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Standard Paper

Adaptions of photosynthesis in sun and shade in populations of some Afromontane lichens

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Abstract

Photosynthetic organisms have evolved a great variety of mechanisms to optimize their use of sunlight. Some of the clearest examples of adaptations can be seen by comparing photosynthesis in different species and in different individuals of the same species that grow under high and low light levels. While the adaptations of sun and shade higher plants have been relatively well studied, much less information is available on the photobionts of lichenized *Ascomycetes*. An important adaptation that can protect photosynthetic organisms from the potentially harmful effects of excess light is non-photochemical quenching (NPQ); NPQ can dissipate unused light energy as heat. Here we used chlorophyll fluorescence to compare the induction and relaxation of NPQ and the induction of electron transport (rETR) in collections of the same lichen species from exposed and from more shaded locations. All species have trebouxioid photobionts and normally grow in more exposed microhabitats but can also be readily collected from more shaded locations. Shade forms display generally higher NPQ, presumably to protect lichens from occasional rapid increases in light that occur during sunflecks. Furthermore, the NPQ of shade forms relaxes quickly when light levels are reduced, presumably to ensure efficient photosynthesis after a sunfleck has passed. The maximal relative electron transport rate is lower in shade than sun collections, probably reflecting a downregulation of photosynthetic capacity to reduce energy costs. We also compared collections of pale and melanized thalli from three species of shade lichens with *Symbiochloris* as their photobiont. Interestingly, NPQ in melanized thalli from slightly more exposed microhabitats induced and relaxed in a way that resembled shade rather than sun forms of the trebouxioid lichens. This might suggest that in some locations melanization induced during a temporary period of high light may be excessive and could potentially reduce photosynthesis later in the growing season. Taken together, the results suggest that lichen photobionts can flexibly adjust the amount and type of NPQ, and their levels of rETR in response to light availability.

Key words: chlorophyll fluorescence, photoprotection, photosynthesis, sunfleck, xanthophyll cycle

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Introduction

Plants are adapted to grow in an extraordinarily wide range of light environments, from the deep shade of rainforest understoreys to high light habitats such as deserts and mountain tops (Greer 2022). This is only possible because plants have evolved various mechanisms to optimize their use of sunlight; furthermore, individual species can display great plasticity in their response to changes in light availability. In higher plants, 'sun species' tend to have more components of the photophosphorylation electron transfer chains, increasing their ability to synthesize ATP and NADPH to fix CO_2. This allows them to possess higher activities of Calvin cycle enzymes. The net result is that sun plants typically display higher light-saturation points and maximum rates of photosynthesis. The 'cost' of these adaptations is that sun leaves tend to have high respiration rates, probably because of increased maintenance costs associated with, for example, the higher activities of photosynthetic cytochromes and enzymes.

In addition to balancing light availability with investment in systems involved in photophosphorylation and carbon fixation, plants must protect themselves when the amount of light absorbed exceeds that which can be used. Excess light energy can result in elevated levels of reactive oxygen species (ROS) which can cause photo-oxidative damage (Roach & Krieger-Liszkay 2019). In the short term, a common tolerance mechanism involves increasing the dissipation of excess energy absorbed as heat using non-photochemical quenching. Quenching can be expressed as qN, or the proportion of energy absorbed dissipated as heat, or in absolute terms as NPQ (Liu *et al.* 2019). qN has two main components, qE and qI (Kalaji *et al.* 2017). The component qE, or fast relaxing energy dependent quenching, represents quenching that is relaxed during the first 200 s of darkness with a relaxation half-time of *c.* 30 s. This parameter is influenced by low lumen pH and the xanthophyll cycle (Gilmore 2004). In absolute terms, this quenching corresponds to NPQ_{fast}. The second component, qI, or slow relaxing quenching, is generally thought to be caused by photoinhibition, and represents the fraction of quenching that

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takes from *c.* 8–10 min or longer to relax. In absolute terms, this quenching corresponds to NPQ_{slow}. However, it is now realized that photoinhibition is only one of the many processes responsible for slow relaxing quenching (Liu *et al.* 2019). In higher plants, as might be intuitively predicted, sun plants typically have three to four-fold larger pools of xanthophyll cycle pigments than shade plants, and as a result higher NPQ (Demmig-Adams 1998; Mathur *et al.* 2018). Furthermore, growing individual species of higher plants at increasing light levels increases pigment pool size and NPQ (Demmig-Adams *et al.* 2020). A possible exception to this trend may be plants that grow in fluctuating light levels. For example, exposing *Arabidopsis* to an identical total dosage of light, supplied under either constant or fluctuating conditions, induces higher NPQ in plants receiving fluctuating light (Alter *et al.* 2012). Results suggest that *Arabidopsis* has only a limited ability to utilize short sunflecks, and constitutively high NPQ may be needed in such plants. However, in environments where light levels change rapidly, excessive NPQ reduces the efficiency of photosynthesis when light returns to lower levels (Murchie & Ruban 2020). In such environments, the rapid relaxation of NPQ may confer a selective advantage (Kromdijk *et al.* 2016).

For lichens, different species, and even different populations within one species, can display sun and shade forms (Piccotto & Tretiach 2010). Typically, as for higher plants discussed above, lichens from exposed sites tend to have higher light saturation points and higher maximum rates of photosynthesis than those growing in more shaded microhabitats. Furthermore, lichen photobionts have developed extensive mechanisms to protect themselves from the effects of high light stress (Beckett *et al.* 2021*b*). Tolerance mechanisms to long-term (over a range of weeks to months) light stress include the synthesis of cortical light screening pigments by the fungal symbiont (Solhaug & Gauslaa 2012). In the shorter term, as for higher plants, photobionts can reduce high light stress by thermally dissipating excess light using NPQ (Demmig Adams *et al.* 1990). However, unlike the typical pattern found in higher plants, sun lichens do not always display higher NPQ than those growing in the shade. For example, Vráblíková *et al.* (2006) showed that NPQ in *Xanthoria parietina* (L.) Th.Fr from an exposed site increases from early spring until the summer solstice. Intuitively this is consistent with a greater need for photoprotection in the season with the highest solar irradiance and is consistent with most of the higher plant literature. However, MacKenzie *et al.* (2002) found greater levels of photoprotection in *Lobaria pulmonaria* (L.) Hoffm. in spring than in late summer. Similarly, Veres *et al.* (2020) found generally higher NPQ in shaded rather than exposed soil crust lichens. There are various explanations for these differences. First, probably only relatively few lichens growing in shade microhabitats experience uniformly low light. For example, lichens growing on the trunks of trees are exposed to rapidly changing light levels because gaps in the canopy vary depending on diurnal variations in the angle of sunlight, tree architecture and movement of the tree branches. Lichens in such habitats experience rapidly changing levels of irradiance; the relatively brief periods that lichens are exposed to high light levels are known as 'sunflecks'. As for higher plants growing under these conditions, photobionts may need at least some constitutive NPQ to protect themselves against photoinhibition. Second, sun lichens may use a variety of mechanisms to protect themselves against high light in addition to NPQ, for example upregulation of ROS scavenging enzymes, or enzymes involved in the PSII repair cycle (Beckett *et al.* 2021*b*). Third, recent evidence suggests that NPQ (and levels of xanthophyll cycle pigments) in free-living algae may be involved in tolerance to stresses other than light (Fernández-Marín *et al.* 2021). Irrespective of the reasons, for lichens no simple correlation seems to exist between NPQ and light availability.

