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Effects of $UVC_{254\,nm}$ on the photosynthetic activity of photobionts from the astrobiologically relevant lichens *Buellia frigida* and *Circinaria gyrosa*

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Abstract: In the past decade, various astrobiological studies on different lichen species investigated the impairment of viability and photosynthetic activity by exposure to simulated or real space parameters (as vacuum, polychromatic ultraviolet (UV)-radiation and monochromatic UVC) and consistently found high post-exposure viability as well as low rates of photosynthetic impairment (de Vera *et al.* 2003, 2004a; 2004b; de la Torre *et al.* 2010; Onofri *et al.* 2012; Sánchez *et al.* 2012, 2014; Brandt *et al.* 2014). To achieve a better understanding of the basic mechanisms of resistance, the present study subdued isolated and metabolically active photobionts of two astrobiologically relevant lichens to $UVC_{254\,nm}$, examined its effect on photosynthetic activity by chlorophyll *a* fluorescence and characterized the UVC-induced damages by quantum yield reduction and measurements of non-photochemical quenching. The results indicate a strong impairment of photosynthetic activity, photoprotective mechanisms and overall photobiont vitality when being irradiated in the isolated and metabolically active state. In conclusion, the present study stresses the higher susceptibility of photobionts towards extreme environmental conditions as UVC-exposure, a stressor that does not occur on the Earth. By comparison with previous studies, the present results highlight the importance of protective mechanisms in lichens, such as morphological–anatomical traits (Meeßen *et al.* 2013), secondary lichen compounds (Meeßen *et al.* 2014) and the symbiont's pivotal ability to pass into anhydrobiosis when desiccating.

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Introduction

Lichens represent a unique and evolutionary successful mode of life. These symbiotic associations colonize harsh ecological niches and tolerate extreme environmental conditions (Kappen 1988), including high levels of photosynthetically active radiation (PAR) from 400 to 700 nm and ultraviolet radiation (UVR) from 200 to 400 nm (Lange 1992; Nybakken *et al.* 2004), infrequent water supply, extreme drought, heat and cold (Kappen 1993). Such conditions are found in alpine, polar and arid habitats (Lange 1992) as the Antarctic Dry Valleys (Onofri *et al.* 2007; Sun *et al.* 2010) or the Atacama Desert (McKay *et al.* 2003). Their key to success is the symbiotic nature formed by at least two organisms, a heterotrophic mycobiont and a photoautotrophic photobiont which is a eukaryotic green alga or a cyanobacterium. Lichen thalli represent sophisticated structures and confer physiological features none of the isolated symbionts reveals. The adaptive potential of lichens to extreme environments constitutes the interest of astrobiologists in this peculiar symbiosis (de Vera *et al.* 2010) that consequently became a model for astrobiology (Sancho *et al.* 2008). Substantial progress was made to study their resistance towards extraterrestrial environments, including simulations of space vacuum, Mars atmosphere and UVR (de Vera *et al.* 2003, 2004a, b, 2008, 2010; de la Torre *et al.* 2007, 2010; de Vera & Ott 2010; Sánchez *et al.* 2012), of meteorite impacts (Stöffler *et al.* 2007; Horneck *et al.* 2008), but also in space missions as LICHENS II, LITHOPANSPERMIA and STONE on BIOPAN 5/6, and LIFE on EXPOSE-E (de la Torre *et al.* 2007, 2010; Sancho *et al.* 2007; Raggio *et al.* 2011; Onofri *et al.* 2012; Scalzi *et al.* 2012; Brandt *et al.* 2014). Encouraged by the high viability after space exposure, the current BIOMEX-mission will expose the lichens *Buellia frigida* and *Circinaria gyrosa* for 12–18 months to space and simulated Mars conditions in EXPOSE-R2 on the outside platform of the Russian service module Zvezda of the International Space Station (ISS).

