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Symbiosis as a successful strategy in continental Antarctica: performance and protection of *Trebouxia* photosystem II in relation to lichen pigmentation

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Abstract Lichens as symbiotic associations consisting of a fungus (the mycobiont) and a photosynthetic partner (the photobiont) dominate the terrestrial vegetation of continental Antarctica. The photobiont provides carbon nutrition for the fungus. Therefore, performance and protection of photosystem II is a key factor of lichen survival. Potentials and limitations of photobiont physiology require intense investigation to extend the knowledge on adaptation mechanisms in the lichen symbiosis and to clarify to which extent photobionts benefit from symbiosis. Isolated photobionts and entire lichen thalli have been examined. The contribution of the photobiont concerning adaptation mechanisms to the light regime and temperature conditions was examined by chlorophyll *a* fluorescence and pigment analysis focusing on the foliose lichen *Umbilicaria decussata* from North Victoria Land, continental Antarctica. No photoinhibition has been observed in the entire lichen thallus. In the isolated photobionts, photoinhibition was clearly temperature dependent. For the first time, melanin in *U. decussata* thalli has been proved. Though the isolated photobiont is capable of excess light protection, the results clearly show that photoprotection is significantly increased in the symbiotic state. The closely related photobiont of *Pleopsidium chlorophanum*, a lichen lacking melanin, showed a higher potential of carotenoid-based excess light tolerance. This fact discriminates the two photobionts of the same *Trebouxia* clade. Based on the results, it can be concluded that the successful adaptation of lichens to continental Antarctic conditions is in part based on the physiological potential of the photobionts. The findings provide information on the success of symbiotic life in extreme environments.

Keywords Terrestrial ecosystems · Isolated photobionts · Photoprotection · Secondary metabolites · Photosynthesis

Abbreviations

ANOVA	Analysis of variance
chl *f*	Chlorophyll fluorescence
DEPS	De-epoxidation state of the xanthophyll pool
DMSO	Dimethyl sulfoxide
GANOVEX	German Antarctic North Victoria Land Expedition
HPLC	High-performance liquid chromatography
MY	Malt–yeast
NPQ	Non-photochemical quenching
PAM	Pulse-amplitude modulation
PAR	Photosynthetically active radiation
PPFD	Photosynthetically active photon flux density
PS	Photosystem
ROS	Reactive oxygen species
SLC	Secondary lichen compound
TOM	*Trebouxia* organic medium
UVR	Ultraviolet radiation

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Introduction

The vegetation of continental Antarctica is sparse and consists of highly specialized organisms (Robinson et al. 2003; Hughes et al. 2006; Convey 2010). In this harsh environment, lichens dominate the terrestrial macrovegetation (Kappen 2000). These symbiotic consortia of a fungus (mycobiont) and a photosynthetic partner (photobiont) can, in general, be described as primary colonizers of extreme environments (Chen et al. 2000). The poikilohydric lifestyle of lichens is characterized by anabiosis, the physiologically inactivated state of dehydrated lichen thalli. In particular, in the state of anabiosis, many lichen species show considerable tolerance against abiotic stressors, enabling them to survive under environmental conditions which might be lethal to other organisms (Meeßen et al. 2013). The conditions consist of sustainable life in hot and cold deserts, in alpine regions, but also survival under most extreme parameters such as ultraviolet (UV) irradiation (Sánchez et al. 2014), freezing (Kappen and Lange 1972; Harańczyk et al. 2012), space exposure (Onofri et al. 2012) and even acetone rinsing (Gauslaa and Solhaug 2004).

Lichens are highly resistant to extreme temperatures, a feature that substantially supports the success of these organisms in cold environments such as the ice-free areas of the Antarctic continent and the adjacent islands (Kappen and Lange 1970, 1972; Kappen 1973; Schlensog et al. 2003). While lichens are often highly resistant to temperature extremes in the anabiotic state, photosynthetic activity of polar lichens has been detected at subzero temperatures (Kappen et al. 1996).

Lichen colonization occurs in microniches on the Antarctic continent. The microclimatical conditions of these sites are determined by abiotic factors such as rock topography (Jahns and Fritzler 1982). Such microniches for colonization by various lichen species can be minimum sized rock surface fissures. Microniches in rock fissures are characterized by meltwater supply and therefore, at least temporarily improve water availability. The occurrence of meltwater depends on topography of the respective site, substrate structure and the amount of snow and ice close by. Meltwater availability may be erratic compared to the diurnal change of air humidity (Schroeter et al. 2011). In general, the lichen Umbilicaria decussata colonizes rock sites of different size characterized by fissures and especially at continental sites supported by meltwater. Water availability strictly controls lichen activity due to the poikilohydric nature of these organisms (Green and Lange 1995). At continental Antarctic sites, meltwater supply is especially crucial for lichen activity (Kappen et al. 1998). Metabolic activity and especially photosynthetic primary production in continental Antarctica are primarily limited by water availability (Kappen 1985; Gjessing and Øvstedal 1989; Kappen and Breuer 1991; Schroeter et al. 1992).

At habitats of continental Antarctica, lichens live under the conditions of a frigid desert (Huiskes et al. 2006). The physiologically active period of lichens can be characterized by high light intensity (Kappen et al. 1998; Schroeter et al. 2010). Photoprotective mechanisms of the lichen symbiosis are crucial to avoid photodamage which would reduce photosynthetic productivity in the short vegetation period. Lichens of maritime Antarctica mainly depend on air humidity and dehydrate during times of excess insolation (Schlensog et al. 2003). Photoprotection in the hydrated state seems to be a prerequisite for the meltwater-activated lichens at continental sites.

For metabolically active, moist thalli of the continental Antarctic lichen species Umbilicaria aprina, considerable tolerance to high light intensities was detected by Kappen et al. (1998). Schlensog et al. (2003) proposed that the reflectance and absorbance of the U. aprina thallus cortex are the key factors of irradiation tolerance in this lichen. The shading capacity of lichen cortices reduces the light intensity experienced by the symbiotic photobionts substantially, compared to the ambient light (Ertl 1951; Büdel and Lange 1994).

Green algae of the genus Trebouxia, which are the most common among lichen photobionts, are specialized to a lichenized lifestyle. It has still not been clarified to which extent they can survive aposymbiotically under natural conditions (Wornik and Grube 2010). Meanwhile, there is a lot of experience in cultivation of lichen symbionts, especially Trebouxia photobionts (Schaper and Ott 2003; Sadowsky and Ott 2012). To compare the performance of photobionts in the lichen thallus to their isolated cultured state is a prerequisite for the knowledge on special adaptation mechanisms of the photobiont. Photobiont adaptations are especially interesting when low photobiont diversity is detected in a lichen community of a definite environment. Special characteristics of photobionts might be based on adaptation mechanisms to the respective environment (Sadowsky and Ott 2012; Domaschke et al. 2013). Clade S Trebouxia photobionts (Helms et al. 2001) dominate in macrolichens throughout Antarctica (Romeike et al. 2002).

