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Subtropical lichens from the Afromontane can display rapid photosynthetic acclimation to simulated climate change

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Abstract

Afromontane forests are an important part of the KwaZulu Natal region of southern Africa, having a distinctive flora with a high proportion of endemic species, and lichens are keystone members. Unlike other continental areas, KwaZulu Natal climate change is predicted to increase rainfall and cloudiness. In the present study, hydrated Afromontane lichens from both exposed and shaded microhabitats were given either constant [100 µmol(photon) m^{-2} s^{-1}] or fluctuating [0, 200, 0 µmol(photon) m^{-2} s^{-1}] light for 8 h a day for 3 d and changes monitored in nonphotochemical quenching (NPQ) and rates of photosynthetic electron transport. In sun but not shade collections, NPQ strongly increased following treatment with constant and fluctuating light. It seems likely that CO_2 fixation may be reduced in moist thalli, and the increase in NPQ may reduce ROS formation during exposure to light while hydrated. Sun lichens can readily modify their NPQ in response to increased cloudiness and rainfall expected in KwaZulu Natal.

Keywords: chlorophyll fluorescence; photobionts; reactive oxygen species; stress.

Introduction

Climate change is already affecting species, including lichens, in all ecosystems (Stanton *et al.* 2023). A direct and obvious effect of climate change is an increase in temperature (Abbass *et al.* 2022), and interestingly, lichen photobionts appear to be rather poorly adapted to temperature shifts (Nelsen *et al.* 2022). A recent modeling study indicated that at current rates of temperature increase, lichens will need to migrate impossibly fast to maintain their current temperature optima. The implication is that in the future, extinctions may become common (Mallen-Cooper *et al.* 2023). Significant geographical biases exist in current studies of lichens and climate change (Stanton *et al.* 2023). Most studies have been carried out on lichens in temperate or boreal environments where most lichen researchers have historically been based. Very few studies have been carried out in Africa (Maphangwa *et al.* 2012).

Global warming is predicted to not only increase mean temperatures but also to cause climatic shifts such as a reduction in cloud cover over most continental areas (Liu *et al.* 2023). However, in some regions such as

Highlights

- Hydrated lichens were treated with constant and fluctuating light in the laboratory
- In sun but not shade collections, NPQ strongly increased following treatment
- Increases in NPQ may reduce ROS formation during exposure to light while hydrated

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Abbreviations: F_O – minimal fluorescence yield of the dark-adapted state; F_O' – minimal fluorescence yield of the light-adapted state; F_M – maximal fluorescence yield of the dark-adapted state; F_M' – maximal fluorescence yield of the light-adapted state; F_S – steady-state fluorescence yield; F_V – variable fluorescence; F_V/F_M – maximal quantum yield of PSII photochemistry; lk – the light intensity at which PAR saturation sets in; NPQ – nonphotochemical quenching; q_E – energy-dependent quenching; q_I – photoinhibitory quenching; rETR – relative electron transport rate; ROS – reactive oxygen species; α – initial slope of the light curve; Φ_{PSII} – effective quantum yield of PSII photochemistry.

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the Kwa-Zulu Natal region in southern Africa, cloudiness and average rainfall in general will likely increase (Hart *et al*. 2018, Pinto *et al*. 2022). The first effect of this change will be an increase in the period lichens are hydrated and therefore metabolically active. Second, lichens will be subjected to an increase in light fluctuations due to "cloud flecks" (Morales and Kaiser 2020). Increases in the time lichens are hydrated and increases in light fluctuations seem particularly likely to occur in species that grow in exposed microhabitats, *e.g*., rock faces that rapidly dry after sunrise and presently experience rather steady light levels, where ambient radiation only changes slowly as a result of diurnal or seasonal changes. The ability of lichens to respond to these changes in their light environment is currently poorly understood.

In all photosynthetic organisms, light stress can occur when the energy they absorb exceeds that which can be used to fix carbon. This energy can end up converting ground-state oxygen to reactive oxygen species (ROS) (Pospíšil 2016). ROS can attack the photosynthetic apparatus, causing photoinhibition and photo-oxidative stress, resulting in less carbon fixation. The photobionts of lichens use a variety of processes to reduce the harmful effects of high light on ROS formation (for review *see* Beckett *et al*. 2021a). A particularly important mechanism is the dissipation of excess light as harmless heat in a process referred to as nonphotochemical quenching (NPQ). Estimating NPQ in lichens with chlorophycean photobionts is relatively simple with standard chlorophyll fluorescence devices (*see* Kalaji *et al*. 2017 for details). Very broadly, it is possible to classify lichens into "sun" or "shade" species. In general, sun species grow in exposed localities, such as on rock faces, bare soil, or the periphery of tree canopies, while shade species grow on forest floors or the trunks of trees. It is not uncommon to find sun and shade species growing meters apart from each other, receiving very different light levels (Cung *et al*. 2021). In some cases, particularly for sun lichens, the same species can be found growing in both types of habitats. Furthermore, on a microhabitat scale, a lichen that needs more sunlight might grow on top of a branch, while one that prefers moisture and shade might grow on the underside of the same branch.