Solar UVR is divided into three main spectral regions: UVA (400–320 nm), UVB (320–280 nm) and UVC (280–200 nm). UVR can destroy chemical bonds (Kovács & Keresztes 2002) and produce reactive oxygen species (ROS, Caldwell *et al.* 1998), causing cytotoxic, mutagenic and necrotic lesions

in organisms (Uchida *et al.* 2010; Yao *et al.* 2011). As the DNA action spectrum sharply increases in UVC (Sass *et al.* 1997), it is one of the most lethal factors in space and imposes a dramatic threat on life (Horneck 1999; Nicholson *et al.* 2005). For instance, Martian surface flux from 200 to 400 nm generates about a thousandfold more biologically effective DNA damage than terrestrial UVR surface flux (Cockell *et al.* 2000; Cockell 2014). UVB/UVC cause direct damage on DNA or indirect damage via ROS-induced oxidative stress (Horneck *et al.* 2006). Most effectively about 260 nm (Takeuchi *et al.* 1996), they induce various photoproducts as cyclobutane pyrimidine dimers and pyrimidine-(6,4)-pyrimidone dimers, as well as hydration of pyrimidines, base-pair deletions and insertions, DNA–protein crosslinks and DNA double-strand breaks (Strid *et al.* 1994; Britt 1999). Such lesions alter DNA structure, shift gene reading frames, lead to genomic instability and hinder replication (Vass *et al.* 2005; Yao *et al.* 2011). As aromatic amino acids strongly absorb at 280 nm (Vass *et al.* 2005), UVB and UVC not only destroy tyrosine, tryptophane and phenylalanine, but also split disulphide bridges (Nasibi & M'Kalantari 2005). These damages impair protein structure and enzyme activity as reported for photosynthetic enzymes such as Rubisco, ATP synthase and violaxanthin de-epoxidase (Nasibi & M'Kalantari 2005; Vass *et al.* 2005). Additionally, UVR destroys double bonds of unsaturated fatty acids, change the chemical properties of phospho- and glycolipids, and affect membrane integrity (Takeuchi *et al.* 1996).

In lichens, several strategies constitute extremotolerance (including UVR-resistance) and thus are of astrobiological interest. Three aspects are most important: (1) *Poikilohydry* allows lichens to tolerate desiccation in the ametabolic state of anhydrobiosis (Kranner *et al.* 2005) and also defies stressors accompanying desiccation as heat, cold and high levels of PAR and UVR (Nybakken *et al.* 2004). In astrobiology, the effect of poikilohydry is stressed by studies on UVC-resistance in anhydrobiotic and in rehydrated, metabolically active lichens (Sánchez *et al.* 2014). (2) *Thallus morphology* adapts lichens to their environment by a broad range of growth types and functional structures. (3) *Secondary lichen compounds* (SLCs) prevent oxidative stress, photoinhibition and biological damage (Solhaug & Gauslaa 2004; McEvoy *et al.* 2006) and play a crucial role in shielding the photobiont against excess PAR and UVR (Ertl 1951). As UVR-protection is most needed when repair mechanisms are inactive (i.e. when desiccated, Lange *et al.* 1999), SLCs are discussed as a key factor to resist the desiccating conditions of space vacuum and Martian atmosphere (de Vera *et al.* 2003, 2004a, b). With focus on astrobiology, the protective potentials of lichen morphology and of SLCs were recently addressed (Meeßen *et al.* 2013, 2014).

Besides the protective effect of SLCs on lichen photobionts, the knowledge on the adaptive potential of isolated photobionts towards abiotic stressors is scarce (Kranner *et al.* 2005; Kranner & Birtić 2005), especially when considering the light- and temperature dependency of the photosynthetic performance of photobionts from extreme habitats as Antarctica (Barták *et al.* 2007). Recent results indicate habitat-specific adaptations of isolated lichen photobionts towards sub-zero temperatures down to −25 °C. They also gave hint to prolonged retention of photosynthetic activity during the desiccation of the photobiont of the extremotolerant Antarctic endemite *B. frigida* (Sadowsky & Ott 2012). Moreover, desiccation and high irradiation are important sources of oxidative stress (Kranner *et al.* 2005). To avoid the formation of ROS in chloroplasts, non-photochemical quenching (NPQ) reduces excess excitation energy at the PS II (Fernández-Marín *et al.* 2010) by the mechanisms of photoinhibition, including active degradation of the D1 protein and zeaxanthin-driven thermal dissipation (Demming-Adams & Adams 1996; Krause & Jahns 2004).

