Interspecific differences in desiccation tolerance of selected Antarctic lichens: Analysis of photosystem II effectivity and quenching mechanisms

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Abstract

Lichens can survive and cope with unsufficient water supply resulting in low intrathalline relative water content. Under such conditions, photosynthesis is negatively affected by different degree of dehydration. In our study, fully hydrated samples of *Xanthoria elegans, Umbilicaria decussata* and *Usnea aurantiaco-atra* were light-acclimated and during following desiccation from a fully hydrated to dry state, steady-state chlorophyll fluorescence (F_S), effective quantum yield of photochemical processes in PSII (Φ_{PSII}), and nonphotochemical quenching (qN) were measured in response to decreasing relative water content (RWC). The three experimental lichen species showed a high desiccation tolerance. The desiccation-induced decrease in Φ_{PSII} was found in *X. elegans*, *U. decussata* and *U. aurantiaco-atra*, at the RWC values below 30%. This is well comparable to the evidence reached in other Arctic/Antarctic lichen species. Interspecific differences in desiccation tolerance of these selected Antarctic lichens, based on the analysis of photosystem II effectivity and quenching mechanisms, were described and discussed.

Introduction

Lichens form a major part of the vegetation and are dominant components of biological soil crusts in polar regions. These symbiotic organisms occur in many ecosystems around the world, also in the most extreme environments, such as Arctic re-

gions and Antarctica, and they are well adapted to desiccation/rehydration cycles. This means that lichens can survive conditions of very low relative water content, and similarly to plants, photosynthesis is the key process that is affected by dehydration. It is well-known that lichen thalli consist of at least two partners: a fungal mycobiont and an algal or cyanobacterial photobiont. Photosynthetic performance of the photobionts exposed to desiccation is species-specific and dependent on thallus structure and morphotype as well. Photosynthesis is not operational in lichen thalli experiencing a high degree of desiccation, but is recovered within a short period after rehydration (Williams et al. 2017).

Principles of lichen response to desiccation/rehydration cycles in lichens has been described by Green et al. (2011). It is well established that under dry atmospheric conditions, lichen thalli desiccate to low water contents and then become dormant, *i.e.* physiologically inactive. Ability to tolerate desiccation as well as activate physiological processes after recovery from dryness seems to be species-specific (Green et al. 2011). In general, the rate of recovery may be related to the rate of the dehydration/rehydration. Species that dry and then hydrate rapidly, as *e.g.* crustose lichens on rock surfaces, recover rapidly. Lichens appear to show less ability to tolerate long periods of desiccation than bryophytes, and even some vascular plants (Green et al. 2011).

To assess the dehydration-induced decline on lichen photosynthetic activity, several methods based on chlorophyll fluorescence have been used. Majority of these methods exploit either (1) fast chlorophyll fluorescence transient approach (*e.g.* Guéra et al. 2016) or (2) slow Kautsky kinetics supplemented with quenching analysis (*e.g.* Marečková et al. 2019). In some cases, other biophysical methods are used to get as supplemental information on lichen performance during desiccation, *e.g.* spectral reflectance indices. Recently, Bednaříková

et al. (2020a) measured photosynthetic parameters related to the fast transient of chlorophyll fluorescence (OJIP) during the desiccation process and related the changes in chlorophyll fluorescence parameters to the relative water content measured gravimetrically. Simultaneously, she measured the change in spectral reflectance parameters (*e.g.*, NDVI, PRI, G, NPCI) during thallus dehydration and related them to RWC as well. Barták et al. (2021) examined the effect of thallus desiccation on primary photosynthetic processes. They investigated the lichen responses to the RWC in their thalli during the transition from a wet (RWC of 100%) to a dry state (RWC of 0%). The slow Kautsky kinetics of chlorophyll fluorescence (ChlF) that was recorded during controlled dehydration (RWC decreased from 100 to 0%) and supplemented with a quenching analysis revealed a polyphasic species-specific response of variable fluorescence and the parameters related to PSII functioning (F_V/F_M, Φ_{PSII}) and the desiccation-induced activation of protective mechanisms (non-photochemical quenching, NPQ).

Proctor et al. (2007) studied the desiccation tolerance in the moss *Polytrichum formosum* (Hedw.) G.L. Sm. and suggested a significant role for the cytoskeleton in desiccation responses. Similar study of their team (Proctor and Smirnoff 2000) found that in desiccation-tolerant bryophytes, recovery of photosynthesis in the sampled air-dried only for a limited period of time (few days) required only limited chloroplast protein synthesis and was substantially independent of protein synthesis in the cytoplasm.

The effect of desiccation and rehydration on the function of PS II has been studied in the desiccation tolerant lichen *Cladonia convoluta* (Lam.) Anders by thermoluminescence in the research of Sass et al. (1996). The authors concluded that thermoluminescence might be used as a sensitive indicator of desiccation-induced PSII inactivation.

Hamerlynck et al. (2002) showed that microenvironmental variation results in different patterns of resource acquisition in this highly desiccation tolerant moss. They suggested that growth in the open imparts greater desiccation tolerance, and the development of a long-lasting slowly reversible photoprotective mechanisms.

Desiccation of thalli brings limitations not only to photophysical but also biochemical processes of photosynthesis. Tuba et al. (1996) reported the negative changes in CO_2 assimilation accompanying limitations in potential photochemical activity (as measured by slow fluorescence). They also reported photosynthetic pigment concentrations changes, and an increase in dark respiration of two desiccation-tolerant lichens *Cladonia convoluta* and *C. furcata* (Huds.) Baumg, and a desiccation-tolerant moss *Tortula ruralis* (Hedw.). The authors stated that small plant size and small cell volume in desiccation-tolerant lichens and mosses, together with rapid recovery of photosynthesis after desiccation, allow the mosses to utilize the small amounts of intermittently available water from brief showers or dew.