For sun lichens, the total amount of light they receive is determined by the angle of the sun (time of day and season) and by cloud cover. However, lichens are poikilohydric and readily dry out, and only carry out carbon fixation when hydrated. In a typical field study of diurnal patterns of photosynthesis in sun lichens, Reiter *et al*. (2008) measured photosynthesis in *Xanthoria elegans* in the high Alps. Often *X. elegans* is hydrated by overnight dew and starts the day with moderate to high water contents. Net photosynthesis starts shortly after sunrise but stops after lichens dry out, typically after *ca*. 3 h. Presumably, lichens from open habitats are exposed to potentially photoinhibitory light levels just before they dry out. However, there are times when even sun species become "supersaturated" and show photosynthetic depression, *e.g*., during heavy rain (Cowan *et al*. 1992). This is because CO_2 diffuses slowly through water-filled intercellular spaces within the upper cortex and algal layer of a lichen thallus. These conditions also promote photoinhibition, because while lichens are still intercepting light, they cannot carry out much carbon fixation. In contrast, shade species typically spend more time hydrated (Pannewitz *et al*. 2003), and experience much lower average light levels. Furthermore, the light levels in these microhabitats are much more variable as a result of diurnal changes in the angle of sunlight, tree architecture, and movements of tree branches in the wind. The relatively brief periods when lichens are exposed to high light levels are known as "sun flecks". Presumably, most of the light stress shade lichens experience will be during the onset of a sun fleck, particularly if thalli are supersaturated.

Our recent surveys of photoprotection in a range of lichens show that the main difference between sun and shade forms is that shade forms possess higher, quickly inducing, and relaxing NPQ (Beckett *et al*. 2021b, Mkhize *et al*. 2022). We suggested higher NPQ may protect shade lichens from the rapid changes in light levels during sun flecks. The present investigation aimed to test the ability of a range of southern African Afromontane and Savannah lichens to adapt their NPQ in response to simulated climate change conditions, specifically longer periods of hydration and fluctuating light. Lichens were subjected to continuous hydration at either moderate fluctuating light levels for 8 h a day for 3 d on a 3 min cycle, or to the same conditions but with constant light. Light levels were adjusted to give the same total dose for both treatments. Originally, we hypothesized that sun lichens currently rarely experience short-term fluctuations in light, and therefore exposure to fluctuating light may increase NPQ. Conversely, shade lichens are already growing in fluctuating light conditions and will not need to change their NPQ response. The duration of periods of relatively bright and dim light varies greatly between habitats, but the average duration of sun flecks in subtropical Afromontane forests is probably *ca*. 2 min (Pallardy 2011). However, here we show that while NPQ in sun lichens does indeed rapidly increase in response to treatment with fluctuating light, similar changes occur when they are exposed to constant light, possibly because when continuously hydrated CO_2 fixation is restricted and therefore ROS formation may increase.

Materials and methods

Lichen material: Sun forms of *Ramalina celastri* (Sprengel) Krog and Swinscow, *Usnea undulata* Stirt., pale and melanized *Crocodia aurata* (Ach.) Link., and shade forms of *Lepraria incana* (L.) Ach. were collected from an Afromontane Forest in the Fort Nottingham Road Nature reserve. Squamules of *Cladonia coniocraea* (Flörke) Spreng and thalli of *Xanthoparmelia conspersa* (Ehrh. ex Ach.) Hale was collected from exposed rock surfaces in savannah vegetation in the Cumberland Nature Reserve just outside Pietermaritzburg. Shade forms of *R. celastri* and *U. undulata* were collected from shaded trees in a small pocket of Afromontane Forest in Queen Elizabeth Park on the outskirts of Pietermaritzburg. The photobionts of these lichens have been reported to belong to the chlorophycean genus *Trebouxia* except for

C. aurata which belongs to *Symbiochloris* (Rambold *et al*. 1998). After collection, lichen material was allowed to air dry between filter paper overnight and then stored at –24°C for a maximum of four weeks.

Chlorophyll fluorescence measurements: Chlorophyll fluorescence was measured using a *PAM 2500* fluorimeter (*Walz*, Effeltrich, Germany) using a red LED. After dark adaptation for 10 min (determined by initial experiments to be optimal) the maximal efficiency of photosystem II (PSII; F_V/F_M) was measured by giving a flash of saturating light of *ca*. 16,500 µmol(photon) m^{-2} s^{-1} for 0.8 s, 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. Occasional thalli with anomalous values of F_V/F_M were discarded. The relative electron transfer rate (rETR) was calculated as: $rETR = 0.5 \times \Phi_{PSII} \times PAR$, where PAR = photosynthetically active radiation and Φ_{PSII} is the effective quantum yield of PSII photochemistry calculated as: $(F_M' - F_S)/F_M'$, where F_M' = maximal fluorescence yield of the light-adapted state and F_S = stable fluorescence signal in the light.

NPQ was calculated using the formula of Bilger *et al.* (1995): $NPQ = (F_M - F_M')/F_M'$. In addition, NPQ was divided into fast and slow relaxing quenching, corresponding approximately to q_E and q_I, respectively, using equations in Kalaji *et al.* (2017): $NPQ_{fast} = (F_M - F_M')/F_M' - (F_M - F_M'')/F_M''$, $NPQ_{slow} = (F_M - F_M'')/F_M''$, where F_M'' = maximum fluorescence after 10 min of darkness.