Previous astrobiological studies showed that even within the thallus photobionts are more susceptible to simulated space and Mars conditions than mycobionts. The photobiont of *Peltigera aphthosa* from habitats moderately exposed to UVR and PAR is more susceptible to $UVC_{254\,nm}$ (0.02–2.02 J cm^{-2}) than those from highly exposed habitats (*B. frigida, Xanthoria elegans*; de Vera & Ott 2010). $UVC_{254\,nm}$ irradiation of *X. elegans* in doses of 5, 10 and 20 kJ m^{-2} induced dose-correlated photoproduct formation in isolated photobionts (≤90 per 10^4 bp) and intact thalli (≤9 per 10^4 bp), whereas photoproducts were not found in the isolated mycobiont (de Vera 2005). Recently, the effect of a $UVC_{200-280\,nm}$ (up to a dose analogue of 67 days in low earth orbit (LEO)) was tested on the photosynthetic activity of the space-tested photobionts of *C. gyrosa* and *Rhizocarpon geographicum* in metabolically active and anhydrobiotic thalli. Both photobionts were proofed to be more resistant in desiccated thalli although the photobiont of *Circinaria gyrosa* (*C. gyrosa*-PB) is more resistant (Sánchez *et al.* 2014).

Based on the results presented above, it is consequent to assess the effect of abiotic stressors on the isolated photobiont and to determine its inherent potential of resisting extreme environmental conditions. In the present study, we tested the photobionts' capacity to protect its photosynthetic apparatus during UVC-exposure and to regenerate afterwards. $UVC_{254\,nm}$-radiation was used for exposure, since it causes direct damage on photosynthesis (Takeuchi *et al.* 1996; Sass *et al.* 1997) and is known as a harmful stressor that is not found on the Earth (Vass *et al.* 2005) but characteristically contributes to space and Martian surface environments (Horneck 1999). We chose the isolated photobionts of *C. gyrosa* and *B. frigida*, which revealed high rates of post-exposure viability in previous astrobiological experiments (de Vera & Ott 2010; Raggio *et al.* 2011; Sánchez *et al.* 2012, 2014; Meeßen *et al.* 2013, 2014) and are also part of the current BIOMEX mission. Both photobionts were irradiated with increasing doses of $UVC_{254\,nm}$ up to 41.7 J cm^{-2} and subsequently allowed to recover for up to 240 h. Its effect on photosynthetic activity was characterized by chlorophyll *a* fluorescence as maximum quantum yield (QY) and induction kinetics. Chlorophyll *a* fluorescence is considered a good indicator of photosynthetic activity (Lüttge & Büdel 2010) and the effect of environmental stresses on photosynthesis (Krause & Jahns 2004) and thus it is widely used to determine the viability of photosynthetic

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organisms after (simulated) space and Mars exposure experiments (de la Torre *et al.* 2007, 2010; de Vera *et al.* 2008, 2010; de Vera & Ott 2010; Raggio *et al.* 2011; Onofri *et al.* 2012; Sánchez *et al.* 2012, 2014; Brandt *et al.* 2014). Since wavelengths below 290 nm (basically UVC) do not reach the surface of the Earth (Takeuchi *et al.* 1996; Vass *et al.* 2005), detailed information on the effect of UVC on photosynthesis is scarce. Therefore the present results may form a valuable basis for further investigations.

Material and methods

Material

Buellia frigida Darb. (1910) is an endemic, crustose lichen frequently colonizing maritime and continental Antarctic habitats. This species preferentially grows on rocks being fully exposed to wind, low temperatures and high irradiation levels during Antarctic summer. It occurs in latitudes down to 84°S and in altitudes up to 2015 m a.s.l. (Øvstedal & Lewis Smith 2001). *B. frigida* samples were collected by S. Ott in 2009/2010 in the vicinity of Gondwana Station at Gerlache Inlet, North Victoria Land (74°38′S, 164°13′E). Dried samples were stored at −25 °C until photobiont isolation. Based on non-coding internally transcribed spacer (ITS) sequences its photobiont was identified as the coccal green alga *Trebouxia* sp. clade S (also referred to *Trebouxia jamesii*; Sadowsky & Ott 2012).

