Photoinhibition of photosynthesis in Antarctic lichen *Usnea antarctica*. II. Analysis of non-photochemical quenching mechanisms activated by low to medium light doses

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Abstract

The paper focus sensitivity of an Antarctic lichen *Usnea antarctica* to photoinhibition studied under controlled laboratory conditions. Main emphasis was given to the analysis of quenching mechanisms, *i.e.* deexcitation pathways of absorbed light energy exploited in non-photochemical processes. Thalli of *U. antarctica* were collected at the James Ross Island, Antarctica (57°52´57´´ W, 63°48´02´´ S) and transferred in dry state to the Czech Republic. After rewetting in a laboratory, they were exposed to medium light intensities (300, 600 and 1000 μmol m^{-2} s^{-1} of photosynthetically active radiation) for 6 h. Before and during photoinhibitory treatments, chlorophyll fluorescence parameters, photoinhibitory (qI), state 1-2 transition (qT), and energy-dependent quenching (qE) in particular were measured to evaluate dose- and time-dependent changes in these parameters. The results showed that among the components forming non-photochemical quenching (qN), qI contributes to the largest extent to qN, while qE and qT contribute less. This finding differs from our earlier studies made in a short term-, and high light-treated *U. antarctica* that found qE together with qI is the most important part of non-photochemical quenching. Possible explanation is that photoinhibition in PS II in *U. antarctica*, when induced by low to medium light, activates qE to only limited extend and for a relatively short time (tens of minutes). With prolonged high light treatment lasting several hours, qE tends to be reduced to the values close to zero and qI then forms a major part of qN.

Key words: photoinhibitory quenching, state1-2 transition quenching, energy-dependent quenching

Abbreviations: F_V/F_M - potential photosynthetic quantum yield of photosystem II, Φ_{PSII} - effective photosynthetic quantum yield of photosystem II, NPQ / qN - non-photochemical quenching, qE - energy-dependent quenching, qI - photoinhibitory quenching, qT - state 1-2 transition quenching

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Introduction

Photoinhibition of photosynthesis is defined as light dependent and slowly reversible retardation of photosynthesis, independent of any developmental change. Functional consequences of photoinhibition of photosynthesis are a reduction in the maximum quantum yields for CO_2 uptake and oxygen evolution (Long et al. 1994). In photosynthetic apparatus, chloroplastic pigment-protein complexes in particular, photoinhibition is understood as any change to photosystem II (PS II) and/or molecular components of photosynthetic electron transport chain that, due to excess light absorbed in chlorophyll molecules, reduce effectivity of photosystem II functioning. Some studies exploiting chlorophyll fluorescence approach, however, have used photoinhibition to mean photooxidative damage to PS II.

In lichens and mosses, due to their poikilohydric nature, photoinhibition is not studied as frequently as in higher plants since unstable and, thanks to environmental factors rapidly changing hydration status of lichen thalli affect photosynthetic processes and thus complicate measurements. Therefore, majority of studies of photoinhibition in the lichens and mosses are made under controlled laboratory conditions when hydration status of lichen thalli is kept constant. Such studies have shown that sensitivity of lichens to photoinhibition is species-specific and related to algal/cyanobacterial photobiont (Demmig-Adams et al. 1990a) and capacity of interconversion of xanthophyll cycle pigments, *i.e.* zeaxanthin formation (Demmig-Adams et al. 1990b). Other factors affecting sensitivity of lichens to photoinhibition are prevailing light environment of the habitat (Gauslaa et Solhaug 1996). Recently, physiological background of photoprotective mechanisms in PS II in photoinhibited chlorolichens is studied (Heber et al. 2000). The studies point out similarities of quenching mechanisms activated in desiccating and photoinhibited lichens (Heber 2008), their symbiotic algae in particular (Wieners et al. 2012).

Several field experiments have been made to study photoinhibition in Antarctica using both gas exchange and chlorophyll fluorescence approach in the field (*e.g.* Kappen et al. 1998). Among them, the study made on Antarctic mosses (Lovelock et al. 1995) pointed out reversible photoinhibition in an Antarctic moss measured at wet state. However, field studies made on Antarctic lichens could hardly distinguish between limitation of photosynthetic processes related to thallus dehydration and progressive photoinhibition because the processes co-occur simultaneously. That was why the photoinhibition of Antarctic lichens is studied under constant thallus hydration in laboratory-based facilities. Lichens from open, sunny habitats have generally a high capacity to cope with a short-term high light stress. In laboratory studies, chlorophyll fluorescence technique is used to determine extent of PS II functioning. Slow chlorophyll fluorescence kinetics supplemented with quenching analysis is used more frequently (*e.g.* Barták et al. 2004, Singh et al. 2013) then fast chlorophyll fluorescence transient (OJIP – see *e.g.* Maksimov et al. 2014). In studies focused on photoinhibition that exploit chlorophyll fluorescence kinetics supplemented with quenching analysis, lichens show a rapid recovery (in terms of hours) of functioning of PS II to pre-photoinhibitory status after termination of high light stress as shown for *Usnea antarctica* in our previous study (Barták et al. 2012).