To determine the induction of rETR and the induction and relaxation of NPQ, thalli were dark-adapted and F_V/F_M was measured. An actinic light of 100 µmol(photon) m^{-2} s^{-1} was then turned on, and saturating flashes were applied at increasing intervals for 11 min. The actinic light was then turned off and relaxation was measured for 10 min, with saturating flashes given at increasing intervals. In initial experiments, we tested the induction and relaxation of NPQ in several species of lichens using a range of light levels. Using light levels much above 100 µmol(photon) m^{-2} s^{-1} tended to cause photoinhibition in some species. Therefore, in all the experiments reported here, the induction and relaxation of NPQ was measured using a light level of 100 µmol(photon) m^{-2} s^{-1}.

Rapid light-response curves (RLC) of rETR were measured by increasing the actinic light in nine small steps of 10 to 20 s each from 0 to 250 µmol(photon) m^{-2} s^{-1} for the sun species or eight small steps from 0 to 190 µmol(photon) m^{-2} s^{-1} for the shade species. The equation derived by Eilers and Peeters (1988) was used to calculate the following parameters: α: initial slope of the light curve, related to maximum yield of photosynthesis; $rETR_{MAX}$: the maximal rETR reached during light curve recording, reflecting the light-saturated capacity of the sample [units: µmol(electron) m^{-2} s^{-1}]; lk: the light intensity at which PAR saturation sets in, estimated by constructing a linear regression of the initial part of the light-response curve and extrapolating it until it hits an rETR value corresponding to the estimate of $rETR_{MAX}$; the light intensity where the two lines intersect is lk [units: µmol(photon) m^{-2} s^{-1}].

Experimental treatments: Frozen material was thawed and then hydrated for 24 h at 15°C under dim [*ca*. 20 µmol(photon) m^{-2} s^{-1}] light in a growth cabinet. Initial fluorescence measurements of the induction and relaxation of NPQ and the induction of rETR were carried out, as well as RLC as described above. In initial experiments, a range of light levels were tested for their suitability for treatment. Even for sun species, exposure to levels greater than 100 µmol(photon) m^{-2} s^{-1} tended to cause some photoinhibition, so we decided to standardize on this level for constant light. Lichens were then exposed moist in open Petri dishes for 8 h a day for 3 d at either 100 µmol(photon) m^{-2} s^{-1} of constant light or to a light fluctuating between 200 µmol(photon) m^{-2} s^{-1} and 3 µmol(photon) m^{-2} s^{-1} (normal laboratory lighting) on a 3-min cycle. The light was supplied by a cool LED panel. Lichens were continually monitored to ensure they did not dry out. If they appeared to dry visibly they were sprayed with distilled water. Every day, after the 8-h exposure, Petri dishes were covered with aluminium foil and returned to the growth cabinet for 16 h. On the morning of the fourth day, lichens were taken from the growth cabinet and fluorescence measurements were again made of the RLC, the induction and relaxation of NPQ, and the induction of rETR.

An additional experiment was conducted with a sun collection of *R. celastri* in which material was treated with constant light for 8 h a day for 7 d. The induction and relaxation of NPQ and the induction of rETR were made first thing in the morning after exposure to light for 0 (freshly hydrated), 1, 2, 3, and 7 d.

Statistics: All data was analysed using two-way repeated measure *ANOVAs* in *R* version *4.4.0*. In addition, where appropriate, pairwise *t*-tests between treatments used were carried out with the *Bonferroni* correction.

Results

Table 1 summarizes the basic characteristics of photosynthesis of freshly collected lichens, derived from the RLCs and the induction and relaxation of NPQ. Comparing sun and shade lichens, both $rETR_{MAX}$ and the PAR where saturation sets in (lk) were more than double in the sun compared with the shade species. Measuring the induction of NPQ by 100 µmol(photon) m^{-2} s^{-1} showed that total NPQ was slightly higher in shade than sun species and that NPQ_{fast} was much higher in the shade than in the sun ones.

Figs. 1 and 2 illustrate the effects of exposure to constant and fluctuating light for 8 h a day for 3 d for the sun collections of lichens. Exposure of *Ramalina celastri* and *Xanthoparmelia conspersa* to constant or fluctuating light generally increased NPQ (Fig. 1*A*,*D*; Table 2). In *Cladonia coniocraea*, constant light increased NPQ while fluctuating light had little effect (Fig. 1*B*). While the constant light had little effect in *Usnea undulata*, fluctuating light increased NPQ (Fig. 1*C*). In melanized *Crocodia aurata* neither light treatment had much effect on NPQ (Fig. 1*E*). Different species showed a variety of patterns of induction

Table 1. Summary of photosynthetic parameters of sun and shade collections of the lichens. 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 (lk), and the maximal relative electron transport rate ($rETR_{MAX}$). Nonphotochemical quenching (NPQ) values were obtained by illuminating dark-adapted lichens with light at 100 µmol(photon) m^{-2} s^{-1} and measuring the time course of the induction of NPQ for 11 min, and the subsequent relaxation of NPQ for 11 min after switching off the light. Values are given as ± SE, n = 10.