Circinaria gyrosa Sohrabi (2012) was recently revised from *Aspicilia fruticulosa* (Sohrabi 2012). It originates from continental deserts and arid areas of Eurasia, Northern Africa, Middle Asia and North America. *C. gyrosa* is adapted to harsh abiotic conditions, such as heat, drought and high levels of solar UVR (Sancho *et al.* 2000). Samples were collected in 2010 from clay soils in high basins of Central Spain, Guadalajara, Zaorejas, 1260 m a.s.l. (40°45′40″N, 02°12′08″E). The samples were collected and kept in dark and dry conditions until photobiont isolation. In the present study, the *C. gyrosa*-PB was identified by ITS sequences as *Trebouxia* sp.

Methods

Isolation and cultivation

Cell clusters of both lichens' photobionts were isolated from thallus sections according to the method of Yoshimura *et al.* (2002), pre-cultured on solid *Trebouxia* organic medium (TOM, Ahmadjian 1967) for about 2 months at 12 °C under a 14 h daytime photosynthetic photon flux density (PPFD) of 20 µmol m^{-2} s^{-1} to increase biomass and assure purity (Rubarth Apparate GmbH, Germany). Finally, the photobiont cells were transferred to 75 ml of liquid TOM for further cultivation. The cultures were shaken at 85 rpm for 6 weeks at 12 °C under 12 h daytime PPFD of 15–25 µmol m^{-2} s^{-1}.

Identification of the C. gyrosa*-PB*

For ITS sequence-based identification of the photobiont (as described in Romeike *et al.* 2002), genomic DNA was isolated from 100 mg of fresh algae by homogenization (cooled mortar and pistill under N_2) and the DNeasy® Plant Mini Kit (according to the manufacturer's instructions, QIAGEN GmbH). The genomic DNA was checked on quality and quantity by NanoDrop® ND-2000 (Peqlab GmbH) and the internal transcribed spacer regions 1 and 2 were amplified by PCR with the KOD Hot Start High Fidelity DNA Polymerase Kit, 5% (v/v) dimethyl sulfoxide and cycling conditions suited for a target size of 500–1000 bp (according to the manufacturer's protocol, Novagen GmbH). The primers used were Al1500bf and LR3. Amplified DNA targets were purified with the QIAquick® Purification Kit (QIAGEN GmbH) and sequenced at GATC GmbH. The resulting sequences were identified by BLASTN and BLASTX searches at the NCBI database (http://blast.ncbi.nlm.nih.gov/) as *Trebouxia* sp.

Irradiation and photosynthetic activity measurement

Sample preparation: For every irradiation dose with UVC, 1.00, 0.67, 0.33 and 0.10 ml of homogeneous and comparably dense photobiont culture suspension (with about 12.0×10^6 and 10.7×10^6 cells ml^{-1} for the photobionts of *B. frigida* and *C. gyrosa*, respectively) were transferred to the surface of sterilized polyvinyldene difluoride membrane filters of c. 1 cm^2 (Durapore®, Millipore, Germany, pore size of 0.44 µm), which were located on TOM-agar plates with 18 replicates for each set-up. The algal cells were kept overnight at room temperature (RT) to acclimatize and subsequently irradiated.

Irradiation with UVC: Since drought preconditions cells to UVR-stress (Nasibi & M'Kalantari 2005; Vass *et al.* 2005) and PAR ameliorates the effects of UVR (Strid *et al.* 1994), the exposure conditions were designed to be permanently wet and non-irradiated with PAR. The UVC-irradiation was performed in an air circulation cabinet at RT (Mühlenkamp Reinraumtechnik, Germany) equipped with a HNS 30W G13 G30T8/OF UVC lamp (Puritec®, Osram, Germany, >93% emission at 254 nm, irradiance of 1.1 W m^{-2} at 1 m). The $UVC_{254\,nm}$ irradiation under the clean bench ranged between 455 and 487 µW cm^{-2} after 20 min pre-run (UVP UVX dosimeter, sensor 25, λ = 254 nm).

Doses of UVC and recovery periods

For testing the dose-dependent effect of UVC on the photobionts' photosystem II (PS II) irradiation periods of 0.25, 0.5, 1, 2, 3, 4, 5, 12 and 24 h were chosen besides a non-irradiated control. These periods represent accumulated $UVC_{254\,nm}$ doses of 0.43, 0.87, 1.7, 3.5, 5.2, 6.9, 8.7, 20.8 and 41.7 J cm^{-2}. To prevent drought stress during irradiation the samples were kept wet by frequent supply with sterilized tab water. The measurements of chlorophyll *a* fluorescence (see below) were performed directly before and after the irradiation period as well as after recovery periods of 0, 1, 2, 24, 48, 120 and 240 h. Light induction curves (LICs) were measured after $UVC_{254\,nm}$ doses of 1.7, 8.7 and 41.7 J cm^{-2} (corresponding to 1, 5 and 24 h) and subsequent recovery periods of 0, 24, 48, 120 and 240 h. All recovery periods were performed under normal cultivation conditions as described before.