Desiccation tolerance and desiccation-induced limitation of primary photosynthetic processes is species-specific (Colesie et al. 2018) and vary in different periods of growing season. Ruchika et al. (2020) studied the seasonal variation of the chlorophyll *a* fluorescence parameters and the activities of antioxidantive enzymes in desiccation-tolerant moss species *Syntrichia ruralis*. They concluded that seasonal variation could be due to fluctuations in conditions of their habitat, duration of light intensity, temperature and precipitation.

Cho et al. (2020) assessed the desiccation-induced photosynthetic performance of the fruticose lichen *Cladonia borealis* S. Stenroos and *Usnea* sp. under natural and laboratory conditions. The authors paid attention to light as an interacting factor. To mimic the desiccation-rehydration responses, the authors measured the changes in the PSII photochemistry in lichens under the average light intensity of dawn light and daylight and the desiccating conditions of its natural microclimate. Compared to sun-adapted *Usnea sp.*, the photosynthetic performance *C. borealis* evaluated by chlorophyll fluorescence of exhibited shade-adapted lichen features.

Until now, photosynthetic response of Antarctic lichens has been investigated by chlorophyll fluorescence parameters (typically by potential end effective quantum yields) in many species addressing the lichen response to a variety of environmental factors (*see* Table 1) including thallus desiccation due to severe water stress.

To our best knowledge, sensitivity/resistance of primary photochemical processes in PSII of *Usnea* genus representatives has never been investigated. Therefore, we focused on *Usnea aurantiaco-atra* (Jacq.) Bory, and the species response to controlled desiccation. We hypothesized that the response would be similar to the frequently-investigated species such as *Xanthoria elegans* (Link) Th. Fr. (Barták et al. 2007) and *Umbilicaria decussata* (Vill.) Zahlbr. (Kosugi et al. 2018) that we included in or study as well.

Physiological processes in *U. aurantiaco-atra*, resistance to desiccation in particular, has been studied rather scarcely. Majority of studies addressed the species response to other stress factors. Balarinová et al. (2014) focused on the sensitivity of *Usnea antarctica* Du Rietz and *U. aurantiaco-atra* to photoinhibition. Hájek et al. (2016) showed interspecific differences in the critical temperature for primary photochemical processes of photosynthesis.

Species	Factor	ChlF_par	Source
Buellia frigida Darb.	Drought and cold stress	F_V/F_M	Sadowsky & Ott (2012)
	Temperature and drought stress	F_V/F_M	Backhaus et al. (2015)
Circinaria gyrosa Sohrabi, Sipman, Volk. John & V.J. Rico	Temperature and drought stress	F_V/F_M	Backhaus et al. (2015)
Cladonia borealis S. Stenroos	Light stress	Φ_{PSII}, F_M	Cho et al. (2020)
Dermatocarpon polyphyllizum (Nyl.) Blomb. & Forssell	Thallus temperature decrease	Φ_{PSII}, OJIP	Marečková et al. (2019)
	Drought stress	Φ_{PSII}, F_S, qN	Mishra et al. (2019)
	Temperature and drought stress	Φ_{PSII}, OJIP	Bednaříková et al. (2020a)
	Low and high temperature	OJIP	Bednaříková et al. (2020b)
Fulgensia bracteata (Hoffm.) Räsänen	Drought and cold stress	F_V/F_M	Sadowsky & Ott (2012)
Himantormia lugubris (Hue) I.M. Lamb	Drought stress	Φ_{PSII}, F_S, qN	Barták et al. (2021)
	Different parts of talli	$F_M´$, Φ_{PSII}	Sancho et al. (2020)
Lecania brialmontii (Vain.) Zahlbr.	Drought stress	Φ_{PSII}, F_S, qN	Barták et al. (2021)
Leptogium puberulum Hue	Drought stress	Φ_{PSII}, F_S, qN	Barták et al. (2018)
Mastodia tessellata (Hook. f. & Harv.) Hook. f. & Harv.	Temperature	$F_M´$, $F_0´$, ETR, q_N	Smith et al. (2001)
Parmelia saxatilis (L.) Ach.	Drought stress	Φ_{PSII}, F_S, qN	Barták et al. (2021)
Physconia muscigena (Ach.) Poelt	Drought stress	Φ_{PSII}, F_S, qN	Barták et al. (2018)
Placopsis antarctica D.J. Galloway, R.I.L. Sm. & Quilhot	Drought stress	Φ_{PSII}, F_S, qN	Barták et al. (2021)
Pleopsidium chlorophanum (Wahlenb.) Zopf	Drought and cold stress	F_V/F_M	Sadowsky & Ott (2012)
Ramalina terebrata Hook. f. & Taylor	Drought stress	Φ_{PSII}, F_S, qN	Barták et al. (2021)
Rhizoplaca melanophthalma (DC.) Leuckert & Poelt	Drought stress	Φ_{PSII}, F_S, qN	Barták et al. (2018)
Umbilicaria antarctica Frey & I.M. Lamb	*In vivo / in situ*	Φ_{PSII}, F_S, $F_M´$	Raggio et al.(2016)
	Temperature	F_V/F_M, Φ_{PSII}, qN	Barták et al. (2007)
	Light stress	Φ_{PSII}, OJIP	Balarinová et al. (2014)
	Drought and cold stress	F_V/F_M	Sadowsky & Ott (2012)
Umbilicaria aprina Nyl.	*In vivo / in situ*	PPFD	Schroeter et al. (2010)
	Light stress	F_V/F_M	Kappen et al. (1998)
Umbilicaria cylindrica (L.) Delise	Cold stress	F_V/F_M, Φ_{PSII}	Hájek et al. (2016)