Species	ETR_{max}	lk	α	NPQ_{fast}	NPQ_{slow}	Maximum NPQ
Sun collections						
Ramalina celastri	20.5 ± 1.4	79 ± 5	0.26 ± 0.01	0.32 ± 0.04	0.27 ± 0.08	0.59 ± 0.09
Cladonia foliacea	32.5 ± 11.4	139 ± 49	0.28 ± 0.08	0.44 ± 0.29	0.57 ± 0.03	1.01 ± 0.09
Usnea undulata	26.8 ± 1.9	99 ± 9	0.27 ± 0.01	0.37 ± 0.02	0.22 ± 0.01	0.59 ± 0.04
Xanthoparmelia conspersa	34.1 ± 12.1	135 ± 48	0.25 ± 0.09	0.48 ± 0.08	0.27 ± 0.03	0.75 ± 0.09
Crodia aurata (melanized)	7.9 ± 2.4	22 ± 6.6	0.36 ± 0.11	0.81 ± 0.11	0.32 ± 0.02	1.13 ± 0.12
Mean	24.4 ± 4.8	95 ± 21	0.28 ± 0.02	0.48 ± 0.09	0.33 ± 0.06	0.81 ± 0.11
Shade collections						
Ramalina celastri	17.1 ± 1.1	66 ± 5	0.26 ± 0.01	0.67 ± 0.08	0.44 ± 0.05	1.10 ± 0.11
Lepraria incana	4.0 ± 0.5	12 ± 1	0.31 ± 0.05	0.67 ± 0.02	0.13 ± 0.02	0.80 ± 0.12
Usnea undulata	20.5 ± 1.5	74 ± 7	0.38 ± 0.01	0.58 ± 0.03	0.23 ± 0.02	0.96 ± 0.04
Crocodia aurata (pale)	6.9 ± 2.1	16 ± 5	0.45 ± 0.14	0.77 ± 0.13	0.18 ± 0.07	0.94 ± 0.18
Mean	12.1 ± 4.0	42 ± 16	0.35 ± 0.04	0.67 ± 0.04	0.25 ± 0.07	0.95 ± 0.06

and relaxation of NPQ. For example, for *Usnea undulata* relaxation was almost linear (Fig. 1*C*), while in *Crocodia aurata* relaxation was almost perfectly hyperbolic (Fig. 1*E*). Induction of rETR at 100 µmol(photon) m^{-2} s^{-1} was very fast usually but it was slower in melanized *C. aurata* (Fig. 1*E*). For all species, the light treatments had little effect on the induction of rETR (Fig. 1, Table 3), which was confirmed by RLCs (Fig. 2).

Exposure of shade collections of lichens to constant and fluctuating light had much less effect on NPQ (Fig. 3), with light effects not being significant for *R. celastri* and *L. incana* and only just significant for *U. undulata* and *Crocodia aurata* (Table 2). Induction of rETR at 100 µmol(photon) m^{-2} s^{-1} indicated that the light treatments caused some photoinhibition in *L. incana* and the pale form of *Crocodia aurata* (Fig. 3*B*,*D*). RLCs from shade species were generally consistent with these results (Fig. 4). In *L. incana*, the light treatments appeared to induce slight hardening against photoinhibition at the higher light levels used when constructing the RLCs (Fig. 4*B*).

NPQ in a sun collection of *R. celastri* treated with constant light at 100 µmol(photon) m^{-2} s^{-1} for 8 h a day initially rapidly increased after 1 d, and thereafter progressively increased further until 7 d (Fig. 5*A*). Induction of rETR and the RLCs indicated that the light treatment caused slight photoinhibition (Fig. 5*B*,*C*).

Discussion

In the present study, we tested whether lichens can modify their NPQ in response to simulated climatic shifts, specifically increases in rainfall and cloudiness expected to occur in the Afromontane forests of southeastern South Africa. Here we artificially simulated predicted climate change by treating hydrated lichens with fluctuating or constant light in the laboratory for 8 h a day for 3 d. While even in this short period NPQ greatly increases in sun lichens, NPQ increases in response to not only fluctuating light but to continuous constant light conditions. The high NPQ in shade forms we reported in our earlier studies may be more linked with a need to photoprotect lichens from ROS formation during exposure to light while hydrated, which can reduce carbon fixation while photophosphorylation continues. In contrast to the effect on sun lichens, NPQ in shade lichens is little affected by the laboratory treatments. Probably, our treatments create conditions similar to those of the normal microhabitats of shade lichens. Irrespective of the reasons for the increases in NPQ, it is clear that NPQ can change in response to simulations of the increases in rainfall and cloudiness expected in KwaZulu Natal as a result of climate change.

Characteristics of PSII activity in freshly collected sun and shade lichens: In general, the characteristics of PSII activity in freshly collected material of sun and shade lichens found here are similar to those reported by Beckett *et al*. (2021b) and Mkhize *et al*. (2022). Compared to sun forms, shade forms display generally higher NPQ and possess a greater proportion of their NPQ as NPQ_{fast} (Table 1), probably corresponding to q_E or xanthophyll cycle-based quenching. Similar to reports from higher plants (Greer 2024), the maximal rETR rates were lower in shade than in sun collections, probably reflecting a downregulation of photosynthetic capacity to reduce energy costs. These differences were also evident in collections of the same species of *Ramalina celastri* and *Usnea undulata* from sun and shade localities. However, in melanized and pale thalli of *Crocodia aurata* from more exposed and shaded microhabitats, respectively, NPQ induced and relaxed similarly (Figs. 1*E*, 3*D*). Based on growth measurements, Gauslaa and Goward (2020)