The photosynthetic response of various isolated Antarctic photobionts to drought and cold showed different patterns. These differences between photobionts could be correlated with the environmental conditions of the corresponding lichen species rather than to their phylogenetic position in the genus Trebouxia (Sadowsky and Ott 2012). The environmental conditions inside the thallus normally differ considerably from those outside the thallus. In particular, hydration and light regime for the photobiont are determined by thallus structure and secondary lichen compounds (SLCs) inside the thallus (Sadowsky et al.

2012; Meeßen et al. 2013). Environmental adaptations of lichen symbionts can be regarded as adaptations toward the symbiotic lifestyle. Besides other factors, especially the physiological potential of the isolated symbionts determines the prerequisites for a successful symbiotic lifestyle in respective environments. Therefore, to clarify possible benefits of lichen symbiosis, the performance of symbiotic and aposymbiotic forms should be compared with experiments, as it has been done in the present study.

Materials and methods

Lichen material

Samples of *Umbilicaria decussata* (Vill.) Zahlenbr. (1942) and *Pleopsidium chlorophanum* (Wahlenb.) Zopf (1855) were collected during the 2009/2010 German Antarctic North Victoria Land Expedition (GANOVEX) X in Terra Nova Bay, North Victoria Land, continental Antarctica. Both lichens grew on a sun-exposed rock surface.

All lichen thalli were air-dried and deep-frozen (-25 °C) until photobiont isolation. Both photobionts belong to clade S *Trebouxia* (Brandt 2011; taxonomy after Helms et al. 2001).

Frozen lichen thalli were reactivated prior to the chlorophyll fluorescence analysis. The reactivation procedure was as follows: Entire lichen thalli were sprayed with water and stored at 12 °C in sealed vials (to prevent dehydration) in the dark for 24 h, followed by 14 h of light (20 µmol photons m^2 s^{-1}, fluorescent light source) at the same temperature.

Symbiont isolation and cultivation

Photobionts were isolated from lichen thalli according to Yoshimura et al. (2002) from thallus fragments. Axenic photobiont cultures on *Trebouxia* organic medium agar (TOM) with 1 % glucose (Ahmadjian 1967) were kept at low light intensity (20 µmol photons m^2 s^{-1}; diurnal cycle with 10 h of darkness) and 12 °C in a growth chamber (Rubarth Apparate GmbH, Germany). For pigment isolation and chlorophyll fluorescence experiments, the photobionts were transferred to 1 cm^2 nitrocellulose filter disks on TOM-agar growing in the growth chamber for 4 weeks. Mycobionts were isolated from ascospores and cultivated on malt–yeast (MY) medium under the same conditions as the photobionts.

Chlorophyll fluorescence

Chlorophyll *a* fluorescence (chl *f*) was determined by using a Mini-PAM (pulse-amplitude modulated) fluorimeter (Walz Mess-und Regeltechnik, Germany) according to

Maxwell and Johnson (2000). The parameter $(F_m - F_0)/F_m = F_v/F_m$ (maximum quantum yield of photosystem II) was measured in the dark after 20 min of dark acclimation of the samples by applying a saturating light pulse. Quantum yield of photosystem II (dF/F'_m) in the light-acclimatized state was calculated as $(F'_m - F_t)/F'_m$, and non-photochemical quenching (NPQ) of maximum fluorescence was calculated as $(F_m - F'_m)/F'_m$. Slow chl *f* induction was performed by applying constant illumination on the dark-acclimatized samples for 12 min, followed by 20 min of dark relaxation. Eight samples of photobionts and, due to material limitations, four samples of lichen thalli were used per treatment. The effects of temperature and light intensity on quantum yield and NPQ were analyzed by two-way ANOVA ($\alpha = 0.05$). A post hoc test (Tukey–Kramer multiple comparisons) was applied to detect significant reduction in photosystem II quantum yield after illumination and recovery.

Maximum quantum yield of freshly reactivated lichens was compared between the two species *Umbilicaria decussata* and *Pleopsidium chlorophanum* by an unpaired two-sided *t* test ($\alpha = 0.05$). The checked null hypothesis H_0 was that there were no differences between the lichen species.

Pigment analysis

Photobiont pigments were determined from acetone extracts by HPLC as described in Sadowsky and Ott (2012). The de-epoxidation status of the xanthophyll pool (DEPS) was calculated from the xanthophyll concentrations (zeaxanthin + antheraxanthin)/(violaxanthin + antheraxanthin + zeaxanthin) according to Vráblíková et al. (2004). The effects of temperature and photobiont origin on DEPS were analyzed by two-way ANOVA ($\alpha = 0.05$; $n = 4$). A Tukey–Kramer multiple comparisons post hoc test was applied to detect pairs of significantly differing values.

Photospectrometric determination of *Umbilicaria decussata* thallus and mycobiont pigments from acetone, methanol and dimethyl sulfoxide (DMSO) extracts as well as testing on melanin was performed according to Meeßen et al. (2013). Synthetic melanin (>97 %, Sigma-Aldrich) was used as a reference. Despite the test on acetone solubility, the homogenized samples were pre-rinsed with acetone prior to the melanin testing. Mycobiont pigment extraction in DMSO was performed with native and acetone pre-rinsed mycobiont material.

Microclimate at lichen sites

Air, substrate and thallus temperature as well as relative air humidity and light intensity (photosynthetically active photon flux density, PPFD) were measured with sensors

attached closely to lichen thalli near the Gondwana station at Terra Nova Bay, North Victoria Land. Air humidity and temperature were measured with combined temperature/ humidity probes (HMP 35 A/TH, Vaisala, Finland). Substrate temperature was measured by thermistors (FF-U-V5-0 and FM-SU-VS5-0, Grant, UK). PPFD was measured using a quantum Sensor (Li-190SA, Licor, USA). Data were recorded on loggers (Grant and Eltek, 1000 series). The measurement was taken from January 4 to February 3 at a site of *Umbilicaria decussata*.