The main aim of this study is to compare the negative effects of short- and long-term exposition of *Usnea antarctica* caused by high light using a chlorophyll fluorescence approach. In previous paper (Barták et al. 2012), we focused on negative effects of a short-term photoinhibitory

treatment on PS II, F_V/F_M, Φ_{PSII} in particular. In the follow-up study, we paid attention to the activation of physiological mechanisms forming non-photochemical quenching of absorbed light energy. We hypothesised that photoinhibitory quenching (qI) would be gradually activated with the time of photoinhibitory treatment. We also expected dependency of qI on light dose, *i.e.* extent of qI, its proportion to qN should increase with photoinhibitory light dose. We also hypothesised that contribution of state 1-2 transition (qT), and energy-dependent quechning (qE) to qN would be much lower than that of qI. For experimental photoinhibitory treatment, we have chosen low to medium light intensities so that critical light under which *U. antarctica* activates mechanisms resulting in qI increase could be identified. In contrast to other studies made on Antarctic lichens (*e.g.* Barták et al. 2003) that focused rather short-term photoinhibitory treatment and light doses about 2000 µmol m^{-2} s^{-1}, we used low to medium light intensities and photoinhibitory treatment as long as 6 h.

Material and Methods

Before experimental HL treatment, thalli of *U. antarctica* (*see* Fig. 1) were rehydrated from dry state by regular spraying (each 12 h) by a demineralized water for 72 h. The thalli were placed into Petri dishes between two small sheets of paper, kept at 5°C and exposed to PAR of 10 µmol m^{-2} s^{-1} and sprayed each 12 h. For experiments, thalli showing highest values of effective quantum yield of photosynthetic processes in PS II (preexperiment, data not shown) were selected.

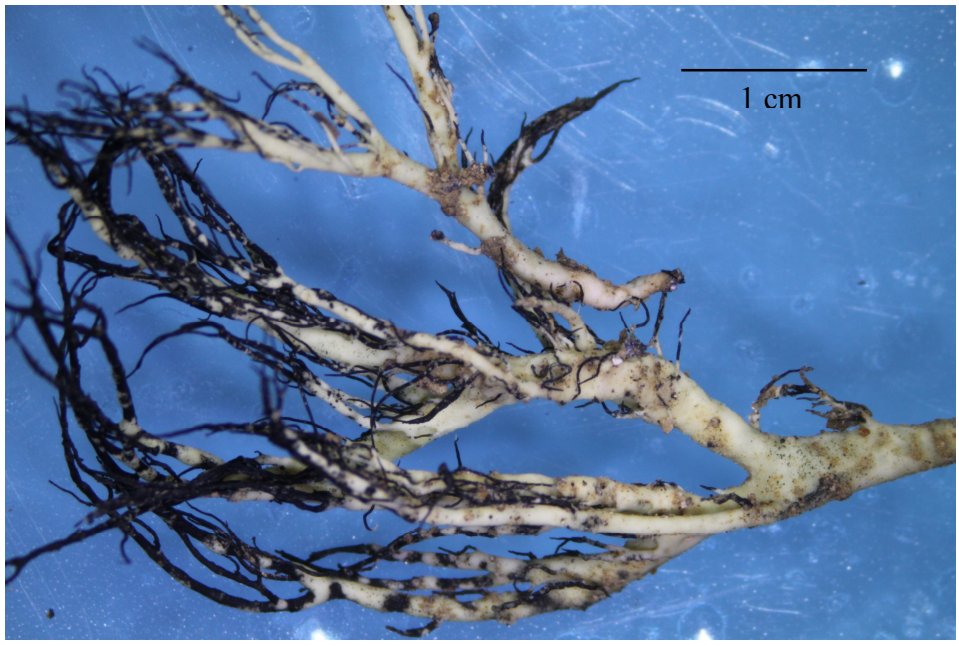

Fig. 1. Detailed photo of *Usnea antarctica* – a lichen with fruticose thallus morphology. Photo by M. Barták.