Umbilicaria decussata (Vill.) Zahlbr.	Drought and cold stress	F_V/F_M	Sadowsky & Ott (2012)
	Light stress	F_V/F_M, Φ_{PSII}, qN	Barták et al. (2003)
	Light stress	F_V/F_M	Kosugi et al. (2018)
Usnea antarctica Du Rietz	Cold stress	F_V/F_M, Φ_{PSII}	Hájek et al. (2016)
	Light stress	F_V/F_M, Φ_{PSII}, qN	Barták et al. (2003)
	Low and high temperature	OJIP	Bednaříková et al. (2020a)
Usnea aurantiaco-atra (Jacq.) Bory	*In vivo / in situ*	PPFD	Schroeter et al. (2010)
	In vivo / in situ	$F_M{'}$, Φ_{PSII}, ETR	Schroeter et al. (2021)
	Light stress	$\Phi_{PSII,}$ OJIP	Balarinová et al. (2014)
	Cold stress	F_V/F_M, Φ_{PSII}	Hájek et al. (2021)
	Cold stress	F_V/F_M, Φ_{PSII}	Hájek et al. (2016)
Usnea lambii (Imshaug) Wirtz & Lumbsch	Drought and cold stress	F_V/F_M	Sadowsky & Ott (2012)
Usnea sphacelata R. Br.	Cold stress	F_V/F_M, Φ_{PSII}	Hájek et al. (2021)
Xanthoparmelia somloensis (Gyeln.) Hale	Ribitol, cold stress	F_V/F_M, Φ_{PSII}, qN	Hájek et al. (2009)
Xanthoria elegans (Link) Th. Fr.	Drought stress	Φ_{PSII}, Fs, qN	Barták et al. (2018)
	Temperature	F_V/F_M, Φ_{PSII}, qN	Barták et al. (2007)
	Low and high temperature	OJIP	Bednaříková et al. (2020b)
Xanthoria mawsonii C.W. Dodge	*In vivo / in situ*	Φ_{PSII}	Pannewitz et al. (2006)

Table 1. Overview of the studies focused on the estimation of photosynthetic processes as affected by environmental factors in lichens by the chlorophyll fluorescence parameters.
Note: ChlF_par - Chlorophyll fluorescence parameter.

Hájek et al. (2016) exposed lichen thalli of *Usnea antarctica*, *Usnea aurantiaco-atra*, and *Umbilicaria cylindrica* (L.) Delise to linear cooling from +20 to −50°C at a constant rate of 2°C min^{-1}, and measured two chlorophyll fluorescence parameters by a modulated fluorimeter.

Recently, Beltrán-Sanz et al. (2022) investigated the potential effects of climate change on the metabolic activity and net primary photosynthesis. Long-term monitoring of chlorophyll fluorescence parameters in the field was combined with photosynthetic performance measurements in laboratory experiments in order to establish the daily response patterns under biotic and abiotic factors at micro- and macro-scales.

In our study, we focused on the response of chlorophyll fluorescence parameters of the species to desiccation in order to evaluate its resistance and compare to other lichen species investigated so far by the same or similar method. We hypothesized, that critical water content at which primary photosynthetic processes are fully inhibited would be comparable to the lichens growing in semidry to dry habitats. However, we expected some differences in critical point (critical relative water contents in a thallus) related to complexity of branching pattern of *U. aurantiaco-atra* thalli.

Material and Methods

Sampling and handling the lichen material

Thali of *Xanthoria elegans* and *Umbilicaria decussata* were collected at the James Ross Island (Antarctica) during the Czech Antarctic expedition in the 2021/2022 austral summer season. Collection sites were located on the northern deglaciated part of the Ulu Peninsula in the vegetation oases formed below the Berry Hill mesa. The lichens were sampled from the altitude ranging from 50 to 150 m a.s.l. and the following sites (*X. elegans*: 63° 48' 10" S, 57° 49' 20" W; *U. decussata*: 63° 48' 28" S, 57° 50' 41" W). Thalli of *U. aurantiaco-atra* were collected from the Nelson Island (South Shetlands Islands), 62°14' 41.72" S, 59° 00' 00.98" W.

Method description

In this study, we used laboratory measurements based on the slow Kautsky kinetics (KKs) method supplemented with saturation pulses in dark- and light-adapted states. Chlorophyll fluorescence parameters were measured by the below-described method based on the study of Barták et al. (2004, 2018).

Fully hydrated samples (4.0 cm^2 segments of thallus) were light-acclimated and during following desiccation from a fully hydrated to dry state, steady-state chlorophyll fluorescence (F_S), effective quantum yield of photochemical processes in PSII (Φ_{PSII}), and nonphotochemical quenching (qN) were measured by a PAM-2000 fluorometer (H. Walz, Germany) repeatedly every 5 min. in response to decreasing relative water contents (RWC). For the formulas, according to which the chlorophyll fluorescence parameters were calculated, see below. Samples were let dry under room conditions (25°C, RH of 70%, 20 µmol m^{-2} s^{-1} PAR). Dehydration time varied between 5 and 8 h according to the

After collection, the thalli of the three experimental lichen species were desiccated under natural outdoor conditions, and then stored in dark at 5°C. After the transfer, the dry lichen samples were stored in a refrigerator under at 2°C. Before laboratory measurements, the thalli were rewetted at 5°C by demineralized water for 24 h. During the rewetting period, the thalli were exposed to dim light of 5 µmol m^{-2} s^{-1} of photosynthetic active radiation. Then the best five samples per species were selected for the experiments using the highest value of F_V/F_M as an indicator of sample vigor.