Results

Microclimate

Air, substrate and thallus temperature (Fig. 1) at the site of *Umbilicaria decussata* followed the same time course and showed similar values. At this site, the air temperature slightly exceeded the substrate and thallus temperature. Thallus temperature exceeded the substrate temperature during the daily temperature maximum. The diurnal rhythm was naturally correlated with the changing insolation (Fig. 2). Maximum photosynthetic photon flux density

(PPFD) of c. 1600 μmol photons m^{-2} s^{-1} from sunlight was reached at c. 1 p.m. during the time when the measurement site was snow free. Maximum temperature >20 °C was reached about 2 h later. In the middle of the night, when PPFD was as low as c. 50 μmol photons m^{-2} s^{-1}, temperatures of air, substrate and *U. decussata* thalli dropped to 0 °C. Air humidity was generally too low for lichen rehydration but reached c. 60 % relative humidity during the night. Air humidity during daytime was generally <20 % (Fig. 2). The measurement site was snow covered from January 22 until the end of the measurement. During snow cover, thallus and substrate temperatures were around or slightly below 0 °C (Fig. 1a). PPFD was reduced to a daily maximum of c. 200–400 μmol photons m^{-2} s^{-1}. Relative air humidity (measured above the snow) rose up to c. 90 % (Fig. 2a).

Chlorophyll fluorescence

Entire lichen thalli ($n = 4$) were successfully reactivated and showed maximum quantum efficiencies (F_v/F_m) of 0.61 ± 0.04 (*Umbilicaria decussata*) and 0.66 ± 0.07 (*Pleopsidium chlorophanum*), respectively. No significant difference of the F_v/F_m values of these two lichens could

Fig. 1 Microclimate of an *Umbilicaria decussata* site: temperatures. *X* axis represents the time; **a** whole measurement period from January 4 to February 3, 2010; **b** detail (January 16/17). Snow coverage of the site occurred from January 22 to the end of the measurement. The sensor for air temperature remained snow free
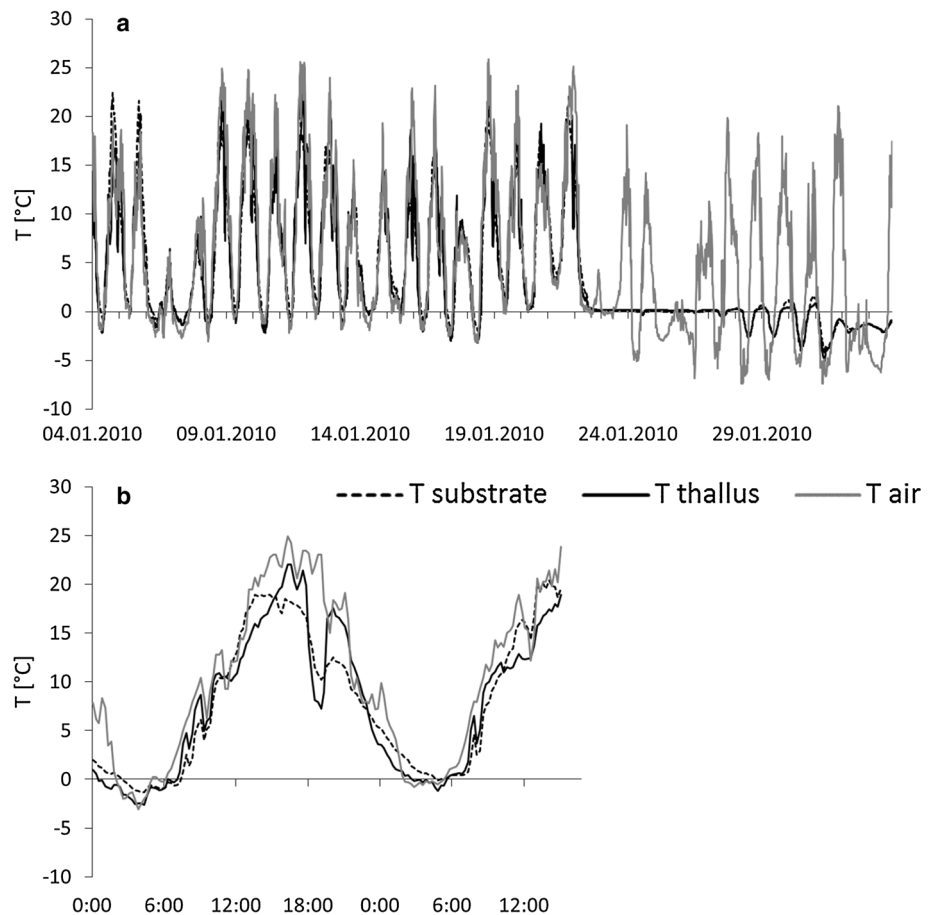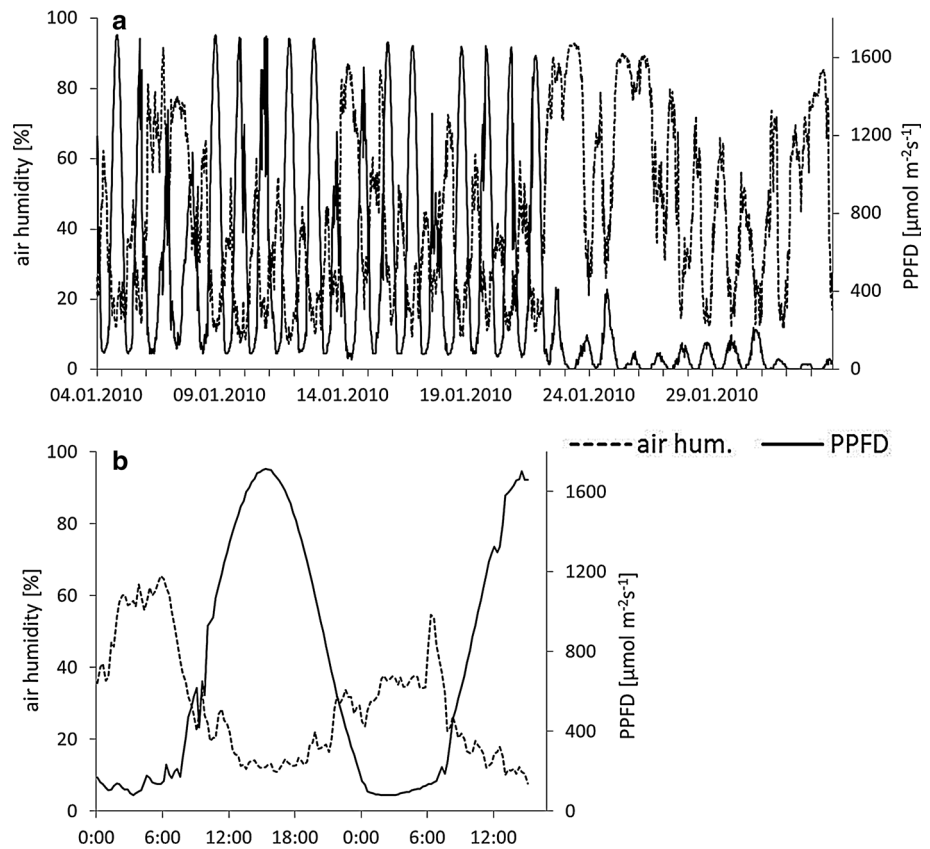
Fig. 2 Microclimate of an *Umbilicaria decussata* site: relative air humidity and photosynthetically active photon flux density (PPFD). *X* axis represents the time; **a** whole measurement period from January 4 to February 3, 2010; **b** detail (January 16/17). Snow coverage of the site occurred from January 22 to the end of the measurement

be detected directly after the reactivation procedure (unpaired two-sided *t* test, $p = 0.19$, $\alpha = 0.05$).