Long-term photoinhibitory treatment

In the long-term experiment, three different irradiances of 300, 600 and 1000 μmol m^{-2} s^{-1} of photosynthetically active radiation were used. Photoinhibitory treatment was provided by a cold LED light source (Technical University, Brno, Czech Republic). Wet *U. antarctica* thalli were placed into a Petri dish with an outer jacket cooled by ice grains so that thallus temperature was kept constant at 5ºC (measured by a HOBO thermocouple and datalogger, Onset Computers, USA) during photoinhibitory treatment. Similarly to previous experiments (Barták et al. 2003, Barták et al. 2012), lichen thalli were oriented horizontally in the Petri dish, *i.e.* perpendicularly to incident light. Individual thalli were arranged in parallel, in such a way that between-thalli shading was avoided. The thalli were exposed to the above-specified light doses for 360 min. Within the period, chlorophyll fluorescence parameters were measured repeatedly (8 times) so that time courses of individual chlorophyll fluorescence parameters (*see* below) characterizing lichen responses to the three experimental light treatment could be evaluated.

Chlorophyll fluorescence parameters

Before Chl fluorescence measurements, individual *U. antarctica* thalli were placed into a predarkening clip and kept in dark for 10 min. to reach full reoxidation of PS II core. For chlorophyll fluorescence measurements, a PAM-2000 (Heinz Walz, Germany), was used. To derive chlorophyll fluorescence parameters, non-photochemical quenching and its components in particular, a method of slow Kautsky kinetics supplemented with saturation pulses was used (*see* Fig. 2) - for details *see* Roháček et al. (2008). To evaluate components of non-photochemical quenching, repetitive pulses of saturation light were applied in 30 s interval, after actinic light was switched off. For F_M'', the last saturation pulse applied after 300 s in dark was used. These measurements were taken repeatedly. To assess the effect of dose and duration of photoinhibitory light treatment on non-photochemical quenching of absorbed light energy in PS II, and its components qE, qI, and qT (for definition, *see* Krause et Weis 1991) were evaluated. For qE, qI, and qT calculation, Eqns. 3-5 (Roháček 2002, 2010) were used.

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

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

$$q_E = 2 * (F_M'' - F_0'') - (F_M' - F_0')/(F_M - F_0) \qquad \textit{Eqn.}\ 3$$

$$q_I = (F_M - F_0) - (F_M'' - F_0'')/(F_M - F_0) \qquad \textit{Eqn.}\ 4$$

$$q_T = q_N - q_E - q_I \qquad \textit{Eqn.}\ 5$$

where F_0 / F_0' is minimum (background) chlorophyll fluorescence induced by a weak light in dark-/light-adapted sample, F_M is maximum chlorophyll fluorescence reached during saturation pulse applied on dark-adapted sample, F_M' is chlorophyll fluorescence level reached during a saturation pulse applied on light-adapted sample (actinic light on), F_M'' is chlorophyll fluorescence level reached during saturation pulse applied on sample after switching off actinic light. For calculations of qN, NPQ and qI during photoinhibitory treatment, initial (prephotoinhibitory) F_M value of was used (Barták et al. 2003). For F_M'' value, the last saturation pulse was applied after the sample was for 300 s in dark was used.

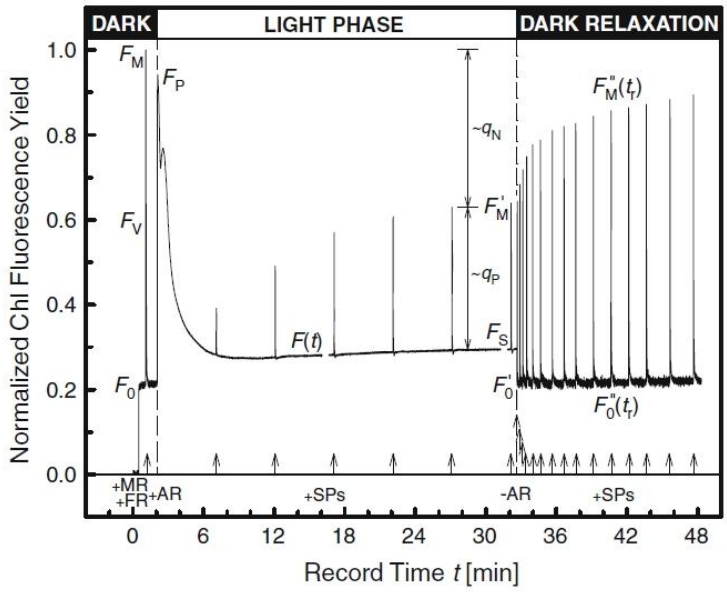

Fig. 2. Slow chlorophyll fluorescence curve with indication pulses and values of chlorophyll fluorescence used in calculations of non-photochemical quenching and its components (qI, qT, and qE). *Source*: Roháček (2010).