sample characteristics (dimension, weight, complexity of the thallus structure, *etc*). During the desiccation time, the sample weight was measured using a Mettler scale (Mettler AE-100, Germany) in 5 min. interval as well. Relative water content (RWC) was calculated from the weight of the thallus recorded during the desiccation after subtraction of dry weight of the sample (oven dried at 70°C for 24 h). Particular chlorophyll fluorescence parameters were calculated as according to the following equations:

$$\Phi_{PSII} = (F_M' - F_S) / F_M' \qquad \textit{Eqn. 1}$$

$$qN = (F_M - F_M') / (F_M - F_0) \qquad \textit{Eqn. 2}$$

where F_M is the maximum chlorophyll fluorescence recorded on a dark-adapted sample (5 min.) after application of a saturation pulse, F_M' is the maximum chlorophyll fluorescence recorded on a light-adapted sample after application of a saturation pulse, F_S is the steady-state fluores-

cence in a light-adapted sample, and F_0 is the minimum chlorophyll fluorescence.

Chlorophyll fluorescence parameters were plotted against RWC for each parameter giving desiccation response curves.

Data processing

Each dependence of the chlorophyll fluorescence parameters in all repeats was plotted against RWC as a discrete dependence of values with an interval of 3-5% of RWC.

For the effective quantum yield of photochemical processes in PSII and variable chlorophyll fluorescence we used double normalization to present these parameters in the interval between 0 and 1.

To calculate the points (point 1 – P1, *see* Fig. 1 for their definition), we approximated the values at different stages of

The curves were analyzed as regards the RWCs at which the important points (P1 and P2) were distinguished on the curves (*see* below Data processing).

desiccation (usually the range of 50-90% and 15-30%) by linear regression and defined as the intersection of these functions. Also, in the case of Φ_{PSII}, we determined critical RWC, *i.e.* the value at which this parameter is zero, as the intersection of the linear dependence and the abscissa (point 2 – P2 - critical RWC).

At addition we defined the slope of the linear function between the initial value of Φ_{PSII} (when RWC 100%) and the value of the parameter at the turning point, and expressed in units of Φ_{PSII} normalized (P3-1).

Results

Effective quantum yield declined with progressive dehydration from fully wet (RWC = 100%) to a dry state (RWC = 0%) in a majority of the studied species as shown in Fig. 1. Maximum Φ_{PSII} values were found at the full hydration state (RWC = 90–100%) for all studied species. During desiccation, Φ_{PSII} remained unchanged in *Umbilicaria decussata* in a wide range of RWC (100–20%) and then declined sharply to 0, at the same time in the case of *Xanthoria elegans*, Φ_{PSII} decreased slightly in the range of 100–30% RWC and then decreased rapidly according to the S-curve. Such behavior is also characteristic of the desiccation of *Usnea aurantiaco-atra*, but with a greater variation of Φ_{PSII} values.

As shown in the Table 2, the RWC for P1 reached 20% for *U. aurantiaco-atra* and *X. elegans*, which is significantly higher than the corresponding value for *U. decussata* ($13.5\pm4.0\%$).

Also, the difference between critical RWCs (point P2) for *U. decussata* and *U. aurantiaco-atra* is statistically significant ($p < 0.05$). (This point means the value of RWC, at which Φ_{PSII} is 0).

As for the dependence of F_S on the RWC, the interspecies differences for all three studied objects are also noteworthy. Similar to the previous parameter, we used the double normalization for presentation and comparison of our data. Relations of F_S to the RWC for *U. decussata* and *X. elegans* show a similar shape, gradually decreasing during desiccation.

In all studied species, F_S exhibited a biphasic course with desiccation. The first phase was typified by a constant-rate gradual decrease in F_S from fully wet (RWC = 100%) to a partially dehydrated state (typically 30–40% RWC) and then the second phase with rapid decrease in F_S.

In the case of *X. elegans* there were two phases distinguished with for F_S desic-

cation-induced decline, *i.e.* before and after the P1 was reached (at about 50% RWC). P1 for the other two species was observed at other values during desiccation, close to 30% RWC for *U. auran-tiaco-atra* and close to 20% RWC for *U. decussata*. The differences in RWCs in which the P1 was achieved were found statistically significant ($p < 0.05$) between the three experimental species.
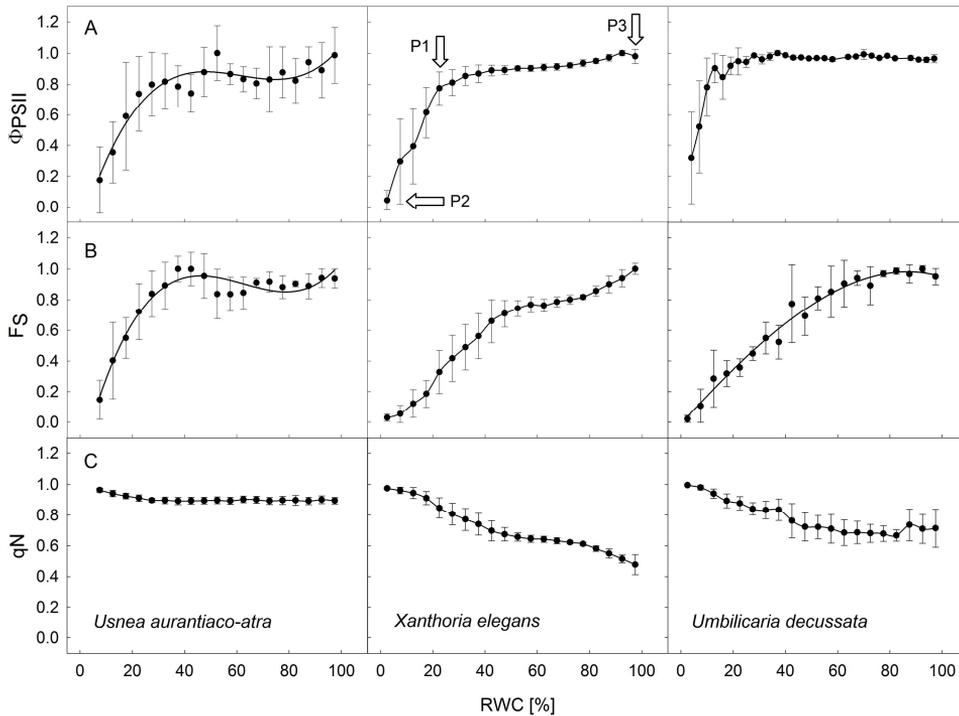
Fig 1. Maximum value-normalized desiccation response curves of effective quantum yield of PSII (Φ_{PSII}), steady-state chlorophyll fluorescence (F_S), and non-photochemical quenching (qN) recorded in experimental lichen species desiccating from a wet (RWC = 100%) to a dry state (RWC = 0%). Data points represent pooled data of three replicates. Standard deviations of the Φ_{PSII} mean calculated for the RWC classes (100–95%, …, 5–0% RWC) of the mean for all the species. A – effective quantum yield of photochemical processes in PSII (normalized), B – steady-state chlorophyll fluorescence (normalized), C – non-photochemical quenching, P1 – bending point, P2 – critical point, P3 – maximum hydration point.