The reaction of reactivated *U. decussata* thalli to continuous illumination at 4, 12 and 20 °C is shown in Fig. 3, Tables 3 and 4. Actual quantum yield dF/F'_m reached a steady state after <5 min at any applied light intensity (20, 200 and 1000 µmol photons m^{-2} s^{-1}) or temperature except the combination of low temperature and low light intensity (4 °C and 20 µmol photons m^{-2} s^{-1}, Fig. 3a). Saturation of NPQ occurred more slowly. In particular, at 4 °C and 200 µmol photons m^{-2} s^{-1} (Fig. 3b), NPQ did not reach a steady state within the illumination time. By two-factor ANOVA, a significant effect of light intensity and temperature on the steady-state level of quantum yield was detected (Table 1). The effect of light was considerably greater than the effect of temperature.

No significant effect of temperature and light intensity on the regeneration potential of NPQ and PS II quantum yield was detected by two-factor ANOVA (Table 2). The dark relaxation time seemed to be sufficient to regenerate F_v/F_m at any condition applied, which is indicated by the lack of statistically significant differences (Tukey–Kramer post hoc test) between the initial F_v/F_m and the quantum yield values after the light treatment followed by 20 min of dark relaxation (Fig. 3).

The photosynthetic characteristics of the isolated photobionts (Fig. 4; Tables 3, 4) were more effected by the conditions applied as described above, compared to the entire lichen thalli, indicated by lower steady-state levels of dF/F'_m and higher NPQ levels during illumination. In particular, at moderate PPFD combined with 4/12 °C (Fig. 4b, d), and at high PPFD (Fig. 4e, h), NPQ exceeded the values obtained from the lichen thalli and was not saturated after 12 min of illumination. NPQ was not fully regenerated within 20 min at any applied condition except the combination of 20 °C and low light intensity.

By two-factor ANOVA, significant effects of temperature and light intensity variation could be detected, including a pronounced stronger effect of light intensity (Table 1). Additionally, the interactive effect of the two factors was significant.

The quantum yield of PS II reached the initial unstressed value after illumination at up to 200 µmol photons m^{-2} s^{-1} at 12 °C, while at lower temperatures, quantum yield of PS II was significantly reduced after illumination at 200 and 1000 µmol photons m^{-2} s^{-1} (Table 1; Fig. 4). After treatment at 20 °C and 200 µmol photons m^{-2} s^{-1} and a subsequent recovery, quantum yield was only slightly but significantly reduced from 0.57 ± 0.05 to 0.47 ± 0.19. Two-factor ANOVA (Table 2) indicated that only light
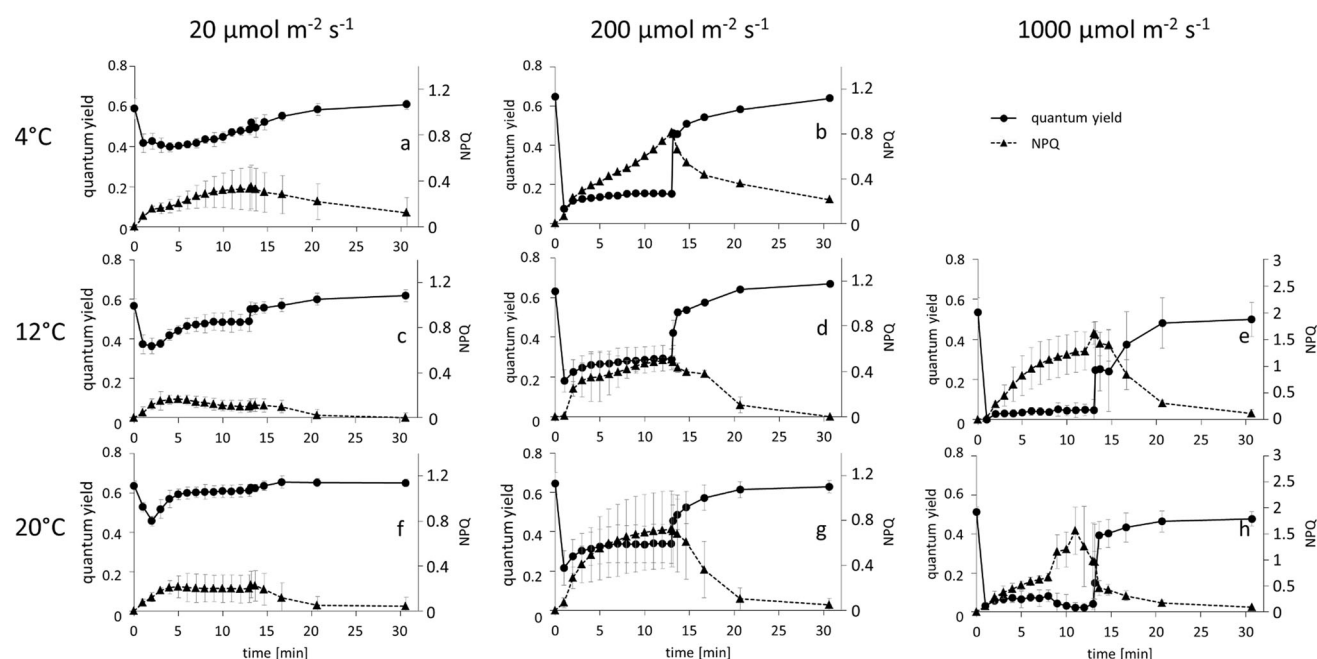
Fig. 3 Quantum yield of photosystem II and non-photochemical quenching (NPQ) derived from slow chlorophyll fluorescence induction in reactivated, dark-acclimatized entire *Umbilicaria decussata* thalli. Actinic light was switched on in minute 2 and switched off in minute 13. Applied temperatures: 4 °C (**a**, **b**); 12 °C (**c–e**); 20 °C (**f–h**). Applied light intensities (photosynthetically active photon flux density, PPFD): 20 μmol photons m^{-2} s^{-1} (**a**, **c**, **f**); 200 μmol photons m^{-2} s^{-1} (**b**, **d**, **g**); 1000 μmol photons m^{-2} s^{-1} (**e**, **h**). Values are presented as mean ± SD. Please note the higher NPQ scale in **e** and **h**

intensity, not the temperature, had a significant effect on dark recovered quantum yield values. By contrast, both factors as well as their interaction significantly affected the variation of NPQ recovery. Again, the light intensity contributed more to the parameter variation than temperature did.