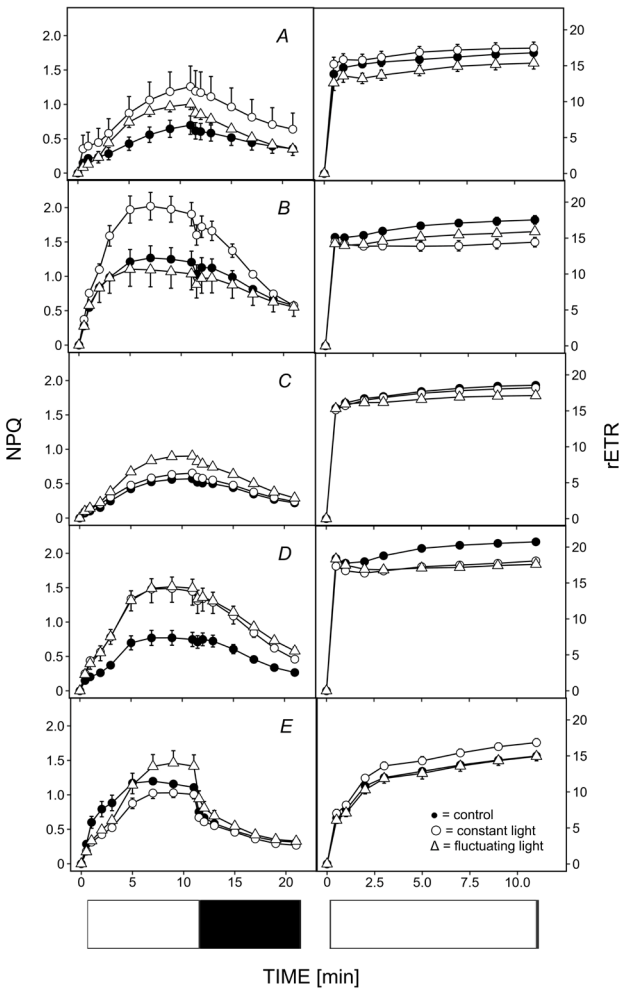

Fig. 1. The effect on nonphotochemical quenching (NPQ) and relative electron transport rate (rETR) of treatment with light at a constant 100 µmol(photon) m^{-2} s^{-1} or light fluctuating between 200 µmol(photon) m^{-2} s^{-1} for 3 min and 3 µmol(photon) m^{-2} s^{-1} for 3 min for 8 h a day for 3 d in sun collections of lichens. (*A*) *Ramalina celastri*; (*B*) *Cladonia coniocraea*; (*C*) *Usnea undulata*; (*D*) *Xanthoparmelia conspersa*; (*E*) *Crocodia aurata* (melanized). The error bars show the mean ± SE (n = 10) when larger than symbol size. The white bars in the row at the bottom of each graph indicate the periods when samples were exposed to light and the dark when samples were exposed to dark.

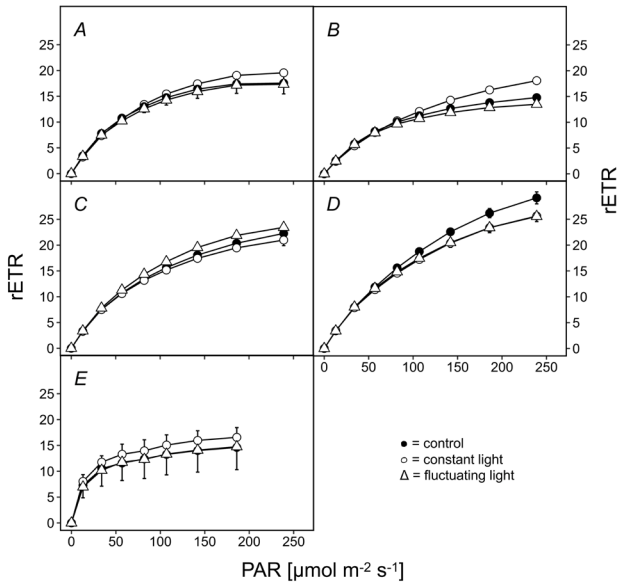

Fig. 2. Rapid light curves (rETR as a function of light level) in sun collections of lichens. (*A*) *Ramalina celastri*; (*B*) *Cladonia coniocraea*; (*C*) *Usnea undulata*; (*D*) *Xanthoparmelia conspersa*; (*E*) *Crocodia aurata* (melanized). The error bars show the mean ± SE (n = 10) when larger than symbol size.

suggested that in *Lobaria pulmonaria* melanic pigments may adjust the light received by the photobionts 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, consistent with the results obtained here. Interestingly, at variance with the present study, Mkhize *et al*. (2022) found that melanized *C. aurata* had higher NPQ than shade forms (pale *C. aurata*), suggesting that in those collections melanization had not normalized light levels. While melanization can be rapid under inducing conditions (Solhaug *et al*. 2003), it is possible that in the material used by Mkhize *et al*. (2022) cortical screening pigments were insufficient for adequate photoprotection Therefore, in the microhabitat occupied by the melanized forms, additional biochemical photoprotective mechanisms such as higher NPQ are required. However, this does not appear to be true for the material used in the present study. Taken together, results from lichens collected freshly from the field suggest that the rates of rETR and the amount and type of NPQ in photobionts can show considerable variation according to differences in light availability.