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Chlorophyll *a* fluorescence measurements

The pre- and post-irradiation photosynthetic performance of PS II was assessed by chlorophyll *a* fluorescence measurements. The activity of PS II of each sample was determined by a Mini-pulse-amplitude-modulated (PAM) fluorometer (Heinz Walz Mess- und Regeltechnik GmbH, Germany) according to Maxwell & Johnson (2000). Maximum quantum yield ($QY_{(Fv/Fm)}$) of PS II was calculated as $F_v/F_m = (F_m - F_0)/F_m$ with F_v is the variable fluorescence yield, F_m is the maximal fluorescence yield and $F_0$is the minimal fluorescence yield (Schreiber *et al.* 1994). F_v/F_m was measured at the photobiont samples by application of a saturating light pulse (c. 5000 µmol photons $m^{-2}\,s^{-1}$) to dark acclimatized samples. The comparison of normalized pre- and post-irradiation $QY_{(Fv/Fm)}$ allowed determining the dose-dependent impact of UVC on the photosynthetic performance of both lichen photobionts and its recovery processes.

By measuring LICs via slow chlorophyll *a* fluorescence of PS II, photochemical and non-photochemical light energy quenching (NPQ) can be observed in the dark-acclimatized state, during light acclimation and subsequent dark relaxation (Roháček 2002). LICs were performed with both photobionts before and directly after $UVC_{254\,nm}$ irradiation, as well as after recovery periods of 24, 48, 120 and 240 h. The UVC doses were 1.7, 8.7 and 41.7 J cm^{-2} and the volume of applied algal suspension was 0.33 ml. For each LIC, an initial measurement in a dark acclimatized sample is followed by 7 min of actinic light exposure (11 µmol photons $m^{-2}\,s^{-1}$) and a subsequent relaxation phase of additional 18 min in the dark. Data of 8.7 J cm^{-2} and 48 h of recovery were not presented in this study.

Results

Dose- and recovery-dependent QY measurements

Photobiont of Buellia frigida (B. frigida-PB)

With all four volumes of applied *B. frigida*-PB the maximum quantum yield $QY_{(Fv/Fm)}$ of the PS II decreases with increasing $UVC_{254\,nm}$-exposure for doses between 0.43 and 5.2 J cm^{-2} (corresponding to irradiation periods of 15 min to 3 h, Fig. 1(a)–(d)). For UVC-doses of 6.9–41.7 J cm^{-2} (4–24 h of irradiation) this dose-dependent correlation is not given. For all applied algal suspension volumes, the $QY_{(Fv/Fm)}$ does not decrease with doses ≥5.2 J cm^{-2} (Fig. 1(a), (c) and (d)) and the dose–effect correlation is not observed at UVC-doses beyond 6.9 J cm^{-2}.

The thickness of the applied algal layer was c. 0.25 mm in case of 1.00 ml application volume, corresponding to 10–12 layers of algal cells with an average diameter of 20–25 µm. For algal suspensions of 0.10 ml per filter the applied algal layer is thinnest. Consequently, the mutual shading effect of algal cells is least and the effect of UVC on the photosynthetic activity of PS II is most pronounced (Fig. 1(a)). Immediately after irradiation with UVC-doses from 0.43 to 41.7 J cm^{-2} the maximum QY is reduced from 74 to 3% of the pre-exposure control $QY_{(Fv/Fm)}$. With higher algal suspension volumes the $QY_{(Fv/Fm)}$ is less reduced. When 0.33 ml of algal suspension is applied the average $QY_{(Fv/Fm)}$ for doses between 5.2 and 41.7 J cm^{-2} is c. 42% of the control value. Under these conditions a minimum of 36% is reached after experiencing 5.2 J cm^{-2}. After the highest dose (41.7 J cm^{-2}, Fig. 1(b)) the $QY_{(Fv/Fm)}$ is reduced to c. 46%. At samples with 0.67 and 1.00 ml of applied suspension the average $QY_{(Fv/Fm)}$ for doses ≥5.2 J cm^{-2} is c. 54%. Both $QY_{(Fv/Fm)}$ minima are c. 52% at 8.7 J cm^{-2} and c. 55% after 41.7 J cm^{-2} (Fig. 1(c)–(d)).