In the case of *U. aurantiaco-atra*, dependence of F_S (normalized) on RWC differed from other species studied in this experiment. During desiccation from 100 to 30% of the relative water content, did not show any sign of decrease, even a slight increase in the RWC range of 30–50%.

Desiccation response curve of non-photochemical quenching (qN) showed a biphasic course in all three species. In final phase of desiccation, qN showed a higher rate of increase than at the early phase of desiccation at the RWC range declining from 100 to 40%. However, species specific value of RWC was distinguished for the change in the rate of qN increase (*X. elegans* 50%, *U. decussata* 30%). In the case of *U. aurantiaco-atra* this parameter increased by about 10%. The RWC for

the P1 point for *X. elegans* differed significantly when compared with other species and reached the value of approximately 45% RWC (p < 0.05).

Fig. 2 shows the dependences of the F_S/F_M' on RWC. In general, the dependences of this parameter are similar for all species. A relative change in this parameter for *U. decussata* is approximately 70% of initial value, for *X. elegans* it is about 50% and for *U. aurantiaco-atra* it is close to 30% (based on final value of this parameter at RWC close to 0%).

	Usnea aurantiaco-atra			*Xanthoria elegans*			*Umbilicaria decussata*		
	P(1) RWC (%)	P(2) RWC (%)	P(3-1) $\Phi_{PSII}(n)$	P(1) RWC (%)	P(2) RWC (%)	P(3-1) $\Phi_{PSII}(n)$	P(1) RWC (%)	P(2) RWC (%)	P(3-1) $\Phi_{PSII}(n)$
$\Phi_{PSII}(n)$	**20.5** ±2.7	**8.4** ±3.6	**0.210** ±0.149	**21.5** ±5.5	**4.4** ±3.2	**0.144** ±0.105	**13.5** ±4.0	**2.8** ±0.6	**-0.012** ±0.029
$F_S(n)$	**30.5** ±2.6	nm	nm	**48.2** ±5.6	nm	nm	**20.8** ±6.6	nm	nm
qN	**26.3** ±4.9	nm	nm	**45.5** ±6.8	nm	nm	**19.8** ±7.6	nm	nm
F_S/F_M'	**20.4** ±2.9	nm	nm	**19.9** ±8.1	nm	nm	**14.1** ±3.7	nm	nm

Table 2. Parameters derived from the desiccation response curves. Data points represent pooled data of three replicates. Standard deviations of the Φ_{PSII} were calculated for the RWC classes (100–95%, …, 5–0% RWC). *Key to the abbreviation*: nm – not measured.
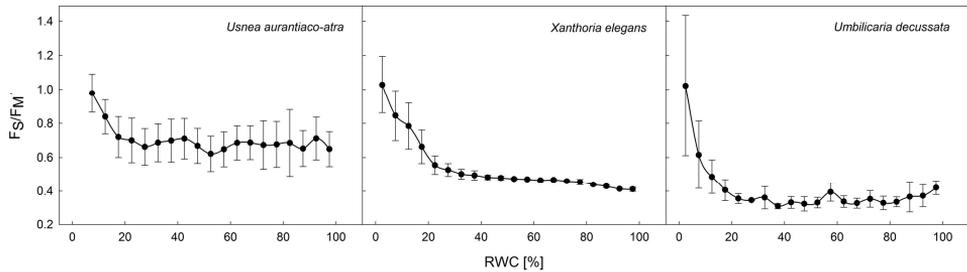
Fig. 2. The ratio of steady-state chlorophyll fluorescence (F_S), to maximum chlorophyll fluorescence reached after the saturation pulse applied in light-adapted state (F_M') as dependent on relative water content (RWC).