In sun forms both constant and fluctuating light treatments increase NPQ: The main aim of the present study was to test the ability of lichens to respond to the general increases in rainfall and cloudiness expected to occur in KwaZulu Natal (Hart *et al*. 2018, Pinto *et al*. 2022). These changes are more likely to affect lichens growing in exposed habitats than those in shaded woodlands. In woodlands, thalli already remain hydrated for long periods and are subject to variable light levels due to sun flecks. Here, we simulated predicted climate change by treating lichens for 8 h a day for 3 d with constant light at 100 µmol(photon) m^{-2} s^{-1} or light fluctuating between 200 and 3 µmol(photon) m^{-2} s^{-1} on a 3-min cycle. We originally hypothesized that exposing sun lichens to fluctuating light would have a greater effect on NPQ than constant light, as photobionts may need protection from the sudden increases in light levels that occur at the start of light fluctuations. Interestingly, results showed that in sun lichens both treatments increase NPQ; constant moderate light was as effective at increasing NPQ as fluctuating light (Figs. 1, 2; Tables 2, 3). Indeed, for the sun form of *Ramalina celastri* and *Cladonia coniocraea* increases in NPQ were greater in material given constant rather than fluctuating light (Fig. 1*A*,*B*). In the field, photosynthesis

Table 2. Repeated measure two-way *ANOVA* on the induction and relaxation of nonphotochemical quenching (NPQ) in sun and shade lichens. For each species, ten disks were measured at intervals during induction in the light and relaxation in the dark following the switching on light at 100 µmol(photon) m^{-2} s^{-1} as indicated in Figs. 1 and 3. In addition, pairwise *t*-tests between the three treatments used were carried out using the Bonferroni correction for *p*-values. **P*<0.05; ***P*<0.01; ****P*<0.001; *****P*<0.0001.

	Light treatment	Time	Interaction	Control *vs* constant	Control *vs* fluctuating	Constant *vs* fluctuating
Sun species						
Ramalina celastri	*	****	****	****	****	*
Cladonia coniocraea	****	****	****	****	****	**
Usnea undulata	***	****	****	0.315	****	****
Xanthoparmelia conspersa	**	****	****	****	****	0.658
Crocodia aurata (melanized)	0.124	****	****	*	0.588	**
Shade species						
Ramalina celastri	0.744	****	0.915	0.620	0.244	0.097
Lepraria incana	0.284	****	*	0.540	0.137	**
Usnea undulata	*	****	****	0.317	****	*
Crocodia aurata (pale)	*	****	****	0.530	***	**

Table 3. Repeated measure two-way *ANOVA* on the induction of relative electron transfer rate (rETR) in sun and shade lichens. For each species, ten disks were measured at intervals during induction rETR following the switching on light at 100 µmol(photon) m^{-2} s^{-1} as indicated in Fig. 1 and 3. In addition, pairwise *t*-tests between the three treatments used were carried out with the Bonferroni correction for *p*-values. **P*<0.05; ***P*<0.01; ****P*<0.001; *****P*<0.0001.

	Light treatment	Time	Interaction	Control *vs* constant	Control *vs* fluctuating	Constant *vs* fluctuating
Sun species						
Ramalina celastri	0.053	****	0.098	0.975	0.309	*
Cladonia coniocraea	**	****	****	**	***	***
Usnea undulata	0.537	****	0.053	1.000	1.000	1.000
Xanthoparmelia conspersa	0.062	****	****	0.192	1.000	0.256
Crocodia aurata (melanized)	0.197	***	*	0.367	1.000	0.206
Shade species						
Ramalina celastri	0.862	****	0.605	1.000	1.000	1.000
Lepraria incana	***	****	****	****	***	0.061
Usnea undulata	0.156	****	*	1.000	0.234	0.652
Crocodia aurata (pale)	*	****	***	0.111	**	0.857

in sun forms is often confined to a few hours in the early morning when thalli are wet from overnight dew and have not been desiccated by the sun (Lange 2003). For lichens, which normally grow under these conditions, the NPQ of freshly collected material is generally low, appearing less important in photoprotection than in shade collections (Table 1) (Mkhize *et al*. 2022). Presumably, protection under these conditions is provided by other mechanisms such as a cyclic or pseudocyclic electron flow around PSI, or the PSII repair cycle (Beckett *et al*. 2021a, 2023). Recent evidence from the related free-living alga *Chlamydomonas reinhardtii* suggests that pseudocyclic electron flow remains active in constant light and can reduce excess H_2O_2 production from PSII (Pfleger *et al*. 2024). In contrast to their normal field conditions, in the present experiments, sun lichens were kept on moist filter paper, more or less fully hydrated throughout the 3 d of treatment. While CO_2 fixation is often depressed in fully saturated thalli, due to limitations in CO_2 diffusion through intercellular water to the photobiont cells (Lange and Green 1996), photophosphorylation continues to occur even in fully saturated thalli. For example, a field study by Leisner *et al.* (1997) measured both CO_2 fixation and rETR in the sun lichen *Lecanora muralis*. These workers found "higher than expected ETR in the supersaturated condition, where photosynthesis was very depressed