The post-irradiation recovery of the PS II was assessed by measuring the $QY_{(Fv/Fm)}$ after 1, 2, 24, 48, 120 and 240 h (Fig. 1 (a)–(d)) of cultivation under favourable growth conditions (as described for photobiont culture). After irradiation with 0.43 and 0.87 J cm^{-2} the $QY_{(Fv/Fm)}$ decreases remarkably in the recovery period between 48 and 240 h. With 0.33, 0.67 and 1.00 ml of photobiont suspension the $QY_{(Fv/Fm)}$ consistently drops by c. 30% (Fig. 1(b)–(d)). In case of 0.10 ml applied suspension, the decrease of $QY_{(Fv/Fm)}$ is even more distinctive (Fig. 1(a)). The decrease starts after 24 h and drops by c. 50% of the pre-exposure control value and it also occurs after a UVC-dose of 1.7 J cm^{-2}. At 0.67 and 1.00 ml applied algal suspension the $QY_{(Fv/Fm)}$ drops even below the respective $QY_{(Fv/Fm)}$ values of the highest dose. Moreover, the higher suspension volumes of 0.67 and 1.00 ml reveal an insignificant but consistent regeneration of $QY_{(Fv/Fm)}$ by c. 10% for most irradiation doses ≥5.2 J cm^{-2} within 240 h of post-irradiation cultivation (Fig. 1(c) and (d)).

Photobiont of Circinaria gyrosa

The results with the *C. gyrosa*-PB are comparable with those of the *B. frigida*-PB although some minor differences are observed. In general, the post-exposure $QY_{(Fv/Fm)}$ of the *C. gyrosa*-PB also decreases in parallel to the applied UVC-dose ≤3.5 J cm^{-2} (compare Fig. 1(a)–(d) with 1(e)–(h)). As in the *B. frigida*-PB, a significant dose-dependent effect on $QY_{(Fv/Fm)}$ is not observed for doses ≥5.2 J cm^{-2}. However, in contrast to *B. frigida* the highest irradiation dose of 41.7 J cm^{-2} always results in the lowest post-irradiation $QY_{(Fv/Fm)}$.

Post-exposure $QY_{(Fv/Fm)}$ of the *C. gyrosa*-PB are 6, 29, 40 and 47% when irradiated with 41.7 J cm^{-2} in algal suspension volumes of 0.10, 0.33, 0.67 and 1.00 ml. By trend, these $QY_{(Fv/Fm)}$ values are slightly lower when compared with the respective values of the *B. frigida*-PB, which are 3, 46, 55 and 55%. Again, the UVC-imposed decline of PS II activity is less distinct the denser and thicker the algal layer is. In 0.10 ml samples of the *C. gyrosa*-PB UVC-doses from 0.43 to 41.7 J cm^{-2} lead to a dose-dependent reduction of $QY_{(Fv/Fm)}$ from 72 to 6% of the normalized pre-exposure $QY_{(Fv/Fm)}$ control. The obtained data are comparable to those obtained with the *B. frigida*-PB. At samples with 0.33 ml of applied photobiont cells and doses between 5.2 and 41.7 J cm^{-2} the average $QY_{(Fv/Fm)}$ is c. 39% of the control $QY_{(Fv/Fm)}$ with a minimum of 32% after experiencing 8.7 J cm^{-2} and of 29% after the highest dose of $UVC_{254\,nm}$ (Fig. 1(f)). At samples with 0.67 and 1.00 ml of applied photobiont suspension and doses ≥5.2 J cm^{-2} (Fig. 1 (g)) the average $QY_{(Fv/Fm)}$ is c. 50% with a minimum of 40 and 47%, respectively, after the highest dose (Fig. 1(g)–(h)).