Recently, Beckett *et al.* (2021*a*) compared various photosynthetic parameters in a range of sun and shade lichens using chlorophyll fluorescence, and Fig. 1 illustrates a summary of this survey. Unlike results from higher plants, shade lichens displayed higher NPQ than sun species (Fig. 1A). Consistent with the proposed benefits of rapid relaxation outlined above, a large proportion of the NPQ in shade species relaxed quickly on transition from light to dark (Fig. 1A). As discussed above, these species grow in habitats where they experience rapidly changing light levels such as sunflecks, and rapid relaxation of NPQ will enable them to efficiently utilize the lower light levels available after a sunfleck has passed. However, more consistent with results from higher plants, rETR saturated at lower light intensities in shade than sun species (Fig. 1B & C), and shade species displayed lower maximum rates of rETR. These observations suggest that a general downregulation of photosynthetic capacity is likely to occur at lower light levels. While the survey of Beckett *et al.* (2021*a*) compared different lichen species, our preliminary work suggested that similar differences can occur within the same species. Mkhize *et al.* (2022) compared the induction of rETR, and the induction and relaxation of NPQ in sun and shade populations of *Ramalina*. Similar to the results summarized in Fig. 1, compared with the sun population, the shade population displayed higher but faster relaxing NPQ and lower maximum rates of rETR. Anecdotal evidence suggests that while shade species are only rarely found in sunny microhabitats, sun species can often be much more readily collected from shaded habitats. Apparently, no comprehensive survey has been carried out on the induction and relaxation of NPQ in different collections of the same species of lichen growing in exposed and shaded habitats. The main aim of the work presented here was to compare the induction and relaxation of NPQ and the induction of rETR in a range of Afromontane lichens that generally grow in sunny sites with shade collections of the same species.

In an additional study, we compared the induction and relaxation of NPQ in melanized and pale thalli of three shade lichens. The melanized and pale forms were collected growing close to each other but typically melanized forms were found in more exposed microhabitats. Gauslaa & Goward (2020) suggested that in *Lobaria pulmonaria* melanic pigments may adjust the light received by the photobiont beneath the screening upper cortex to rather uniform levels, for example across a gradient in tree canopy openness. The implication would be that photosynthetic parameters such as NPQ should not differ between pale and melanic thalli. However, Gauslaa & Goward (2020) also point out that melanin formation is rapid under inducing conditions (Solhaug *et al.* 2003), but it is unknown how fast fungal melanins are removed when pigmented thalli experience lower light levels. Our second aim was to test whether long-lasting excess melanins can cause photobionts to adopt the characteristics of those from shade lichens.

Materials and Methods

Sun and shade collections of lichen material

Heterodermia leucomelos (L.) Poelt, *Parmotrema perlatum* (Huds.) M. Choisy, *Ramalina celastri* (Spreng.) A. Massal. and

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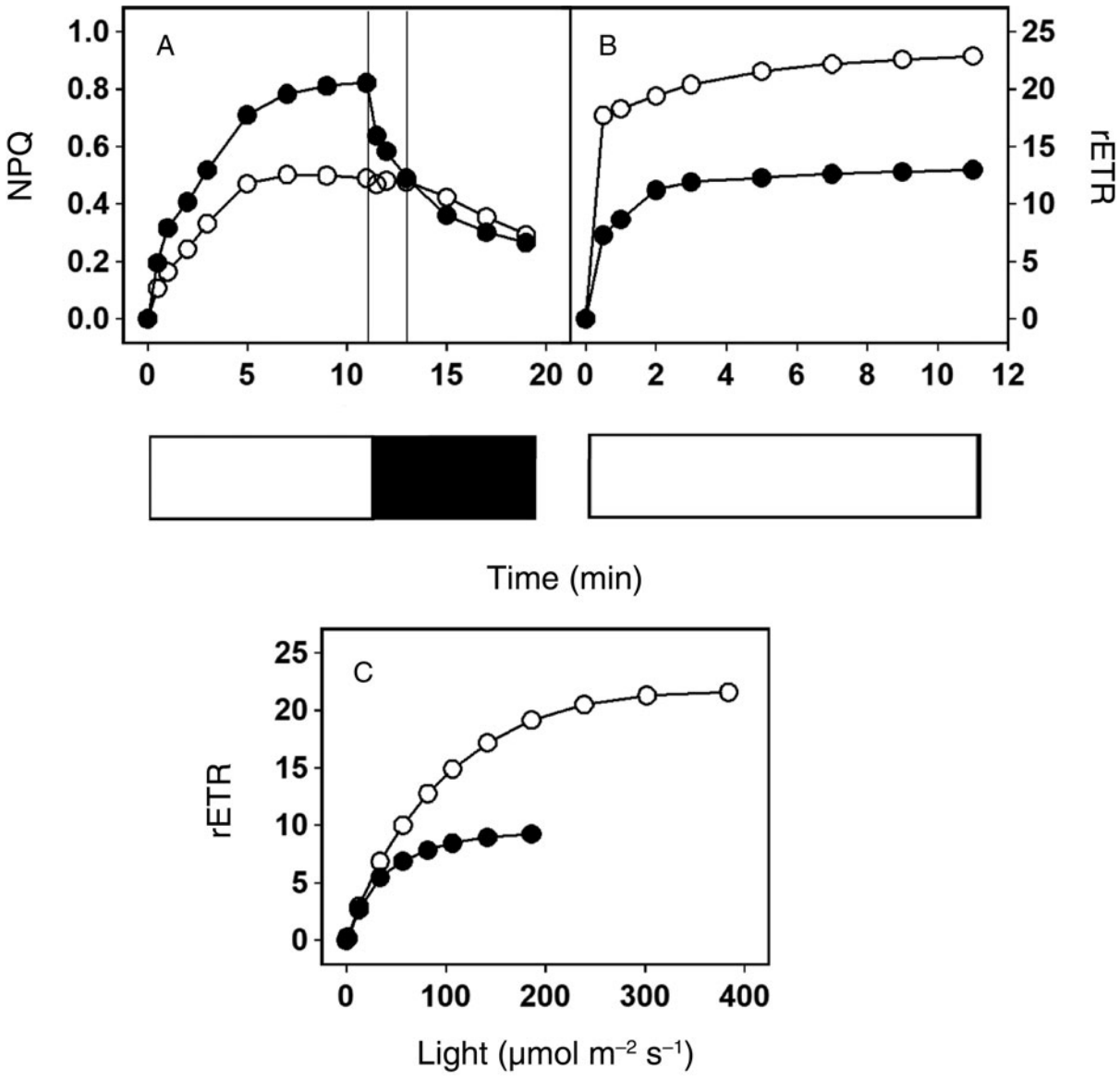