Statistical data analysis

Time courses of chlorophyll fluorescence parameters were processed by an analysis of variance (ANOVA, Statistica, StatSoft, Inc., USA). Statistical significance was evaluated by a Post-hoc test (Newman-Keuls) on 95% level of significance.

Results and Discussion

As expected, potential quantum yield of PS II photochemical processes (F_V/F_M) decreased in an exponential manner (*see* Fig. 3) with time of exposition to photoinhibitory light. The highest decrease of F_V/F_M was found in the 1000 μmol m^{-2} s^{-1} treatment throughout the whole exposition time. Irrespective of treatment, final F_V/F_M value was found as low as 0.22-0.35 indicating substantial photoinhibition of PS II after 6 h-lasting light treatment. For *Usnea antarctica*, earlier study of Barták et al. (2003) reported substantial decrease of F_V/F_M found immediatelly after a short-term photoinhibitory treatment, as well as their fast recovery. In the study, fast phase of recovery (lasting typically 30 min.) was attributed to structural changes in PS II and LHCs and the effects of antioxidative mechanisms. Slow phase of recovery (lasting from tens to hundreds of minutes) was attributed to resynthetic processes in a thylakoid membrane that repair damaged components of PS II an LHCs. Long-term photoinhibition exploiting the exposition of wet lichen thalli to high light for the periods longer than 1 h, has been applied in Central European (Barták et al. 2008) but not yet in Antarctic lichens.