Pigment analysis

NPQ of the photobionts was correlated with activation of the violaxanthin cycle as indicated by increased de-epoxidation state of the xanthophyll pool (DEPS) under moderate illumination (Fig. 5; Table 6). DEPS did not reach the relaxed initial state after 20 min of dark relaxation. By two-factor ANOVA, it could be shown that the illumination treatment and the photobiont origin (species level) both had a significant effect on the DEPS values. However, the contribution of light intensity was greater than that of photobiont species (Table 5). Furthermore, Tukey–Kramers post hoc test revealed that the photobiont of *Pleopsidium chlorophanum* showed significantly higher DEPS than the unstressed control after 5 min of irradiation. DEPS of the *Ubmilicaria decussata* photobiont was significantly elevated after 10 min (Fig. 5; Table 6).

Brownish thallus extracts of *Umbilicaria decussata* revealed absorption maxima of chlorophylls in the blue and red light, but also absorption due to other substances in the

blue light and UV spectrum (Fig. 6). In the DMSO extract, the spectrum is characterized by increasing absorption in the blue and UV region toward a maximum at c. 270 nm (Fig. 6a). Extracted compounds of the isolated mycobiont showed a similar spectrum in DMSO and a brown coloration. It could be shown by chemical testing (Table 7) that the absorbance increasing with lower wavelengths is due to melanin present in both the thallus and the cultivated mycobiont. A blue light-absorbing compound of the thallus and the isolated mycobiont could be detected in the acetone extract. Acetone pre-rinsed mycobiont material extracted in DMSO showed reduced absorbance in the blue and near UV spectrum. Acetone and methanol extracts (Fig. 6b, c) of the lichen and mycobiont showed absorption peaks at c. 320 nm, but no brown coloration.

Discussion

Pigmentation

Light-screening pigments are a diverse class of secondary lichen substances which are often located in the cortex (Dietz et al. 2000). Some are able to protect against excess visible (photosynthetically active radiation, PAR) light, but most are UVR screening substances (Solhaug et al. 2003). A known UVR screening compound in *Umbilicaria*

Table 1 Two-factor ANOVA of the effects of temperature and light intensity on the steady-state quantum yield and maximum NPQ values obtained in the light-acclimatized state of *Umbilicaria decussata* photobionts and entire lichen thalli

Factor	Sum of squares	Degrees of freedom	Mean square	F	p value	H_0 rejected?
Steady-state photobiont quantum yield						
Temperature	0.03	2	0.02	39.55	2.26E−11	Yes
Light	0.83	2	0.41	991.69	7.16E−44	Yes
Interaction	0.05	4	0.01	27.90	1.13E−12	Yes
Within	0.02	55	0.00			
Total	1.98	63	0.03			
Maximum photobiont NPQ						
Temperature	0.65	2	0.33	8.15	7.94E−04	Yes
Light	13.91	2	6.96	173.14	1.84E−24	Yes
Interaction	1.37	4	0.34	8.54	1.96E−05	Yes
Within	2.21	55	0.04			
Total	37.09	63	0.59			
Steady-state lichen quantum yield						
Temperature	0.05	2	0.02	7.94	2.39E−03	Yes
Light	0.55	2	0.28	92.57	9.97E−12	Yes
Interaction	0.03	4	0.01	2.51	6.94E−02	No
Within	0.07	23	0.00			
Total	1.43	31	0.05			
Maximum lichen NPQ						
Temperature	0.07	2	0.04	0.17	8.46E−01	No
Light	0.96	2	0.48	2.22	1.31E−01	No
Interaction	0.17	4	0.04	0.20	9.36E−01	No
Within	4.95	23	0.22			
Total	9.58	31	0.31			

Significance level $\alpha = 0.05$. Null hypothesis H_0: no significant effect of the factor

decussata thalli is gyrophoric acid (Øvstedal and Lewis Smith 2001). Fungal melanins absorb PAR and especially UVR. Melanic compounds are also widespread among lichens (Beckett et al. 2012). Melanin-pigmented lichen cortices can serve as effective barriers against different harmful effects of excess insolation (Nybakken et al. 2004; Meeßen et al. 2013). This study presents the first proof of melanin in *Umbilicaria decussata*. The pigment may contribute substantially to the protection of the photobiont when the metabolically active *U. decussata* thallus is exposed to sunlight. The Antarctic endemic lichen *Buellia frigida* may also profit from its protective melanin-pigmented cortex. The *B. frigida* mycobiont, as that of *U. decussata*, also produces melanin in the isolated and cultured state, and it has been hypothesized that this pigmentation may protect the development of new-formed fungal tissue in *B. frigida* from UV radiation (Meeßen et al. 2013).

Microclimate

Continuous variations in air, substrate as well as thallus temperature measured at microsites of *U. decussata* for 24 h for 4 weeks from below 0 °C until about 20 °C occurred (Fig. 1). The relatively high temperatures are accompanied by increasing light intensities at noon (Fig. 2) when the thallus in general is dry (Ott in prep.). Because of low air humidity (Fig. 2), no dew fall occurred. Schlensog et al. (2013) analyzed a variety of plants and cryptogams of Léonie Island, Antarctic Peninsula and southern maritime Antarctic. In its more xeric microhabitat on Léonie Island, Marguerite Bay, *U. decussata* was moistened by precipitation of rain and snow during the measuring period exclusively, resulting in an erratic activity pattern and the shortest total activity time of that study.