Fig 1. Induction and relaxation of non-photochemical quenching (NPQ) (A), and induction of relative electron transport rate (rETR) (B), for five sun (open symbols) and five shade (closed symbols) species of lichens in response to light at 100 μmol m^{-2} s^{-1}. C, rETR as a function of light intensity in five sun (open symbols) and five shade (closed symbols) species of lichens. Vertical lines on the plots delimit NPQ during the first 2 min of darkness. White and black sections in the boxes at the base of the plots indicate the time periods when samples were exposed to light or darkness respectively. Data taken from Beckett *et al*. (2021*a*).

Usnea undulata Stirt. were collected from Fort Nottingham, KwaZulu Natal, South Africa. The area is a small patch of Afromontane forest and occurs at altitudes between 1500 and 1600 m; the climate is characterized by warm, wet summers and dry, cold (down to freezing temperatures) winters. Lichens were collected from the small tree *Leucosidea sericea* Eckl. & Zeyh. Sun collections were made from minor twigs at the periphery of the canopy (the more normal microhabitat of these species), while shade collections were made a few metres away, from deep inside the canopy on main branches or tree trunks. *Xanthoparmelia conspersa* (Ehrh. ex Ach.) Hale was collected at Queen Elizabeth Park Nature Reserve, KwaZulu Natal, South Africa (altitude *c*. 850 m) on exposed and shaded rocks. *Xanthoria parietina* was collected on the outskirts of Kazan, Russian Federation on exposed and shaded sides of the same silver birch trees (*Betula pendula* Roth). Lichens were cleaned; generally they were collected dry, but if moist they were allowed to dry overnight between sheets of filter paper at laboratory temperature. Lichens were stored dry at 4 °C in a refrigerator for up to 2 weeks. The photobionts of these lichens have been reported to belong to the chlorophycean genus *Trebouxia* (Rambold *et al*. 1998). For uniformity, and to recover from any field stress, before the start of each experiment all material was initially hydrated by spraying with distilled water followed by moist storage for *c*. 24 h in dim light (20 μmol photons m^{-2} s^{-1}) at 12 °C.

Melanized and pale collections of lichen material

Crocodia aurata (Ach.) Link was collected from Fort Nottingham, KwaZulu Natal, South Africa growing on *Leucosidea sericea*. *Lobaria pulmonaria* and *L. virens* (With.) J. R. Laundon were collected from the trunks of oak trees in an old forest in Langangen,

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Norway. All three species tend to grow in shaded habitats and all possess the photobiont *Symbiochloris*. Melanized material was collected from slightly more exposed microhabitats, close to the pale thalli. Material was prepared for experimentation in the same way as the sun and shade collections of the Trebouxoid lichens.

Chlorophyll fluorescence measurements

Chlorophyll fluorescence was measured using a PAM 2500 fluorometer (Walz, Effeltrich, Germany) using the red LED throughout. After a dark adaptation period of at least 10 min, the maximal efficiency of photosystem II (PSII; F_v/F_m) was measured, where F_m = maximum fluorescence and F_v = variable fluorescence or ($F_m - F_o$), with F_o = minimal fluorescence yield of the dark-adapted state. Thalli with anomalous values of F_v/F_m were discarded. Rapid light response curves of relative electron transport rates (rETR) were measured by increasing the actinic light in 11 small steps of 10 to 20 s each, from 0 to 475 µmol photons m^{-2} s^{-1} (at 12, 33, 56, 81, 106, 141, 185, 238, 301, 383 and 475 µmol photons m^{-2} s^{-1}) with saturating flashes at the end of exposure to each light level. The rETR was calculated as:

$$\text{rETR} = 0.5 \times \Phi\text{PSII} \times \text{PAR}$$

where PAR = photosynthetically active radiation and ΦPSII is the effective quantum yield of PSII photochemistry calculated as (F_m' – F_t)/F_m (where F_m' = maximal fluorescence yield of the light-adapted state and F_t = stable fluorescence signal in the light).

The equation derived by Eilers & Peeters (1988) was used to calculate the following parameters:

α: the initial slope of the rapid light curve, related to the effective quantum yield of PSII electron transport under light limited conditions (units: electron photon^{-1}).
$rETR_{MAX}$: the maximal relative ETR reached during light curve recording, reflecting the light saturated capacity of the sample (units: µmol electrons m^{-2} s^{-1}).
lk: the light intensity at which PAR saturation sets in. This is estimated by constructing a linear regression of the initial part of the light response curve and extrapolating it until it hits an ETR value corresponding to the estimate of $rETR_{MAX}$. The light intensity where the two lines intersect is lk (units: µmol photons m^{-2} s^{-1}).

To determine the induction of rETR, and the induction and relaxation of NPQ, thalli were dark-adapted for 10 min and F_v/F_m measured; thalli with anomalous values were discarded. An actinic light of 100 µmol photons m^{-2} s^{-1} was then turned on, and saturating flashes applied at increasing intervals for 11 min. The actinic light was then turned off and relaxation measured for 8 min, with saturating flashes given at increasing intervals. NPQ was calculated using the formula of Bilger *et al.* (1995):

$$\text{NPQ} = (F_m - F_m')/F_m'$$

In addition, NPQ was divided into fast and slow relaxing quenching, corresponding approximately to qE and qI respectively, using equations in Kalaji *et al.* (2017):

$$\text{NPQ}_{fast} = (F_m - F_m')/F_m' - (F_m - F_m'')/F_m''$$

$$\text{NPQ}_{slow} = (F_m - F_m'')/F_m''$$

where F_m'' = maximum fluorescence after 8 min of darkness.