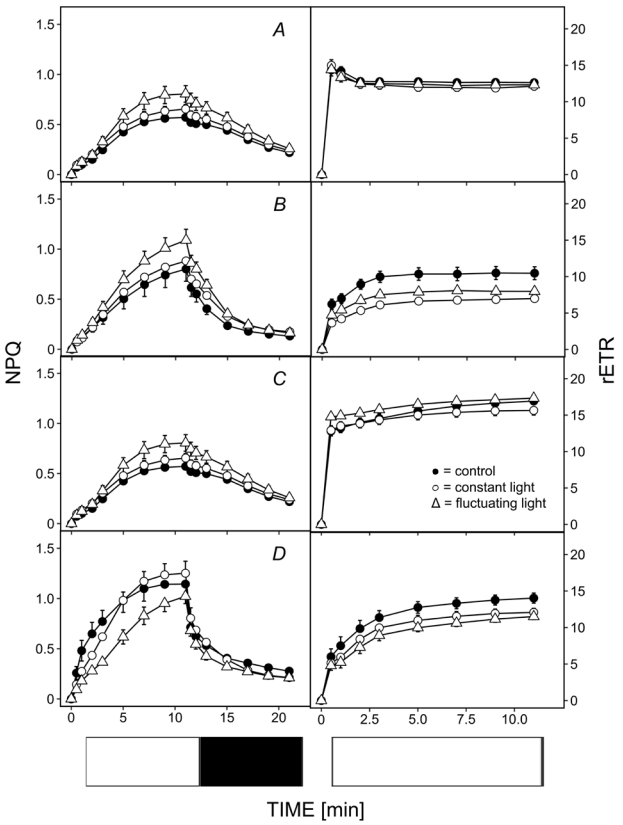

Fig. 3. The effect on nonphotochemical quenching (NPQ) and relative electron transport rate (rETR) of treatment with light at a constant 100 µmol(photon) m^{-2} s^{-1} or light fluctuating between 200 µmol(photon) m^{-2} s^{-1} for 3 min and 3 µmol(photon) m^{-2} s^{-1} for 3 min for 8 h a day for 3 d in shade collections of lichens. (*A*) *Ramalina celastri*; (*B*) *Lepraria incana*; (*C*) *Usnea undulata*; (*D*) *Crocodia aurata* (pale). The error bars show the mean ± SE (n = 10) when larger than symbol size. The white bars in the row at the bottom of each graph indicate the periods when samples were exposed to light and the dark when samples were exposed to dark.

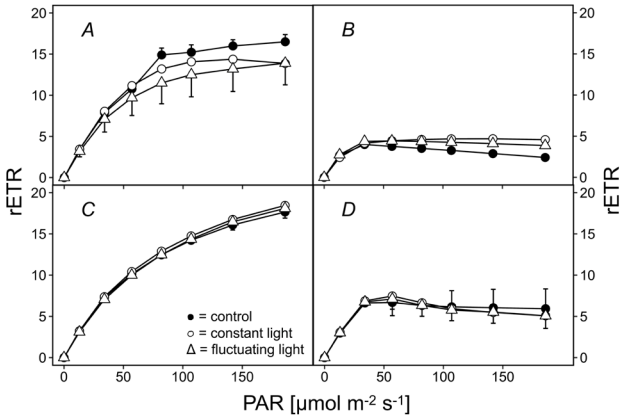

Fig. 4. Rapid light curves (rETR as a function of light level) in shade collections of lichens. (*A*) *Ramalina celastri*; (*B*) *Lepraria incana*; (*C*) *Usnea undulata*; (*D*) *Crocodia aurata* (pale). The error bars show the mean ± SE (n = 10) when larger than symbol size.

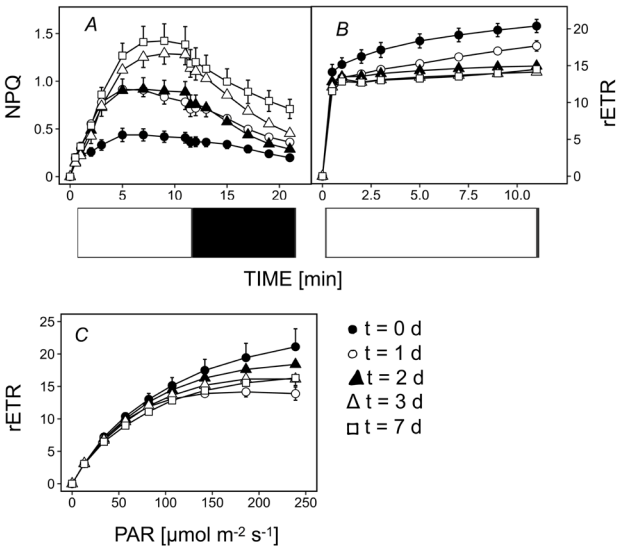

Fig. 5. The effect on nonphotochemical quenching (NPQ) (*A*), relative electron transport rate (rETR) (*B*), and rapid light curves (*C*) in a sun collection of *Ramalina celastri*, freshly collected material after exposure to 100 µmol(photon) m^{-2} s^{-1} for 8 h a day for up to 7 d. The error bars show the mean ± SE (n = 10) when larger than symbol size. The white bars in the row at the bottom of each graph indicate the periods when samples were exposed to light and the dark when samples were exposed to dark.

due to high diffusion resistances". These conditions will promote ROS formation, and these authors suggested photorespiration provides some quenching. Although this may partly be true (Timm and Eisenhut 2023), it seems likely photoprotection will be further improved by increased NPQ. Taken together, our results suggest that one of the main reasons, why our treatments increase NPQ in sun forms, is to reduce the risk of ROS formation in hydrated thalli exposed to even moderate light. The implication is that sun lichens can adapt to the wetter conditions predicted due to climate change.