As a cosmopolitan lichen (Øvstedal and Lewis Smith 2001), a broad ecological amplitude may be beneficial for *U. decussata* at habitats in North Victoria Land. Romeike et al. (2002) compared the hydration-dependent physiological activities of endemic and cosmopolitan lichen species across the maritime Antarctic until Alexander Island south of the maritime Antarctic, Antarctic Peninsula. In general, cosmopolitan species displayed broader ecological amplitudes. In particular, the bipolar lichen *Stereocaulon alpinum* showed adaptation toward high light intensity combined with high thallus humidity. As *U.*

Table 2 Two-factor ANOVA of the effects of temperature and light intensity on the recovered quantum yield and NPQ values obtained after light-acclimation and subsequent dark relaxation for 20 min of *Umbilicaria decussata* photobionts and entire lichen thalli

Factor	Sum of squares	Degrees of freedom	Mean square	F	p value	H_0 rejected?
Recovered photobiont quantum yield						
Temperature	0.00	2	0.00	0.01	9.88E−01	No
Light	0.12	2	0.06	12.13	4.32E−05	Yes
Interaction	0.05	4	0.01	2.27	7.38E−02	No
Within	0.28	55	0.01			
Total	1.56	63	0.02			
Recovered photobiont NPQ						
Temperature	0.23	2	0.12	29.66	1.83E−09	Yes
Light	0.64	2	0.32	81.36	3.69E−17	Yes
Interaction	0.93	4	0.23	59.11	2.83E−19	Yes
Within	0.22	55	0.00			
Total	8.94	63	0.14			
Recovered lichen quantum yield						
Temperature	0.00	2	0.00	0.23	7.98E−01	No
Light	0.00	2	0.00	0.10	9.02E−01	No
Interaction	0.01	4	0.00	0.56	6.93E−01	No
Within	0.06	23	0.00			
Total	0.18	31	0.01			
Recovered lichen NPQ						
Temperature	0.01	2	0.00	0.81	4.59E−01	No
Light	0.00	2	0.00	0.03	9.71E−01	No
Interaction	0.00	4	0.00	0.14	9.67E−01	No
Within	0.09	23	0.00			
Total	0.28	31	0.01			

Significance level $\alpha = 0.05$. Null hypothesis H_0: no significant effect of the factor

decussata, *S. alpinum* grows at cold, alpine sites in the northern hemisphere (Øvstedal and Lewis Smith 2001) and was found in meltwater-fed Antarctic sites. The similar distribution patterns of these two lichen species may cause similar adaption mechanisms on physiology.

Light energy conversion

Excess light energy can lead to the production of cytotoxic reactive oxygen species (ROS) in photosynthetic cells. Lichens can avoid excess light by anabiosis or by shading of the photobionts by a well-developed optically dense cortex. On the other hand, many lichens are highly susceptible to photoinhibition in the dry state. Susceptibility to photoinhibition is especially relevant during the drying process (Gauslaa et al. 2012). In the dry state, dissipation of excess energy can be induced by conformational changes of the photosynthetic apparatus (Heber 2008). As long dry periods with strong insolation may occur at Antarctic habitats, light energy quenching in the dry state may be crucial for lichen survival. While this mechanism can be very effective and is independent of pigment conversions, active photobiont cells can also tolerate excess light by

xanthophyll-based NPQ reactions. Additionally, evidence for pigment-independent chlorophyll fluorescence quenching has been found in desiccated Antarctic lichen photobionts (Sadowsky and Ott 2012). In the illumination experiments concerning isolated and symbiotic photobionts (Figs. 3, 4), NPQ was more rapidly induced at higher temperatures (12 and 20 °C). In particular, in the isolated photobionts, NPQ reached significantly higher values when the temperature was higher at any given light intensity.

Reduction in photosynthetic capacity (photoinhibition) by light stress occurs especially at low temperatures. Active NPQ prevents the photosystem from oxidative damage and avoids long-term (chronic) photoinhibition (Schlensog et al. 2003). The isolated photobionts of *Umbilicaria decussata* (Fig. 4) displayed chronic photoinhibition (detected as incomplete recovery of F_v/F_m) at low temperature (4 °C), even if combined with low or moderate light intensity (20 and 200 µmol photons m^{-2} s^{-1}, respectively). At 12 and 20 °C, chronic photoinhibition could only be observed under high light conditions (1000 µmol photons m^{-2} s^{-1}), despite a minor reduction in quantum yield at 20 °C and 200 µmol photons m^{-2} s^{-1} (Fig. 4). Consequently, it can be hypothesized that the NPQ-based excess
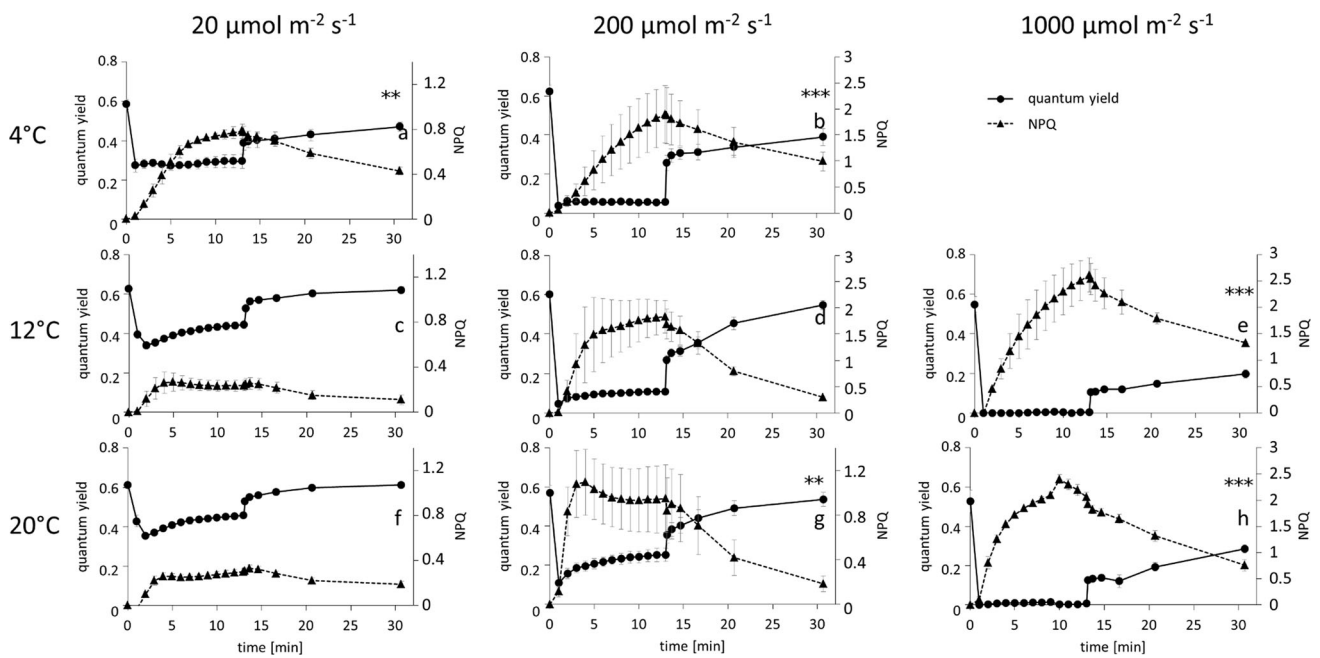
Fig. 4 Quantum yield of photosystem II and non-photochemical quenching (NPQ) derived from slow chlorophyll fluorescence induction in dark-acclimatized isolated, cultured *Umbilicaria decussata* photobionts. Actinic light was switched on in minute 2 and switched off in minute 13. Applied temperatures: 4 °C (**a, b**); 12 °C (**c–e**); 20 °C (**f–h**). Applied light intensities (photosynthetically active photon flux density, PPFD): 20 μmol photons $m^{-2} s^{-1}$ (**a, c, f**); 200 μmol photons $m^{-2} s^{-1}$ (**b, d, g**); 1000 μmol photons $m^{-2} s^{-1}$ (**e, h**). Values are presented as mean ± SD. Please note the higher NPQ scale in **b, d, e** and **h**. *Asterisks* indicate significant differences (Tukey–Kramer multiple comparisons test) between recovered and unstressed quantum yield values: **$p < 0.01$; ***$p < 0.001$