In initial experiments we tested the induction of NPQ using a variety of light intensities, but in a laboratory setting values much above 100 µmol photons m^{-2} s^{-1} tended to cause photoinhibition in some species. However, to test the differences in NPQ and rETR between the sun and shade forms at higher light levels, for *Parmotrema perlatum* and *Ramalina celastri* NPQ and rETR were measured as a function of light intensity (0, 50, 100, 200, 300 and 400 µmol m^{-2} s^{-1}). Material (eight replicates of sun and eight of shade collections) was allowed to equilibrate for 10 min at each light level before readings were taken.

Results

Table 1 summarizes the parameters derived from the rapid light curves, and from the induction and relaxation of NPQ and rETR experiments (Fig. 2). The estimates of the maximum dark-adapted quantum yield of PSII (i.e. F_v/F_m and α) were rather similar in shade and sun collections, although both were usually slightly lower in the sun lichens. Both $rETR_{MAX}$ and the PAR where saturation sets in (lk) were on average much higher in sun compared with shade collections. The only exception was *Heterodermia leucomelos*, where values were slightly higher in shade than sun collections.

Figure 2 compares in detail the induction and relaxation of NPQ and the induction of rETR by light at 100 µmol m^{-2} s^{-1} in shade and sun collections. Differences between the induction of NPQ were rather variable, but NPQ was always higher in sun compared with shade collections, and only in *Xanthoria parietina* was the difference not significant (Table 2). On average, after 11 min NPQ was almost three times as high (Table 1). In general, NPQ relaxed faster in shade than in sun collections, on average after 2 min dropping by 15% in shade and 3% in sun collections. This was confirmed by dividing NPQ into fast and slow relaxing components. In sun collections, NPQ_{slow} was on average *c.* 15% higher than NPQ_{fast}; by contrast, in shade collections NPQ_{slow} was about 25% less than that of NPQ_{fast}. Induction of rETR was rapid, and similar in sun and shade collections. Consistent with the measurements of $rETR_{MAX}$ from the rapid light curves, rETR induced after 11 min was higher in all sun collections except *Heterodermia leucomelos* (Fig. 2B). Generally the higher values of NPQ and lower values of rETR in shade collections observed in the induction/relaxation experiments (Fig. 2) were confirmed when NPQ and rETR were measured over a wider range of light levels for *Parmotrema perlatum* and *Ramalina celastri* (Fig. 3).

In the experiments with melanized and pale collections of shade lichens with *Symbiochloris* as the photobiont, the effective quantum yield of PSII electron transport under light limited conditions (α) was rather similar in melanized and pale collections (Table 3). Both $rETR_{MAX}$ and the PAR where saturation sets in (lk) were on average *c.* 20% higher in melanized forms. In the induction of NPQ experiments (Fig. 4), for *Crocodia aurata* and *Lobaria virens* NPQ was considerably higher in melanized than pale forms, while in *L. pulmonaria* NPQ was initially induced faster in the melanized forms but after 11 min both the pale and melanized forms displayed similar values of NPQ. For all three species, rETR was slightly higher after 11 min in melanized than pale thalli.

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Table 1. Summary of photosynthetic parameters of sun and shade collections of the lichen species. Rapid light curves were used to derive alpha (α), the maximal quantum yield of PSII electron transport under light limited conditions quantum efficiency, the start of light saturation (Ik) and maximal relative electron transport rate ($rETR_{MAX}$). F_v/F_m values (the maximal efficiency of photosystem II) were measured at the start of the rapid light curves. NPQ values (non-photochemical quenching) were obtained by illuminating dark-adapted lichens with light at 100 μmol m^{-2} s^{-1} and measuring the time course of the induction of NPQ for 11 min, and the subsequent relaxation of NPQ for 8 min after switching off the light. Figures are given as ± SE, $n = 10$.

Species	Collection location	F_v/F_m	α	$rETR_{MAX}$	Ik (start of light saturation) (μmol m^{-2} s^{-1})	NPQ after 11 min in the light	NPQ_{fast} (NPQ relaxed after 8 min in the dark)	NPQ_{slow} (NPQ remaining after 8 min in the dark)	% NPQ relaxed after 2 min in the dark
Xanthoparmelia conspersa	Shade	0.71 ± 0.01	0.35 ± 0.01	23.2 ± 1.2	67 ± 3	1.65 ± 0.13	0.85 ± 0.14	0.79 ± 0.09	16
	Sun	0.69 ± 0.01	0.31 ± 0.00	36.6 ± 1.8	120 ± 7	0.84 ± 0.10	0.31 ± 0.09	0.55 ± 0.08	7
Heterodermia leucomelos	Shade	0.67 ± 0.01	0.31 ± 0.00	20.3 ± 1.9	66 ± 6	0.45 ± 0.04	0.17 ± 0.04	0.28 ± 0.05	−3
	Sun	0.64 ± 0.01	0.26 ± 0.01	15.8 ± 1.4	62 ± 6	0.40 ± 0.06	0.19 ± 0.05	0.21 ± 0.04	15
Parmotrema perlatum	Shade	0.67 ± 0.01	0.26 ± 0.02	9.0 ± 0.5	35 ± 2	1.10 ± 0.10	0.72 ± 0.06	0.39 ± 0.06	23
	Sun	0.64 ± 0.01	0.27 ± 0.01	12.9 ± 2.3	49 ± 9	0.83 ± 0.10	0.44 ± 0.08	0.37 ± 0.04	14
Usnea undulata	Shade	0.68 ± 0.01	0.24 ± 0.01	17.2 ± 0.8	72 ± 4	0.63 ± 0.09	0.36 ± 0.06	0.27 ± 0.04	27
	Sun	0.60 + 0.01	0.20 ± 0.01	70.6 ± 11.9	350 ± 54	0.22 ± 1.14	0.16 ± 0.02	0.06 ± 0.02	−12
Xanthoria parietina	Shade	0.70 ± 0.01	0.26 ± 0.01	44.2 ± 2.4	171 ± 11	0.25 ± 0.02	0.09 ± 0.02	0.16 ± 0.01	4
	Sun	0.63 ± 0.01	0.22 ± 0.02	41.2 ± 4.3	173 ± 23	0.22 ± 0.04	0.06 ± 0.01	0.14 ± 0.01	−12
Ramalina celastri	Shade	0.68 ± 0.01	0.24 ± 0.01	12.9 ± 0.8	54 ± 4	1.42 ± 0.15	0.93 ± 0.13	0.49 ± 0.04	25
	Sun	0.66 ± 0.01	0.25 ± 0.01	30.8 ± 2.4	105 ± 10	0.39 ± 0.05	0.14 ± 0.03	0.25 ± 0.03	−5
Mean	**Shade**	**0.69**	**0.28**	**21.1**	**77**	**0.92**	**0.52**	**0.4**	**15**
	Sun	**0.64**	**0.25**	**34.7**	**143**	**0.48**	**0.22**	**0.26**	**3**

