columns). In a comparison of crustose versus more complex three-dimensional growth forms, seven out of the twelve species with the lowest PSII activation at 96% RH were crustose lichens, whereas just one crustose lichen (*R. geographicum*) was among the twelve lichens with the highest activation (Fig. 3e) and the only crust collected in a maritime habitat.

Discussion

Large-scale lichen photobiont distribution has previously been linked to hydrological patterns (Ellis and Coppins 2006; Marini et al. 2011; Peksa and Škaloud 2011), providing some insight into lichen hydration preferences (i.e. ombrophoby/ombrophily). However, the underlying physiological mechanisms that shape photobiont group-specific distributions have been unclear. This study shows that lichen-forming fungi with *Trentepohlia* photobionts can activate PSII by water vapor at significantly lower RH than lichens with the two other photobiont groups, thus providing a functional relationship.

Green-algal photobionts (as well as their free-living counterparts; see Holzinger and Karsten 2013) synthesize group-specific organic molecules such as polyols (Roser et al. 1992; Delmail et al. 2013). For example, trebouxioid and coccomyxoid photobionts produce and supply ribitol to their fungal partner, whereas trentepohlioid photobionts provide erythritol (Richardson et al. 1968). Polyols have osmolytic function, perhaps most importantly in response to osmotic stress (Gustavs et al. 2010). It is suggested that compatible solutes such as polyols may reduce the water potential during desiccation without inhibiting metabolism (Sadowsky et al. 2016). Such a mechanism may explain PSII activity at low water potentials. Additionally, polyols improve drought tolerance of lichens. During desiccation, lichenized algae appear better equipped to dissipate excess light energy in the form of heat, through non-photochemical quenching (NPQ), than their isolated algal counterparts (Kosugi et al. 2009). The mycobiont-produced arabitol functions to reduce sensitivity to photoinhibition via NPQ (Kosugi et al. 2013), revealing that the mycobiont-photobiont symbiosis is integral in minimizing drought stress.

The proximity to the sea, and thus exposure to another ecologically relevant group of osmolytes, the marine aerosols, possibly boosted PSII activation at low relative humidity in both trentepohlioid and trebouxioid photobiont groups (Fig. 3). For the coccomyxoid lichens, the forest site of collected *P. britannica* experienced 5–6 times more natural depositions of Na than the other coccomyxoid species due to its proximity to the open North Sea (Aas et al. 2017). This species performed better than the other species in its group. Our experimental design did not allow for testing the importance of photobiont versus of marine aerosols. However, the proximity to the sea likely affected the ranking of species within photobiont groups (Fig. 3). Salt reduces the osmotic potential and may thus increase the water uptake to allow for basic metabolic processes like PSII activation (Delmail et al. 2013) and photosynthesis (Nash III et al. 1990). Photosynthesis of seashore lichens can be high in saline environments (Smith and Gremmen 2001). The coastal distribution of lichens with *Trentepohlia* is likely tied to their ability to withstand frequent contact with marine aerosols and maintain photosynthesis at low water potentials (see Nash III and Lange 1988; Nash III 1996). Beckett (1995) showed that the coastal trentepohlioid *Roccella montagnei*, when compared with cyano- and trebouxioid lichens, had the lowest water potential, lost turgor pressure at the lowest relative water content (RWC) and had highly elastic cell walls. Similarly, the trentepohlioid *Dendrographa minor* has exhibited detectable CO_2 uptake at water potentials as low as − 38 MPa (Nash III et al. 1990), which is notably lower than those allowing photosynthesis in trebouxioid lichens (Brock 1975) and equivalent to a RH slightly lower than the lowest value used in our experiment (Table 2). *Ramalina capitata* has achieved net CO_2 fixation down to a water potential of − 26.9 MPa (≈ 82% RH) (Pintado and Sancho 2002). RH between 80 and 85% can result in positive gross photosynthesis in comparable trebouxioid lichens (Bertsch 1966; Lange et al. 1990), consistent with the PSII measurements in this study. The salt tolerance of trentepohlioid lichens is certainly beneficial in coastal climates, but high amounts of osmolytes would also be highly favorable in dry, inland environments by allowing water vapor reactivation at low air humidity. Phylogenetic analyses of the Trentepohliales indicate a possible marine origin (Lewis and McCourt 2004); an evolutionary conservation of functional traits from a sea-to-land transition, like enhanced osmoregulation, would allow trentepohlioid lichens to colonize inland low rainfall habitats, as long as water vapor is available. Cultures of isolated lichen photobionts of *Trebouxia* and *Coccomyxa* were also found to grow faster in seawater from the Baltic Sea than in traditional cultivation media (Wieners et al. 2012, 2019).

The coccomyxoid lichens in this study showed the weakest PSII responses to humid air. It is of note that all Coccomyxaceae-associated lichens used here—excluding *B. rufus*—are tripartite (cephalolichens), containing nitrogen-fixing cyanobacteria as a secondary photobiont. Cephalolichens can generally hold more water per thallus surface area than chlorolichens (Gauslaa and Coxson 2011; Gauslaa et al. 2019) and have slower desiccation (Gauslaa et al. 2017). Cephalolichens are intermediate between chloro- and cyanolichens in this respect, and would thus be more specialized to take advantage of both rain and dew as primary water sources. In this perspective, it is apparently a paradox that the only chlorolichen *B. rufus* in the coccomyxoid group became less active than the cephalolichens in the same photobiont group. However, lichen crusts in general exhibited less efficient activation at least at the highest RH (Fig. 3e). The reason for this is unknown, but their intimate connections with their substratum may provide them with non-atmospheric water sources, making them less dependent on humid air.

It is not clear why F_v/F_m after hydration by liquid water declined during the experiment in nearly all species. Perhaps lichens do not tolerate prolonged hydration under low light. The lichen discs first remained fully hydrated for 24 h, underwent a 24 h drying period, then rapidly activated at the various RH treatments. They may therefore have been active for approximately 60 h in the boxes, before recording F_v/F_m at the end. This long treatment possibly resulted in a somewhat lower than normal vitality. Among all lichens, *R. montagnei* had the lowest pre- and post- F_v/F_m, likely due to a reduced viability during specimen transport from S. Africa.

Conclusions

Green algal photobiont type has a clear influence on how a given lichen responds to air humidity. In the present study, lichens with *Trentepohlia* showed higher F_v/F_m in lower RHs than those with Trebouxiophyceae photobionts. The effect of increasing RH on the trebouxioid species varied among species, yet they generally attained the highest F_v/F_m above 95% RH. The coccomyxoid lichens had the weakest overall response to water vapor reactivation, and may be better adapted to exploit liquid water sources.

Author contribution statement NHP, KAS and YG conceived and designed the research. NHP conducted the experiments. NHP and YG analyzed the data. NHP and YG wrote the manuscript. All authors read and approved the manuscript.

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