Table 3 Non-photochemical quenching and photosystem II quantum yield of *Umbilicaria decussata* photobionts and entire lichen thalli under three different temperature conditions

	Dark	20 μmol $m^{-2} s^{-1}$	200 μmol $m^{-2} s^{-1}$	1000 μmol $m^{-2} s^{-1}$
Steady-state photobiont quantum yield				
4 °C	0.61 ± 0.03	0.30 ± 0.04	0.05 ± 0.01	
12 °C	0.59 ± 0.07	0.45 ± 0.02	0.11 ± 0.01	0.00 ± 0.00
20 °C	0.57 ± 0.05	0.46 ± 0.01	0.25 ± 0.03	0.01 ± 0.00
Maximum photobiont NPQ				
4 °C		0.81 ± 0.05	2.00 ± 0.38	
12 °C		0.24 ± 0.05	1.64 ± 0.21	2.14 ± 0.17
20 °C		0.30 ± 0.02	0.95 ± 0.30	2.07 ± 0.05
Steady-state lichen quantum yield				
4 °C	0.64 ± 0.02	0.49 ± 0.02	0.15 ± 0.03	
12 °C	0.55 ± 0.07	0.49 ± 0.05	0.20 ± 0.06	0.05 ± 0.05
20 °C	0.60 ± 0.07	0.63 ± 0.04	0.34 ± 0.10	0.04 ± 0.03
Maximum lichen NPQ				
4 °C		0.44 ± 0.01	0.81 ± 0.35	
12 °C		0.10 ± 0.05	0.41 ± 0.55	1.21 ± 0.82
20 °C		0.21 ± 0.10	0.72 ± 0.34	1.11 ± 0.65

Maximum quantum yield (F_v/F_m) was measured before light treatment in dark-acclimatized thalli, while steady-state quantum yield and maximum non-photochemical quenching (NPQ) were determined after acclimation to the indicated light/temperature conditions. Values are presented as mean ± SD

Table 4 Non-photochemical quenching and photosystem II quantum yield of *Umbilicaria decussata* photobionts as well as entire lichen thalli under three different temperature conditions

	20 µmol m^{-2} s^{-1}	200 µmol m^{-2} s^{-1}	1000 µmol m^{-2} s^{-1}
Recovered photobiont quantum yield			
4 °C	0.47 ± 0.02	0.39 ± 0.03	
12 °C	0.62 ± 0.01	0.55 ± 0.02	0.20 ± 0.02
20 °C	0.61 ± 0.00	0.47 ± 0.19	0.28 ± 0.02
Recovered photobiont NPQ			
4 °C	0.44 ± 0.03	0.97 ± 0.12	
12 °C	0.11 ± 0.05	0.28 ± 0.02	1.12 ± 0.00
20 °C	0.18 ± 0.02	0.16 ± 0.09	0.75 ± 0.07
Recovered lichen quantum yield			
4 °C	0.62 ± 0.01	0.64 ± 0.04	
12 °C	0.62 ± 0.03	0.60 ± 0.09	0.50 ± 0.09
20 °C	0.65 ± 0.01	0.63 ± 0.03	0.48 ± 0.04
Recovered lichen NPQ			
4 °C	0.20 ± 0.01	0.22 ± 0.12	
12 °C	0.00 ± 0.00	0.00 ± 0.00	0.09 ± 0.08
20 °C	0.04 ± 0.06	0.05 ± 0.06	0.09 ± 0.01

Measurements were taken after dark relaxation following the indicated illumination. Values are presented as mean ± SD
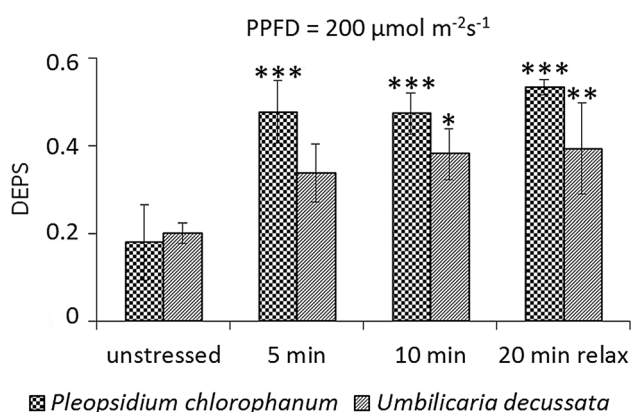

Fig. 5 De-epoxidation state of the xanthophyll pool (DEPS) of isolated photobionts of *Pleopsidium chlorophanum* and *Umbilicaria decussata* at 12 °C. The *first pair of bars* represents the unstressed DEPS of dark-acclimatized photobionts. The *second and third pair of bars* represent the DEPS values of photobionts illuminated at 200 µmol photons m^{-2} s^{-1} for 5 and 10 min, respectively. The last pair of values shows the DEPS after 10 min of illumination, followed by 20 min of dark acclimation. Values are presented as mean ± standard deviation. *Asterisks* indicate significant differences (Tukey–Kramer multiple comparisons test) from values obtained from the respective unstressed photobionts: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

light protection of the isolated *U. decussata* photobiont can effectively prevent photodamage at temperatures of 12 or 20 °C combined with a light intensity which exceeds the cultivation PPFD (at 20 µmol photons m^{-2} s^{-1}) tenfold. A PPFD of 200 µmol photons m^{-2} s^{-1} is in the range of maximum PPFD detected under snow cover in the

measuring period. NPQ can be correlated with the de-epoxidation of the xanthophyll violaxanthin to zeaxanthin which quenches excess excitation energy from photosystem II (Havaux and Niyogi 1999).