Changes in NPQ in sun collections can occur very rapidly. Here, we exposed *Ramalina celastri* to a constant light at 100 µmol(photon) m^{-2} s^{-1} for 8 h a day for up to 7 d, and during this time NPQ progressively increased from *ca*. 0.4 to 1.4 (Fig. 5*A*). The mechanism for the relatively rapid increase in NPQ was not studied here, but in free-living chlorophycean algae, safe dissipation of excess light energy is mediated by light-harvesting complex stress-related (LHCSR) proteins ("q_E") and redistribution of light-harvesting antennae between the photosystems (state transitions or q_T) (Steen *et al*. 2022, Shang *et al*. 2023). While some modulation of NPQ is possible within very short times (Steen *et al*. 2022), in our experimental design, induction and relaxation were measured first in freshly collected material following rehydration overnight under cool conditions in dim light. Second, they were measured on the morning after 1, 2, 3, and 7 d of treatment, again following a night of cool dim conditions. Therefore, the changes we observed reflect relatively short-term rather than instantaneous changes

in NPQ. However, further work is needed to elucidate the precise mechanisms of the rapid changes in NPQ we observed here.

Unlike NPQ, light treatments had little effect on rETR (Figs. 1, 2; Tables 2, 3). We have previously observed that freshly collected material of lichens from shaded microhabitats has lower rates of rETR than material from exposed microhabitats, similar to results from higher plants (Greer 2024). A reduction in rETR probably reflects a downregulation of photosynthetic capacity under generally lower light levels to reduce metabolic expenditure on maintaining unnecessarily high levels of cytochromes and enzymes. In our experimental treatments, the sun lichens at least received lower light levels than they would normally in the field. Theoretically, they could be expected to eventually downregulate rETR to reduce metabolic cost. However, downregulation of rETR was not observed here, presumably because it requires longer times than those needed for the relatively rapid changes in NPQ.

In shade forms constant or fluctuating light treatments have little effect on NPQ: In contrast to the effects on NPQ in sun lichens, the treatment of shade collections with constant and fluctuating light had a small effect (Fig. 3, Table 2). While in *Lepraria incana* and *Usnea undulata* fluctuating light slightly increased NPQ (Fig. 3*B*,*C*; Table 2), general differences were much lesser than for sun species. Perhaps surprisingly, there have been far fewer studies on diurnal variations in water content and photosynthesis in shade than in sun lichens. Intuitively it seems likely that shade lichens will stay hydrated for much longer than typical sun lichens. Pannewitz *et al*. (2003) showed that while even shade *Lobaria pulmonaria* can dry out, thalli remained hydrated for considerable periods. Interestingly, typical maximum light levels when hydrated were 273 µmol(photon) m^{-2} s^{-1} in collections from a slightly more open site, and 86 µmol(photon) m^{-2} s^{-1} in material from a more shaded site. These values are consistent with the light intensities used here. It seems likely that our treatment conditions resemble the microhabitats of shade lichens in the field and therefore had little effect on NPQ, which is already elevated compared with sun collections (Table 1; Beckett *et al*. 2021b, Mkhize *et al*. 2022). While the higher NPQ in shade forms may protect the photobionts from sudden increases in light during sun flecks, as discussed above, it seems equally likely that higher NPQ may be needed to dissipate excess light energy resulting from continued electron flow but also a reduced ability to fix CO_2 in moist thalli.

Constant and fluctuating light has little effect on NPQ in melanized and pale *Crocodia aurata*: *C. aurata* is usually considered a forest "shade" lichen (Galloway 1985), although it may grow in slightly brighter habitats, and under these conditions, the upper cortex becomes melanized. While the light limitation of photosynthesis (lk) occurs at higher light levels in the sun collections than in the shade (Table 1), the general characteristics of photosynthesis in freshly collected material were similar in both forms. Similarly, treating thalli with constant or fluctuating light had little effect on pale or melanized thalli, *i.e*., both collections appeared to behave as shade forms. The only difference is that the treatments mildly inhibited rETR in shade but not sun forms (Figs. 1*E*, 3*D*). As discussed above, it seems likely that in our collections, melanins had normalized light levels between the two forms.

Conclusions: Our earlier work showed that shade lichens are characterized by higher NPQ than sun collections, and we originally hypothesized that the high values of NPQ in shade collections protect from the sudden increases in light levels that occur during sun flecks. However, the results presented here provide no clear evidence that shade lichens possess specific adaptations to fluctuating light. Rather, our results indicate that high NPQ in shade forms is an adaptation to reduce ROS formation that occurs when photophosphorylation continues while thallus oversaturation reduces CO_2 fixation. However, the response of sun lichens to constant and fluctuating light is not always the same (Fig. 1). In future work, we plan to separate the effects of thallus hydration and fluctuating light, for example by testing the effects of light treatments on NPQ in hydrated but not saturated lichens. The normally low values of NPQ displayed by sun lichens can be rapidly increased by treating them with either constant or fluctuating light when moist. Positively, this suggests that the photosynthetic apparatus of Afromontane sun lichens can rapidly adjust to the increases in rainfall and cloudiness predicted to occur in southeastern South Africa.

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