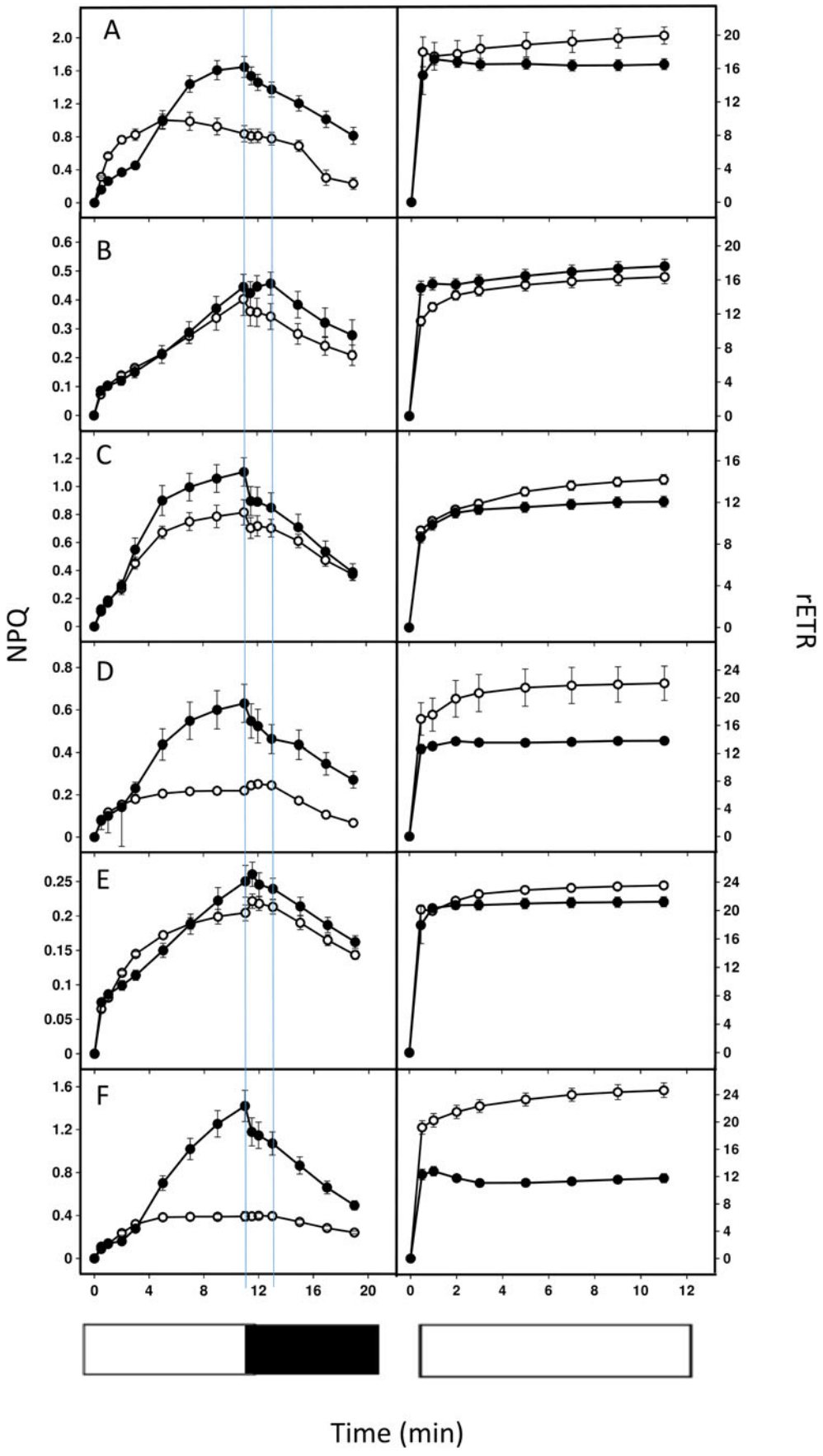

Fig. 2. Induction and relaxation of non-photochemical quenching (NPQ), and induction of relative electron transport rate (rETR) in sun (open symbols) and shade (closed symbols) collections of lichens in response to light at 100 μmol m^{-2} s^{-1}. A, *Xanthoparmelia conspersa*. B, *Heterodermia leucomelos*. C, *Parmotrema perlatum*. D, *Usnea undulata*. E, *Xanthoria parietina*. F, *Ramalina celastri*. Error bars denote the standard error, $n = 10–15$. Vertical lines on the plots delimit NPQ during the first 2 min of darkness. White and black sections in the boxes at the base of the plots indicate the time periods when samples were exposed to light or darkness respectively. In colour online.

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Table 2. Statistical analysis (two-way ANOVA, Microsoft Excel) of the effect on non-photochemical quenching (NPQ) and relative electron transport rates (rETR) of collection location (sun or shade) and time for six lichen species. An actinic light at 100 μmol photons m^{-2} s^{-1} was switched on for 11 min, and then switched off and measurements taken for a further 8 min.