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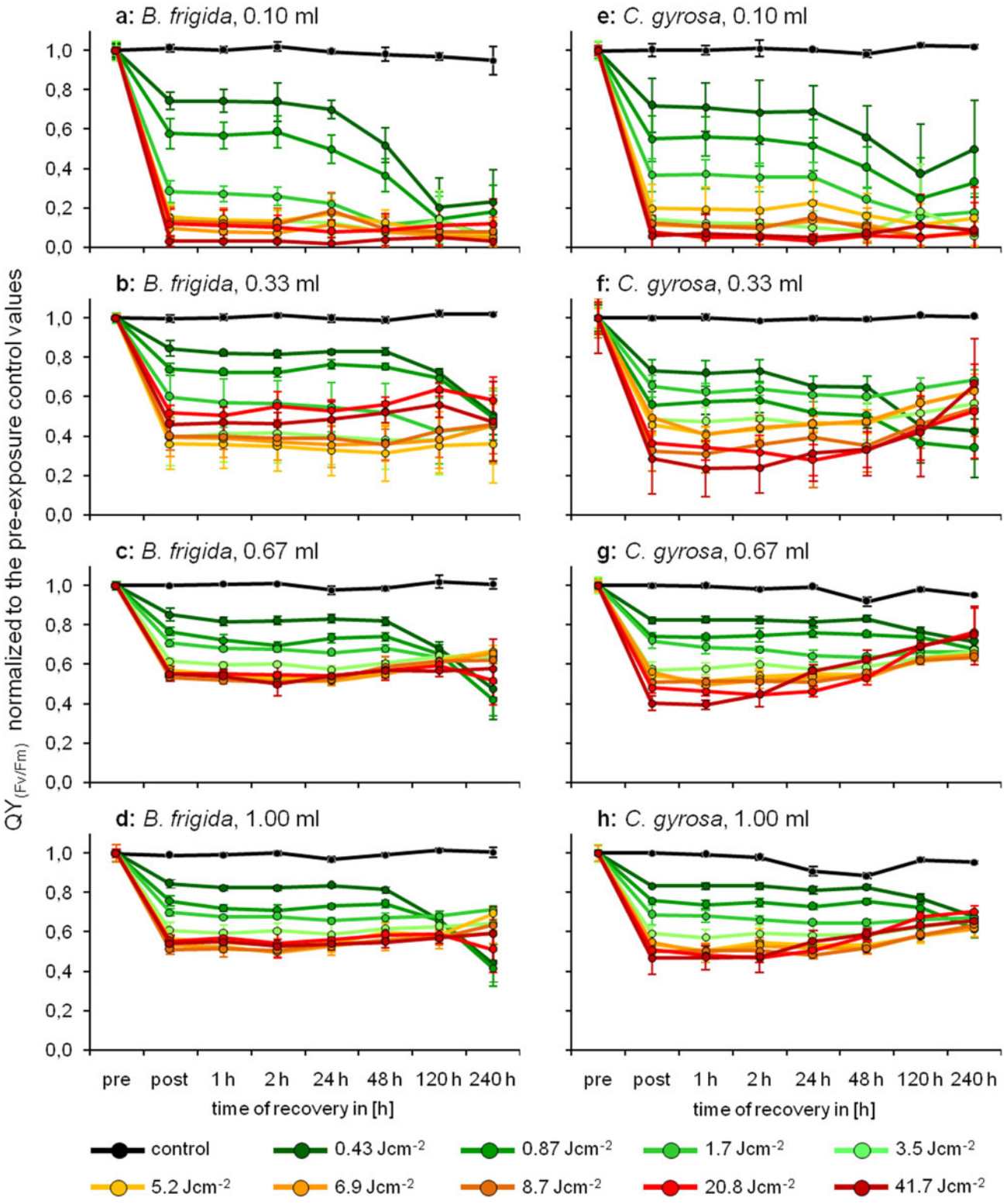

Fig. 1 $QY_{(Fv/Fm)}$ of the *B. frigida*-PB and the *C. gyrosa*-PB after irradiation with $UVC_{254\,nm}$-doses of 0.43, 0.87, 1.7, 3.5, 5.2, 6.9, 8.7, 20.8 and 41.7 J cm^{-2}. Irradiation of filter-applied algal suspension volumes of 0.10 (Fig. 1(a) and (e)), 0.33 (Fig. 1(b) and (f)), 0.67 (Fig. 1(c) and (g)) and 1.00 ml (Fig. 1(d) and (h)), respectively. The $QY_{(Fv/Fm)}$ (*y*-axis) is measured directly before (pre), directly after (post) and after recovery periods of 1, 2, 24, 48, 120 and 240 h (*x*-axis) with mean values ± SD of $n = 18$ replicates.