Discussion

The three experimental lichen species showed a high desiccation tolerance since they exhibited a decline Φ_{PSII} at low intrathalline water contents, *i.e.* at the RWCs below 20% to 30%, as has been reported previously for several Antarctic species (Barták et al. 2021). The decline in Φ_{PSII} in desiccating lichens within the RWC decrease from 20 to 0%, might be attributed to an increasing limitation of photosynthetic processes in PSII in lichen thalli in the final stage of desiccation. Φ_{PSII} refers to PSII performance and linear electron transport from PSII to PSI in chloroplastic

thylakoid membrane. It was shown in previous studies that potential photochemical reactions of photosynthesis in PSII, *i.e.* F_V/F_M declines in a similar manner as Φ_{PSII} in desiccating lichens (*e.g.* Nayaka and Saxena 2014) having a critical RWC at which PSII photosynthetic processes are fully inhibited (point P2 at which F_V/F_M reaching 0) at similar RWC range, *i.e.* 5 – 10%. Such low critical RWC relates to the water potential range from -20 to -30 MPa in majority of lichens (Jupa 2012).

Similarly, Φ_{PSII} decrease found in our study within the RWC values below 30% for *X. elegans*, *U. decussata* and *U. aurantiaco-atra*, is well comparable to the evidence reached in other Arctic/Antarctic lichen species (*see e.g.* Barták et al. 2021). Critical RWC (*see* P2 in Table 1) reached the values below 10%, which supports the idea of high desiccation resistance of the three studied species. Desiccation brought not only decrease in Φ_{PSII} but in steady-state chlorophyll fluorescence (F_S) as well. Desiccation-induced decrease in F_S followed the relation of Φ_{PSII} as dependent on RWC. When F_S is plotted against Φ_{PSII} (not shown here) it gives linear relation in *X. elegans*, *U. aurantiaco-atra* but not in *U. decussata* where the relationship is slightly curvilinear. High correlation coefficients of the above-specified relations ($R^2 = 0.81, 0.81, 0.51$ respectively, $K = 0.62$, 0.87, 0.73) suggest a possibility to use general chlorophyll fluorescence signal (F_S) as an indicator of overall photosynthetic activity in desiccation studies. This approach would allow to measure much more replicates than in the studies exploiting F_V/F_M and/or Φ_{PSII}, however it brings a necessity to measure a sort of calibration measurement of F_S from fully wet to dry state before the experimental measurements. During the desiccation of the lichen thalli, non-photochemical quenching (qN) increased, which indicated an activation of protective mechanisms against oxidative stress in chloroplastic apparatus of symbiotic algae (photobionts). Oxidative stress in desiccating lichens is generated due to excess reactive oxygen species (ROS) formed in PSII during desiccation combined with excess light. Under such conditions, photosynthetic performance of PSII is strongly limited (*see* decline accompanied with qN increase in Fig. 1) and other protective mechanism are activated: (a) increased contents of antioxidants (Kranner et al. 2003), (b) radiationless dissipation of absorbed light energy in inactive RCs PSII (thermal emission from quenching centers according to Heber (2008), and Wieners et al. (2012), (c) dehydration-induced deactivation of PSII (*see* Kosugi et al. 2013), and (d) efficient energy transfer from PSII to PSI (state transition) – Slavov et al. (2013).

An accelerated rate of qN increase was seen at the RWC range 40-30%, *i.e.* at higher RWC vales than the first indication of Φ_{PSII} decrease appeared 10-25%. Our measurements, however, did not allow to determine which of the three components (qE – energy dependent quenching, qT – state transition related quenching or qI – photoinhibitory quenching) played a major role in the increase. To distinguish the three components in desiccating lichens, the method of slow Kautsky kinetics of chlorophyll fluorescence supplemented with quenching analysis should be applied in the follow-up studies similarly to those ones done in mosses (Beckett et al. 2005).

References

BACKHAUS, T., DE LA TORRE, R., LYHME, K., DE VERA, J. and MEEßEN, J. (2015): Desiccation and low temperature attenuate the effect of UVC$_{254\,nm}$ in the photobiont of the astrobiologically relevant lichens *Circinaria gyrosa* and *Buellia frigida*. *International Journal of Astrobiology*, 14(3): 479-488. doi: 10.1017/S1473550414000470

BALARINOVÁ, K., BARTÁK, M., HAZDROVÁ, J., HÁJEK, J. and JÍLKOVÁ, J. (2014): Changes in photosynthesis, pigment composition and glutathione contents in two Antarctic lichens during a light stress and recovery. *Photosynthetica*, 52(4): 538-547.

BARTÁK, M., HÁJEK, J., OREKHOVA, A., VILLAGRA, J., MARÍN, C., PALFNER, G. and CASANOVA-KATNY, A. (2021): Inhibition of primary photosynthesis in desiccating antarctic lichens differing in their photobionts, thallus morphology, and spectral properties. *Microorganisms*, 9(4): 818. doi: 10.3390/microorganisms9040818

BARTÁK, M., HÁJEK, J., VRÁBLÍKOVÁ, H. and DUBOVÁ, J. (2004): High-light stress and photoprotection in *Umbilicaria antarctica* monitored by chlorophyll fluorescence imaging and changes in zeaxanthin and glutathione. *Plant Biology*, 6: 333-341.

BARTÁK, M., VÁCZI, P., HÁJEK, J. and SMYKLA, J. (2007): Low-temperature limitation of primary photosynthetic processes in Antarctic lichens *Umbilicaria antarctica* and *Xanthoria elegans*. *Polar Biology*, 31(1): 47-51.

BARTÁK, M., VRÁBLÍKOVÁ, H. and HÁJEK, J. (2003): Sensitivity of photosystem 2 of Antarctic lichens to high irradiance stress: Fluorometric study of fruticose (*Usnea antarctica*) and foliose (*Umbilicaria decussata*) species. *Photosynthetica*, 41(4): 497-504.