	Xanthoparmelia conspersa		*Heterodermia leucomelos*		*Parmotrema perlatum*		*Usnea undulata*		*Xanthoria parietina*		*Ramalina celastri*		df
	NPQ	rETR	NPQ	rETR	NPQ	rETR	NPQ	rETR	NPQ	rETR	NPQ	rETR	
Sun or Shade	**	**	*	**	**	**	**	**	0.10	**	**	**	1
Time	**	**	**	**	**	**	**	**	**	**	**	**	8
Interaction	**	0.16	0.63	0.21	0.28	0.10	**	**	0.15	0.42	**	**	8

* = $P < 0.01$
** = $P < 0.001$
Error degrees of freedom = 178

Discussion

The main aim of the work presented here was to compare the patterns of NPQ induction and relaxation in collections of lichens from exposed microhabitats with those of the same species from more shaded sites. All species tested here normally grow in more exposed microhabitats, but it is relatively easy to undertake collections of more shaded thalli. Results showed that, generally, shade collections display higher but faster relaxing NPQ, and lower rates of rETR (Fig. 2, Table 1). The differences in NPQ and rETR resemble those we reported earlier for different species of sun and shade lichens (Beckett *et al.* 2021*a*), summarized in Fig. 1. Interestingly, the finding of generally higher values of NPQ in shade than sun forms differs from results usually obtained with higher plants (Demmig-Adams *et al.* 2020). The second part of the study compared collections of pale thalli from three species of shade lichens with melanized thalli of the same species growing nearby but in slightly more exposed habitats. Results showed that patterns of induction and relaxation of NPQ in the melanized forms resemble those from shade lichens (Fig. 4). It seems likely that melanins induced by temporary high light may have long-lasting effects; photobionts under a melanized upper cortex may adopt the characteristics of those from shade lichens. Taken together, results suggest that lichen photobionts can flexibly adjust the amount and type of NPQ and their rETR in response to light availability.

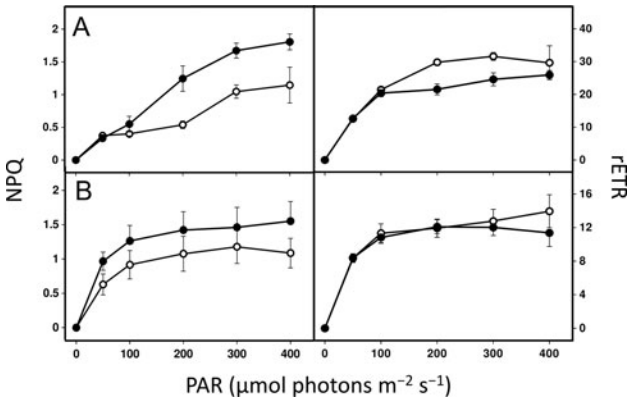

Fig. 3. Non-photochemical quenching (NPQ) and relative electron transfer rate (rETR) as a function of light intensity in sun (open symbols) and shade (closed symbols) collections of *Parmotrema perlatum* (A) and *Ramalina celastri* (B). Error bars denote the standard error, $n = 10–15$.

Rapid light curves

Rapid light curves enable comparison of the parameters of photosynthesis in sun and shade collections of the same species. First, the effective quantum yields of PSII electron transport under light limited conditions of dark-adapted sun and shade lichens are rather similar, whether estimated as α or F_v/F_m (Table 1). In general, both α and F_v/F_m tend to be slightly lower in sun than shade lichens, possibly reflecting some residual stress in the sun populations. These differences might have disappeared if a recovery rehydration period longer than the standard 24 h had been used. Similar results have been found in higher plants (Greer 2022), probably because the efficiency of the light reactions is the same, irrespective of how much light has been received during growth. Second, except for *Heterodermia leucomelos*, the light intensity where saturation of photosynthesis sets in (lk) is lower in the shade collections than in the sun collections (averaging 77 compared with 143 μmol m^{-2} s^{-1}). Third, the average $rETR_{MAX}$, the maximal relative electron transport rate (reflecting the light saturated rate of photosynthesis), is lower in shade than sun collections (21.1 compared with 34.7). As discussed in the 'Introduction', downregulation of photosynthetic capacity in shade plants probably represents an adaptation to save energy on maintaining unnecessarily high levels of cytochromes and enzymes (Greer 2022). Although there are few comparable studies with lichens, Piccotto & Tretiach (2010) obtained similar results from a survey of a range of lichens from contrasting habitats (including some collections of the same species). In the present study, decisions on where to collect sun and shade material were based on careful visual inspection of the study sites and, in general, selections were reflected in the values of lk recorded. Possibly for *Heterodermia*, the differences in light availability of the sun and shade collections were less than visual inspection would suggest, although it is also possible that this species is inherently less plastic. However, in general, lk, or the PAR where saturation starts, appears to be a good quantitative measure of the light regimes of the collection sites of the lichens.

Shade forms display higher NPQ than sun forms

The photosynthetic parameters of the sun and shade collections of lichens differ mainly in their kinetics of induction and dark relaxation of NPQ (Table 1, Fig. 2). In some species, the induction of NPQ appears to be 'biphasic', with the induction kinetics from 0–3 min differing from those from 3–11 min. To understand the reason for these differences at a molecular level would require a

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Table 3. Summary of the photosynthetic parameters of pale and melanized collections of the same lichen species. Rapid light curves were used to derive alpha (α), the maximal quantum yield of PSII electron transport under light limited conditions, the start of light saturation (Ik) and the maximal relative electron transport rate ($rETR_{MAX}$). In separate experiments, dark-adapted lichens were illuminated with actinic light at 100 μmol m^{-2} s^{-1} for 11 min, and the light then switched off and thalli kept in darkness for a further 8 min; non-photochemical quenching (NPQ) was measured at intervals. Figures are given as ± SE, $n = 10$.

Species	Thallus colour	α	$rETR_{MAX}$	Lk (μmol m^{-2} s^{-1})	NPQ after 11 min in the light	% NPQ relaxed after 2 min in the dark
Crocodia aurata	Pale	0.38 ± 0.01	12.9 ± 0.8	49 ± 2	0.85 ± 0.11	53
	Melanized	0.31 ± 0.01	14.7 ± 0.4	54 ± 4	1.51 ± 0.22	57
Lobaria pulmonaria	Pale	0.38 ± 0.02	9.5 ± 1.2	28 ± 5	2.38 ± 0.09	58
	Melanized	0.33 ± 0.10	11.4 ± 0.9	36 ± 4	2.40 ± 0.18	61
Lobaria virens	Pale	0.36 ± 0.02	6.5 ± 0.6	26 ± 8	2.15 ± 0.17	27
	Melanized	0.32 ± 0.10	9.0 ± 0.8	30 ± 4	2.98 ± 0.16	50
Mean	Pale	**0.37**	**9.6**	**34**	**1.79**	**46**
	Melanized	**0.32**	**11.7**	**40**	**2.29**	**56**