The *P. chlorophanum* entire lichen thallus, unlike the *U. decussata* thallus (Meeßen et al. 2013, Fig. 6; Table 6), lacks melanin as a photoprotective metabolite. *P. chlorophanum* contains the UVR protective SLC rhizocarpic acid. Rhizocarpic acid shows little absorption of photosynthetically relevant wavelengths and can thus not be regarded as a protectant against excess PPFD (Hidalgo et al. 2002; Meeßen et al. 2013). Based on its pigmentation, the light-absorbing capacity of the *P. chlorophanum* thallus can be regarded as minor compared to *U. decussata*. The photobionts of *U. decussata* and of *Pleopsidium chlorophanum* belong to clade S *Trebouxia*. Despite the close phylogenetic position and despite the identical culture and experimental conditions of both isolated photobionts, the *P. chlorophanum* photobiont displayed faster activation of xanthophyll de-epoxidation. The photobiont originating from a less shady intrathalline environment may rely on more effective own photoprotection. The xanthophyll-based NPQ can be such a mechanism. Based on the NPQ-relevant xanthophyll composition of the isolated photobionts after illumination (Fig. 5), it can be concluded that the strategy of photoprotection was retained in the isolated, cultured state of the photobionts.

The entire lichen thalli of the study displayed no chronic photoinhibition at any light/temperature condition applied (Fig. 3). Barták et al. (2008) have demonstrated that in the umbilicate lichen *Lasallia pustulata* from a more temperate

Table 5 Two-factor ANOVA of the effect of light/dark treatment and photobiont origin (species) on the DEPS values

Factor	Sum of squares	Degrees of freedom	Mean square	F	p value	H$_0$ rejected?
Treatment	0.37	3	0.12	28.72	4.12E−08	Yes
Species	0.06	1	0.06	14.48	8.60E−04	Yes
Interaction	0.03	3	0.01	2.68	6.93E−02	No
Within	0.10	24	0.00			
Total	0.56	31	0.02			

Photobionts originate from the lichens *Umbilicaria decussata* and *Pleopsidium chlorophanum*. Significance level $\alpha = 0.05$. Null hypothesis H$_0$: no significant effect of the factor

Table 6 DEPS values of isolated photobionts after light treatment (200 µmol m^{-2} s^{-1})

	U. decussata	*P. chlorophanum*
Unstressed	0.20 ± 0.02	0.18 ± 0.02
5 min light	0.34 ± 0.07	0.48 ± 0.07
10 min light	0.38 ± 0.05	0.47 ± 0.06
20 min recovery	0.39 ± 0.02	0.53 ± 0.10

Photobionts originate from the lichens *Umbilicaria decussata* and *Pleopsidium chlorophanum*. Values are presented as mean $\pm$ SD

European site, photosynthetic processes were less influenced by light intensity than by the duration of irradiation. Laboratory experiments of this study indicated efficient use of light energy by moist, active *Umbilicaria decussata* thalli even at high PPFD and low temperatures, compared to the isolated photobiont. This is consistent with findings concerning lichens from across polar regions which show considerable adaptation to low temperatures (Kappen and Lange 1970, 1972). In the isolated state, the *U. decussata* photobiont, among other analyzed *Trebouxia* photobionts from various lichen species, even tolerates long-term freezing without taking serious damage to its photosynthetic capacity (Sadowsky and Ott 2012). NPQ was induced to a lesser extent in the entire *U. decussata* thalli, compared to the isolated photobionts. This may be due to reduced light intensity in the intrathalline algal layer of the lichen, especially caused by melanin pigmentation of the cortex. As the presented experiments show, the examined isolated photobionts are more likely stressed by excess light than by variations in temperature. Therefore, effective sun screening by the mycobiont can be a key feature of successful lichen colonization at continental Antarctic sites.
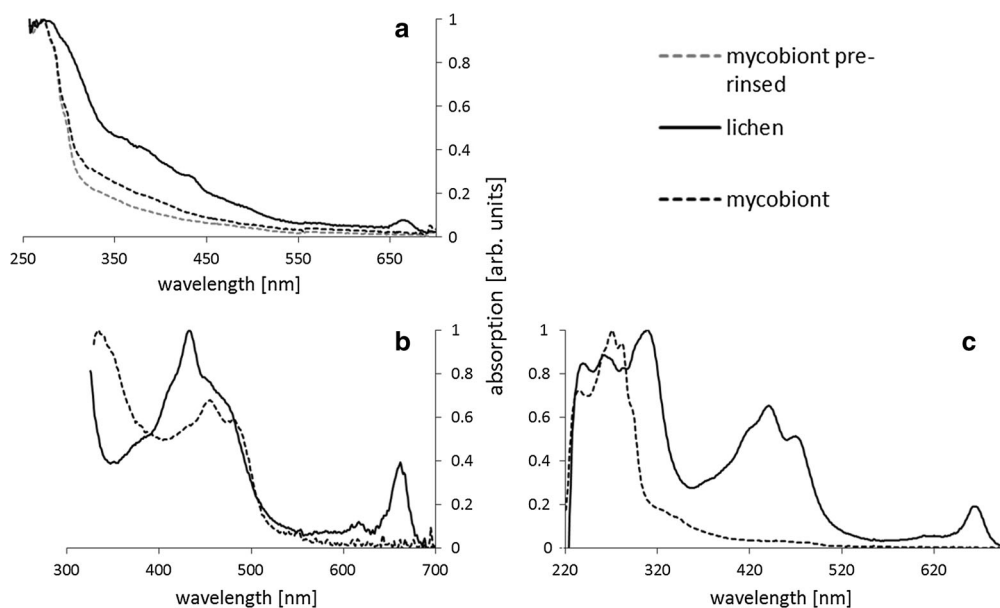
Fig. 6 Absorption spectra of *Umbilicaria decussata* lichen thallus and mycobiont extracts in different solvents. Pre-rinsed mycobiont material was rinsed with acetone prior to the DMSO extraction. Solvents are: **a** DMSO; **b** methanol; **c** acetone. Values are presented as arbitrary units, giving the highest absorption the value 1

Table 7 Chemical characteristics of synthetic melanin compared to acetone pre-rinsed (except test for solubility in acetone) mycobiont and thallus of the lichen *Umbilicaria decussata*

Chemical test	Synthetic	*Umbilicaria decussata*	
	Melanin >97 %	Thallus	Mycobiont
Solubility in			
Water	−	−	−
Methanol	−	−	−
Ethanol	−	−	−
Acetone	−	−	−
Hexane	−	−	−
Ethyl acetate	−	−	−
Toluene	−	−	−
Chloroform	−	−	−
DMSO	+	+	+
0.2 N NaOH	+	+	+
0.2 N NH$_4$OH	+	+	+
Precipitation by			
Alkaline FeCl$_3$	+	+	+
Bleaching in			
H$_2$O$_2$	+	+	+
NaOCl	+	+	+

Positive/negative results are indicated by +/−

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