BARTÁK, M., HÁJEK, J., MORKUSOVÁ, J., SKÁCELOVÁ, K. and KOŠUTHOVÁ, A. (2018): Dehydration-induced changes in spectral reflectance indices and chlorophyll fluorescence of Antarctic lichens with different thallus color, and intrathalline photobiont. *Acta Physiologiae Plantarum*, 40: 177. doi: 10.1007/s11738-018-2751-3

BECKET, R. P., MARSCHALL, M. and LAUFER, Z. (2005): Hardening enhances photoprotection in the moss *Atrichum androgynum* during rehydration by increasing fast- rather than slow-relaxing quenching. *Journal of Bryology*, 27(1): 7-12. doi: 10.1179/174328205X40536

BEDNAŘÍKOVÁ, M., VÁCZI, P., LAZÁR, D. and BARTÁK, M. (2020a): Photosynthetic performance of Antarctic lichen *Dermatocarpon polyphyllizum* when affected by desiccation and low temperatures. *Photosynthesis Research*, 145(2): 159-177. doi: 10.1007/s11120-020-00773-4

BEDNAŘÍKOVÁ, M., FOLGAR-CAMEÁN, Y., KUČEROVÁ, Z., LAZÁR, D., ŠPUNDOVÁ, M., HÁJEK, J. and BARTÁK, M. (2020b): Analysis of K-and L-band appearance in OJIPs in Antarctic lichens in low and high temperature. *Photosynthetica*, 58(SI): 646-656.

BELTRÁN-SANZ, N., RAGGIO, J., GONZALEZ, S., DAL GRANDE, F., PROST, S., GREEN, A., PINTADO, A. and SANCHO, L. G. (2022): Climate change leads to higher NPP at the end of the century in the Antarctic Tundra: Response patterns through the lens of lichens. *The Science of the Total Environment*, 835: 155495. doi: 10.1016/j.scitotenv.2022.155495

CHO, S. M., LEE, H., HONG, S. G. and LEE, J. (2020): Study of ecophysiological responses of the antarctic fruticose lichen *Cladonia borealis* using the PAM fluorescence system under natural and laboratory conditions. *Plants* (*Basel*), 9(1): 85. doi: 10.3390/plants9010085

COLESIE, C., BÜDEL, B., HURRY, V. and GREEN, T. G. A. (2018): Can Antarctic lichens acclimatize to changes in temperature? *Global Change Biology*, 24(3): 1123-1135. doi: 10.1111/gcb.13984

GREEN, T. G. A., SANCHO, L. G. and PINTADO, A. (2011): Ecophysiology of desiccation/rehydration cycles in mosses and lichens. *In*: U. Luttge, E. Beck, D. Bartels (eds.): Plant Desiccation Tolerance. Springer: Berlin/Heidelberg, Germany, pp. 89–120.

GUÉRA, A., GASULLA, F. and BARRENO, E. (2016): Formation of photosystem II reaction centers that work as energy sinks in lichen symbiotic Trebouxiophyceae microalgae. *Photosynthesis Research*, 128: 15-33. doi: 10.1007/s11120-015-0196-8

HÁJEK, J., BARTÁK, M., HAZDROVÁ, J. and FORBELSKÁ, M. (2016): Sensitivity of photosynthetic processes to freezing temperature in extremophilic lichens evaluated by linear cooling and chlorophyll fluorescence. *Cryobiology*, 73(3): 329-334.

HÁJEK, J., HOJDOVÁ, A., TRNKOVÁ, K., VÁCZI, P., BEDNAŘÍKOVÁ, M. and BARTÁK, M. (2021): Responses of thallus anatomy and chlorophyll fluorescence-based photosynthetic characteristics of two Antarctic species of genus *Usnea* to low temperature. *Photosynthetica*, 59(1): 95-105.

HÁJEK, J., VÁCZI, P., BARTÁK, M., SMEJKAL, L. and LIPAVSKÁ, H. (2009): Cryoproective role of ribitol in *Xanthoparmelia somloensis*. *Biologia Plantarum*, 53(4): 677-684.

HAMERLYNCK, E. P., CSINTALAN, Z., NAGY, Z., TUBA, Z., GOODIN, D. and HENEBRY, G. M. (2002): Ecophysiological consequences of contrasting microenvironments on the desiccation tolerant moss *Tortula ruralis*. *Oecologia*, 131(4): 498-505. doi: 10.1007/s00442-002-0925-5

HEBER, U. (2008): Photoprotection of green plants: A mechanism of ultra-fast thermal energy dissipation in desiccated lichens. *Planta*, 228: 641-650.

JUPA, R. (2012): Interspecific differences in photosynthetic efficiency and spectral reflectance in two *Umbilicaria* species from Svalbard during controlled desiccation. *Czech Polar Reports*, 2: 31-41.

KAPPEN, L., SCHROETER, B., GREEN, T. G. A. and SEPPELT, R. D. (1998): Chlorophyll *a* fluorescence and CO_2 exchange of *Umbilicaria aprina* under extreme light stress in the cold. *Oecologia*, 113(3): 325-331.

KOSUGI, M., MIYAKE, A., KASINO, Y., SHIBATA, Y., SATOH, K. and ITOH, S. (2013): Lichens assist the drought-induced fluorescence quenching of their photobiont green algae through arabitol. *In*: Photosynthesis Research for Food, Fuel and the Future. Advanced Topics in Science and Technology in China. Springer, Berlin, Heidelberg, pp. 514–520. doi: 10.1007/978-3-642-32034-7_109

KOSUGI, M., MARUO, F., INOUE, T., KUROSAWA, N., KAWAMATA, A., KOIKE, H., KAMEI, Y., KUDOH, S. and IMURA, S. (2018): A comparative study of wavelength-dependent photoinactivation in photosystem II of drought-tolerant photosynthetic organisms in Antarctica and the potential risks of photoinhibition in the habitat. *Annals of Botany*, 122(7): 1263-1278. doi: 10.1093/aob/mcy139

KRANNER, I., ZORN, M., TURK, B., WORNIK, S., BECKETT, R.P. and BATIČ, F. (2003): Biochemical traits of lichens differing in relative desiccation tolerance. *New Phytologist*, 160: 167-176. doi: 10.1046/j.1469-8137.2003.00852.x

MAREČKOVÁ, M., BARTÁK, M. and HÁJEK, J. (2019): Temperature effects on photosynthetic performance of Antarctic lichen *Dermatocarpon polyphyllizum*: A chlorophyll fluorescence study. *Polar Biology*, 42(4): 685-701.