more sophisticated approach than the one used here. In all cases, NPQ induced after 11 min is higher in shade than sun collections (Fig. 2), on average two times higher (Table 1). In only one species (*Xanthoria parietina*) is the difference not significant (Table 2). This is interesting, because in *Xanthoria* the lk and $rETR_{MAX}$ data derived from the rapid light curves suggest that the bright orange and pale collections do not represent genuine sun and shade forms, respectively (Table 1). It may be relevant that the material of *Xanthoria* used here was the only collection from the generally less bright, cool temperate regions and had the lowest values of NPQ (Table 1). For the other (Afromontane) species used here, it seems likely that in shaded habitats sunflecks can cause light levels to increase very suddenly, potentially causing oxidative stress. Therefore, effective defence mechanisms must be constitutively in place. Higher NPQ in shade collections did not only occur at 100 μmol m^{-2} s^{-1} (the level used to measure induction kinetics), but also occurred when NPQ was measured over a wider range of light levels (Fig. 3). There are a small number of reports from other workers that lichens collected from more shaded habitats may have generally high NPQ (MacKenzie *et al.* 2002; Veres *et al.* 2020). In contrast to these results from lichens, in higher plants sun forms normally have higher NPQ than shade forms (Demmig-Adams *et al.* 2020). Similarly for lower plants, in both filmy ferns and bryophytes, sun forms and species have been reported to possess higher NPQ than those from more shaded sites (Proctor 2003; Proctor & Smirnoff 2015). Furthermore, growing free-living algae under increasing light intensities increases NPQ, probably due to higher pools of xanthophyll cycle pigments (Blommaert *et al.* 2021). The reasons for the differences between lichens and other photosynthetic organisms remain unclear. However, there are reports that suggest that plants growing in shade do not always display low NPQ. For example, Griffiths & Maxwell (1999) found rather similar NPQ in deep shade and sun species of epiphytic bromeliads. As discussed in the Introduction, the typical microhabitats where shade lichens grow are characterized by sunflecks, and high NPQ provides photoprotection from sudden increases in light levels. A further reason for the generally low NPQ of sun forms could be the presence of lichen substances in the upper cortex. Although none of the lichens tested here, except *Xanthoria parietina*, are pigmented, Ndhlovu *et al.* (2022) showed that even unpigmented lichen substances can increase the tolerance of lichens to photoinhibition, apparently by increasing reflectance. It seems likely that sun collections of lichens contain higher concentrations of substances than those growing in shade (Solhaug & Gauslaa 2012), reducing their need for NPQ.

NPQ in shade forms relaxes faster than in sun forms

In addition to shade forms displaying more NPQ than sun forms, a further difference is that NPQ in shade forms tends to relax faster (Fig. 2, Table 1). On average, 15% of NPQ relaxed during the first two minutes of darkness in shade forms, compared with only 3% in sun forms. NPQ has a fast-relaxing component (NPQ_{fast}), corresponding approximately to qE, and a slow-relaxing component (NPQ_{slow}) corresponding to ql (Kalaji *et al.* 2017). The fast-relaxing component relaxes during the first few minutes of darkness and is related to xanthophyll cycle activity, while the slow-relaxing component is caused by photoinhibition and a variety of other processes and takes longer to relax (Gilmore 2004). In sun forms, on average more than half of the NPQ is attributable to qI (Table 1). While shade forms also display significant qI, on average they possess more than double the fast-relaxing component compared to sun forms (Table 1). Unfortunately, there have been few studies that have measured qI and qE in sun and shade populations of the same lichen species to compare with results presented here. However, NPQ has been suggested to play both positive and negative roles in ensuring optimal plant productivity in environments where light levels are rapidly changing (Murchie & Ruban 2020). On the positive side, NPQ delays the onset of photoinhibition by reducing ROS production. On the negative, while not affecting photosynthesis in high light, NPQ can greatly reduce the quantum yield of photosynthesis at lower light levels. In other words, under low light a lichen 'expressing' high NPQ will require a higher irradiance to achieve the same photosynthetic rate as one without it. The implication for lichens could be that, as discussed above, while shade forms growing in habitats subjected to rapidly changing light levels will benefit from high NPQ, they will also benefit from NPQ that relaxes rapidly following transition to low light. Possession of rapidly relaxing NPQ will enable lichens to efficiently utilize the lower light levels available after a sunfleck has passed.

Interestingly, in *Xanthoparmelia* the induction of NPQ during the transition from darkness to light occurs more rapidly in sun compared to shade forms (and to a lesser extent in *Xanthoria*) (Fig. 2). Intuitively, it could be predicted that when growing in a habitat characterized by sunflecks there may be some advantage

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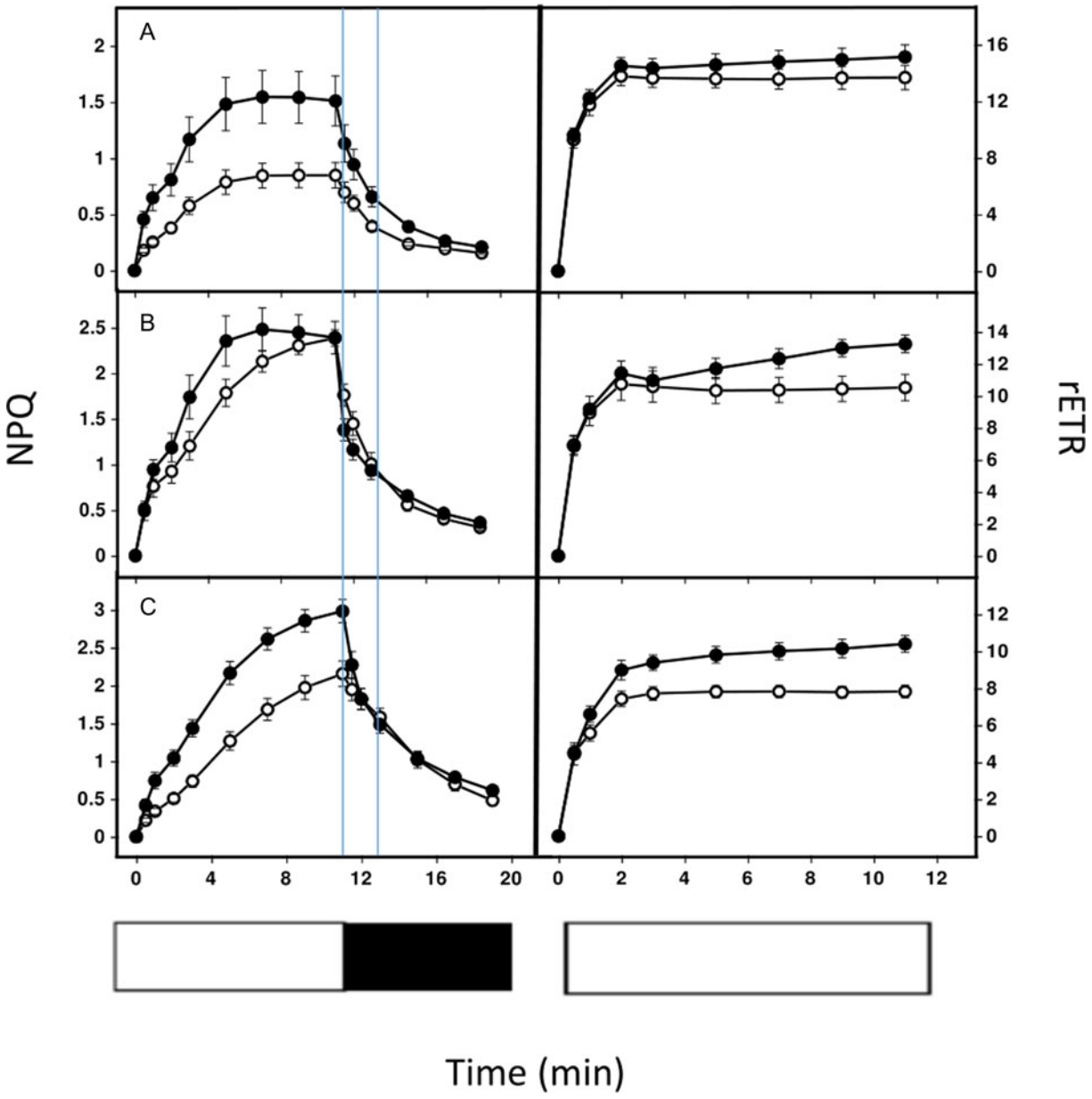