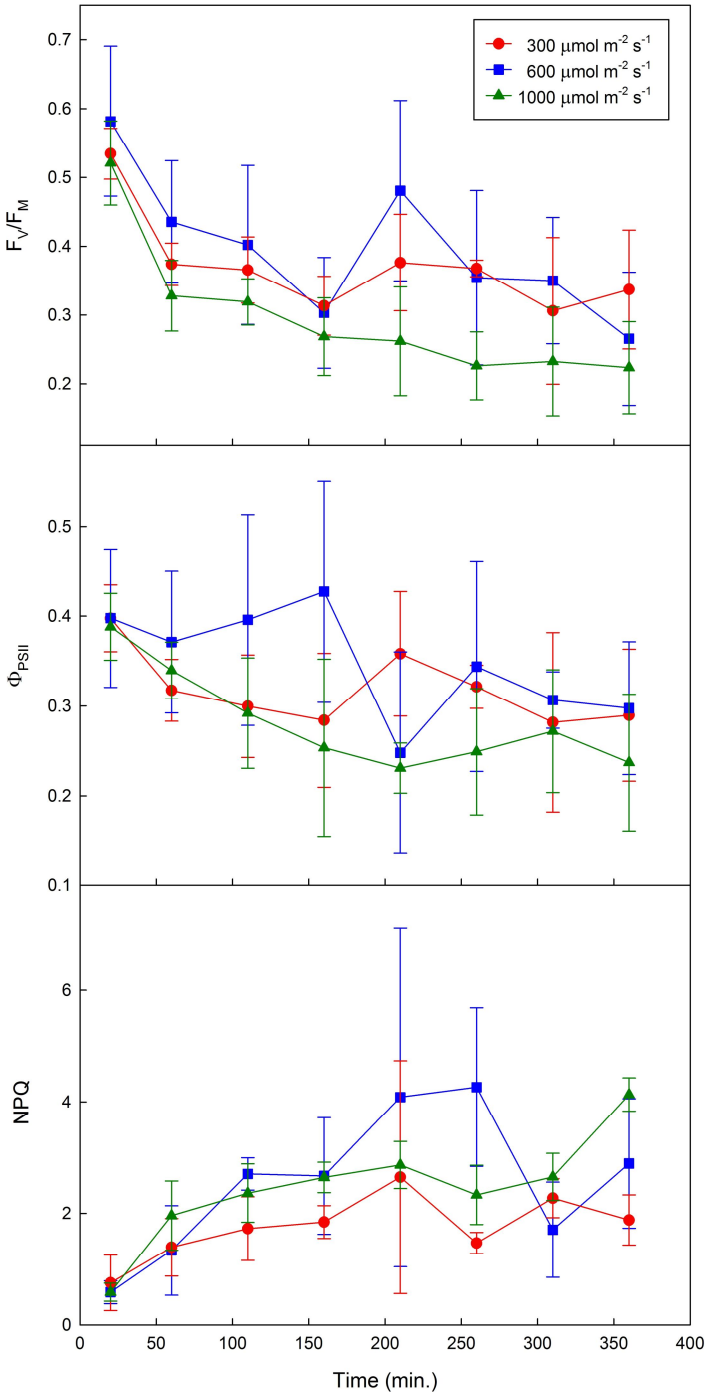

Fig. 3. Time course of F_V/F_M (potential photosynthetic quantum yield of photosystem II), Φ_{PSII} (effective photosynthetic quantum yield of photosystem II) and NPQ (non-photochemical quenching) in *Usnea antarctica* in response to 3 photoinhibitory treatment.