MISHRA, K. B., VÍTEK, P. and BARTÁK, M. (2019): A correlative approach, combining chlorophyll a fluorescence, reflectance, and Raman spectroscopy, for monitoring hydration induced changes in Antarctic lichen *Dermatocarpon polyphyllizum*. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 208: 13-23.

NAYAKA, S., SAXENA, P. (2014): Physiological responses and ecological success of lichen *Stereocaulon foliolosum* and moss *Racomitrium subsecundum* growing in same habitat in Himalaya. *Indian Journal of Fundamental and Applied Life Sciences*, 4: 167-179.

PANNEWITZ, S., GREEN, T., SCHLENSOG, M., SEPPELT, R., SANCHO, L. and SCHROETER, B. (2006): Photosynthetic performance of *Xanthoria mawsonii* C. W. Dodge in coastal habitats, Ross Sea region, continental Antarctica. *The Lichenologist*, 38(1): 67-81. doi: 10.1017/S0024282905005384

PROCTOR, M. C. F., LIGRONE, R. and DUCKETT, J. G. (2007): Desiccation tolerance in the moss *Polytrichum formosum*: Physiological and fine-structural changes during desiccation and recovery. *Annals of Botany*, 99(6): 1243. doi: 10.1093/aob/mcm098

PROCTOR, M. C., SMIRNOFF, N. (2000): Rapid recovery of photosystems on rewetting desiccation-tolerant mosses: chlorophyll fluorescence and inhibitor experiments. *Journal of Experimental Botany*, 51(351): 1695-704. doi: 10.1093/jexbot/51.351.1695

RAGGIO, J., GREEN, T. G. A. and SANCHO, L. G. (2016): In situ monitoring of microclimate and metabolic activity in lichens from Antarctic extremes: A comparison between South Shetland Islands and the McMurdo Dry Valleys. *Polar Biology*, 39(1): 113-122.

RUCHIKA, CSINTALAN, Z. and PÉLI, E. R. (2020): Effect of salicylic acid pre-treatment after long-term desiccation in the moss *Syntrichia ruralis* (Hedw.) Web. and Mohr. *Plants* (*Basel*), 9(9): 1097. doi: 10.3390/plants9091097.

SADOWSKY, A., OTT, S. (2012): Photosynthetic symbionts in Antarctic terrestrial ecosystems: The physiological response of lichen photobionts to drought and cold. *Symbiosis*, 58(1): 81-90.

SANCHO, L., DE LOS RÍOS, A., PINTADO, A., COLESIE, C., RAGGIO, J., ASCASO, C. and GREEN, A. (2020): *Himantormia lugubris*, an Antarctic endemic on the edge of the lichen symbiosis. *Symbiosis*, 82(1): 49-58.

SASS, L., CSINTALAN, Z., TUBA, Z. and VASS I. (1996): Thermoluminescence studies on the function of Photosystem II in the desiccation tolerant lichen *Cladonia convoluta*. *Photosynthesis Research*, 48(1-2): 205-212. doi: 10.1007/BF00041010

SCHROETER, B., GREEN, T. A., PANNEWITZ, S., SCHLENSOG, M. and SANCHO, L. G. (2010): Fourteen degrees of latitude and a continent apart: Comparison of lichen activity over two years at continental and maritime Antarctic sites. *Antarctic Science*, 22(6): 681-690.

SCHROETER, B., GREEN, T. G., PINTADO, A., TÜRK, R. and SANCHO, L. G. (2021): Summer activity patterns for a moss and lichen in the maritime Antarctic with respect to altitude. *Polar Biology*, 44(11): 2117-2137.

SLAVOV, C., REUS, M. and HOLZWARTH, A. R. (2013): Two different mechanisms cooperate in the desiccation-induced excited state quenching in *Parmelia* lichen. *The Journal of Physical Chemistry* B, 117(38): 11326-11336. doi: 10.1021/jp402881f

SMITH, V. R., GREMMEN, N. J. M. (2001): Photosynthesis in a sub-Antarctic shore-zone lichen. *New Phytologist*, 149(2): 291-299.

TUBA, Z., CSINTALAN, Z. and PROCTOR, M. C. F. (1996): Photosynthetic responses of a moss, *Tortula ruralis*, ssp. ruralis, and the lichens *Cladonia convoluta* and *C. furcata* to water deficit and short periods of desiccation, and their ecophysiological significance: A baseline study at present-day CO_2 concentration. *New Phytologist*, 133(2): 353-361. doi: 10.1111/j.1469-8137.1996.tb01902.x

WIENERS, P. C., MUDIMU, O. and BILGER, W. (2012): Desiccation-induced non-radiative dissipation in isolated green lichen algae. *Photosynthesis Research*, 113: 239-247.

WILLIAMS, L., BORCHHARDT, N., COLESIE, C., BAUM, C., KOMSIC-BUCHMANN, K., RIPPIN, M., BECKER, B., KARSTEN, U. and BÜDEL, B. (2017): Biological soil crusts of Arctic Svalbard and of Livingston Island, Antarctica. *Polar Biology*, 40: 399-411. doi: 10.1007/s00300-016-1967-1