Fig. 4. Induction and relaxation of non-photochemical quenching (NPQ), and induction of relative electron transport rate (rETR) in pale (open symbols) and melanized (closed symbols) collections of lichens in response to light at 100 μmol m^{-2} s^{-1}. A, *Crocodia aurata*. B, *Lobaria pulmonaria*. C, *Lobaria virens*. Error bars denote the standard error, $n = 10–15$. Vertical lines on the plots delimit NPQ during the first 2 min of darkness. White and black sections in the boxes at the base of the plots indicate the time periods when samples were exposed to light or darkness respectively. In colour online.

in rapidly inducing NPQ. We can offer no obvious explanation for the rapid induction of NPQ in sun forms of *Xanthoparmelia*. However, even in this species, shade forms display higher overall NPQ, and fast relaxing NPQ comprises a greater proportion of the total (52% for shade compared to 37% for sun, that is NPQ_{fast} / total NPQ after 11 min illumination) (Table 1).

rETR induces rapidly in both sun and shade forms

In contrast to NPQ, rETR is induced very rapidly in all species (Fig. 2). However, there are some suggestions of a biphasic induction of rETR, and some shade forms appear to activate rETR slightly more quickly than sun forms. Presumably, rapid activation is an advantage for populations of lichens that receive much of their solar radiation as sunflecks. In higher plants, after dark-adapted leaves are illuminated, several minutes are required for PSII and PSI to be synchronized for O_2 evolution, NADP reduction and ATP synthesis. Synchronization is associated with several processes at the molecular level (e.g. the phosphorylation of light harvesting complex II) (Kalaji *et al.* 2017). While the differences appear relatively small, there could be some merit in using more sophisticated approaches than the one used here to compare the rates of induction of rETR in sun and shade forms.

Induction and relaxation of NPQ in pale and melanized thalli

Comparisons of the induction and relaxation of NPQ in melanized and pale thalli of members of the same species show that

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melanized forms generally have more NPQ than pale forms (Fig. 4, Table 3). In all species, NPQ increases faster in melanized forms than pale forms. After 11 minutes, NPQ was considerably higher in melanized than pale *Crocodia aurata* and *Lobaria virens*, while in *L. pulmonaria* NPQ was similar. Thus, in general, the induction and relaxation of NPQ in the photobionts of the melanized thalli resemble more closely those of the shade rather than the sun collections in the first part of this study (Fig. 2). However, in all three species, rETR was slightly higher after 11 min in melanized than in pale thalli, and $rETR_{MAX}$ was *c.* 20% higher (Table 3). Furthermore, lk was *c.* 20% higher in melanized than pale thalli. This suggests, for rETR and lk, the photobionts in melanized thalli resemble more closely those of the sun forms described in the first part of this study. Based on growth measurements, Gauslaa & Goward (2020) suggested that in *Lobaria pulmonaria* melanic pigments may adjust the light received by the photobiont beneath the screening upper cortex to rather uniform levels, for example across a gradient in tree canopy openness. The implication would be that photosynthetic parameters, for example NPQ, should not differ between pale and melanic thalli, which was clearly not observed here. However, Gauslaa & Goward (2020) also point out that melanin formation is rapid under inducing conditions (Solhaug *et al.* 2003), but it is unknown how fast fungal melanins are removed when pigmented thalli experience lower light levels. It might be relevant that the study of Gauslaa & Goward (2020) was carried out in inland British Columbia, corresponding to the 'boreal' climatic zone, while the lichens used here were collected from a nemoral boreal region in Norway or South African sub-tropical Afromontane vegetation. Possibly, in the regions we collected our lichens, the light levels of sunflecks are higher, resulting in melanization that may be excessive for some of the year.

Conclusions

The main study here compared the induction and relaxation of NPQ in collections of the same lichen species from exposed and more shaded locations. The lichen species all have trebouxioid photobionts and normally grow in more exposed microhabitats but can readily be collected from more shaded locations. Although there are some differences between species, results gave a rather consistent picture. Shade forms display generally higher NPQ, presumably to protect lichens from occasional rapid increases in light that occur during sunflecks. However, the NPQ of shade forms relaxes quickly when light levels are reduced (relatively more NPQ is qE), presumably to ensure efficient photosynthesis can occur after a sunfleck has passed. Results are rather at variance with data from other photosynthetic organisms, where more usually the NPQ of sun forms is higher than that of shade forms. Interestingly, the *Symbiochloris* photobionts of melanized shade-adapted lichens behave as if they are adapted to relatively lower light levels than pale forms, suggesting that in some locations melanization induced during a temporary period of high light might reduce photosynthesis later in the growing season. While a recent study suggested that lichen photobionts are rather poor in adapting to temperature shifts (Nelsen *et al.* 2022), results presented here suggest that photosynthetic responses to light may be more plastic.

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