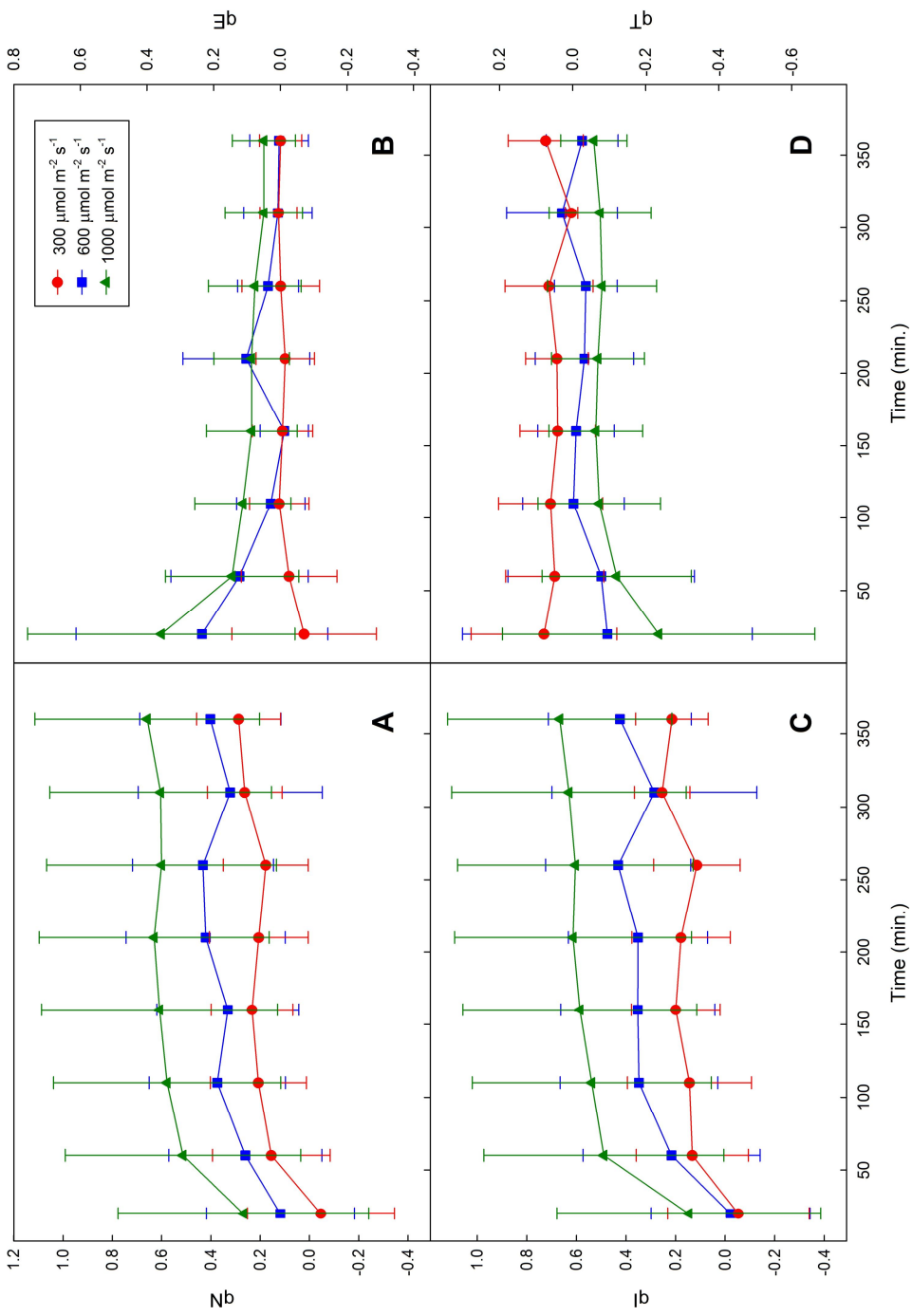

Fig. 4. Time course of qN (non-photochemical quenching), qE (energy-dependent quenching), qI (photoinhibitory quenching) and qT (state 1-2 transition quenching) in *Usnea antarctica* in response to 3 photoinhibitory treatment.

Non-photochemical quenching, both NPQ and qN increased with high light treatment, however the shape of the relationships of NPQ/qN to time of light treatment slightly differed. The rate of initial qN increase was higher than that of NPQ within the first 60 min. of light treatment. In both parameters, more or less equilibrated value was reached after 210 min. exposition to experimental light treatments indicating that such time is required to activate and balance all physiological mechanisms involved into photoprotection of photosynthetic apparatus of lichen symbiotic alga *Trebouxia* sp. Formation of zeaxanthin from violaxanthin is one of them that is considered as an early response of photosynthetic apparatus to high-light stress. It is associated with formation of transthylakoidal pH gradient when PS II are overenergized due to excess light. These changes lead to an increase in energy-dependent quenching (qE). In our study, qE showed generaly increased values only in the first 120 min. of high light treatments (*see* Fig. 4B), then decreased to more or less constant value close to zero, indicating that the light doses used in this study did not cause full and long-term activation of violaxantin to zeaxanthin conversion. Thus, qE-attributed photoprotective mechanisms were not exploited when 300, 600 and 1000 μmol m^{-2} s^{-1} of PAR were used. Such conclusion can be supported by the data of Balarinová et al. (2014) who reported only small change in glutathione content, another photoprotective mechanism, in *U. antarctica* exposed to the same high light treatments. Higher light doses (typically of about 2000 μmol m^{-2} s^{-1} of PAR), however, lead to a dramatic decrease of glutathione content in lichens due to light-dependent glutathione degradation to glutamylcysteine (Barták et al. 2004, Vráblíková et al. 2005).

State one-state two transition quenching (qT) was found more or less constant throughout the period of high light treatments showing the values close to zero (*see* Fig. 4D). This indicated that the light doses used in our study did not cause activation of non-photosynthetic energy transport from PS II to PS I via detached of energized LHCs from PS II. Such mechanism, *i.e.* qT, generally only makes a small contribution to overal non-photochemical quenching and is typical for low light doses specifically (Maxwell et Johnson 2000). In studies devoted to photoinhibition in lichens, qT it is typically evaluated together with photoinhibitory quenching – qI (*i.e.* qT+I, see *e.g.* Barták et al. 2003).

Photoinhibitory quenching (qI) exhibited a rise during high light treatment, most rapid and apparent at 1000 μmol m^{-2} s^{-1} of PAR within the first 60 min. of the treatment (*see* Fig. 4C). At the end of exposition to high light, qI values were found significantly higher (qI = 0.65) in *U. antarctica* treated by 1000 μmol m^{-2} s^{-1} than in other two treatments. These results suggest that the changes in structure and functioning of PS II induced by photoinhibitory treatment were dose- dependent as hypothesized. Irrespective of high light treatment, qI formed dominant part of total qN indicating that the other two mechanisms, *i.e.* qE and qT were much less involved into photoprotection of *U. antarctica* exposed to physiological PAR doses.

Concluding remarks

As shown in this and previous study (Barták et al. 2012), hydrated *U. antarctica* is resistant to both short-term (strong) and long-term (low to medium light) photoinhibitory treatments. Such a resistance might be attributed to effective dissipation of absorbed light energy. The dissipation involves xanthophyll pigments cycle and also zeaxanthin-independent quenching of absorbed light energy by strong sinks lead-

ing to heat dissipation. Another cause for high resistance of *U. antarctica* to photoinhibition is a presence of antioxidative enzymes and substrates in lichen thalli, glutathione in particular (Balarinová et al. 2014, Gasulla et al. 2012). Further research involving fluorometric, biochemical and molecular-biology approaches is required to evaluate contribution and particular importance of (1) energy dissipation and (2) activity of antioxidants to effective photoprotection in *U. antarctica* and/or other Antarctic lichens.

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