As observed in the *B. frigida*-PB, the *C. gyrosa*-PB shows a time-delayed decrease of $QY_{(Fv/Fm)}$ after radiation of UVC of 0.43 and 0.87 J cm^{-2} and after 48 h of regeneration that is not observed in samples exposed to higher doses of UVC. According to the results with the *B. frigida*-PB, such decrease is also found after doses of 1.7 J cm^{-2} and starts at an earlier

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point of the recovery period in suspension volumes of 0.10 ml (Fig. 1(e)). With algal concentrations of 0.10 ml this decrease is c. 30% of the control, but rises again between 120 and 240 h of recovery by about 10%. Such recovery was not observed in the *B. frigida*-PB at similar irradiation doses (Fig. 1(a)). With higher algal suspension volumes, no recovery of $QY_{(Fv/Fm)}$ occurs but its delayed loss at low doses is less pronounced, being 10–15% in samples with suspension volumes of 0.67 and 1.00 ml (Fig. 1(g) and (h)).

The regeneration of photosynthetic activity after UVC-doses ≥5.2 J cm^{-2} during post-exposure cultivation is observed in samples of 0.33, 0.67, 1.00 ml algal concentration but not in samples with 0.10 ml. In contrast to the *B. frigida*-PB, the regeneration is more distinctive in *C. gyrosa*-PB. It ranges between 15 and 40% and reaches a $QY_{(Fv/Fm)}$ recovery rate of 40% even in samples that were exposed to the highest dose (41.7 J cm^{-2}, Fig. 1(f) and (h)).

Slow chlorophyll a *fluorescence induction*

The QY measurements are normalized to the initial, dark acclimatized $QY_{(Fv/Fm)}$ control. With the start of the light exposure the QY decreases and subsequently stabilizes as the effective quantum yield $QY_{(dF/Fm')}$ of PS II. At the end of the light exposure the QY increases rapidly as it regenerates to pre-irradiation levels (after 7 min, refer to the controls (●) in Fig. 2(a)–(d)). Basically, this type of diagram represents physiological processes of PS II light and dark acclimation to face photo-oxidative stress, to reduce formation of ROS imposed by high light conditions and to protect the photosynthetic apparatus: the xanthophyll cycle (induction and relaxation within minutes) protects the photosynthetic apparatus by dissipating excessively absorbed light energy, while the active degradation of the protein D1 in PS II (replacement within hours) reduces the capacity of light energy conversion.

QY of the B. frigida-PB

Directly after irradiating the *B. frigida*-PB with 1.7 J cm^{-2}, $QY_{(Fv/Fm)}$ decreases to c. 55% of the control level $QY_{(Fv/Fm)}$ and with the start of light exposure the $QY_{(dF/Fm')}$ decreases by another 10% (Fig. 2(a)). The latter decrease is comparable with the effect observed in the control. During light exposure, the $QY_{(dF/Fm')}$ basically remains at the same level, but in contrast to the control, the QY does not recover with the end of the light exposure. It decreases to a final QY that is 13% below the initial level. This pattern does not change during 240 h of recovery, as indicated by non-recovering LICs (Fig. 2(a)). Three results indicate damage of the PS II by 1.7 J cm^{-2} of UVC: (1) the initial $QY_{(Fv/Fm)}$ is reduced (in accordance to prior $QY_{(Fv/Fm)}$-results), (2) the $QY_{(Fv/Fm)}$ does not recover to the initial level during the LICs, (3) the recovery period of 240 h does not lead to a significant restoration of the photosynthetic capacity of PS II, as would be indicated by rising quantum yields. Directly after irradiating the photobiont with the highest UVC-dose, the $QY_{(Fv/Fm)}$ drops to c. 25% of the control and again by another 10% with the start of light exposure (Fig. 2(b)). Compared to the data of 1.7, 41.7 J cm^{-2} impose a stronger effect on the PS II: the initial $QY_{(Fv/Fm)}$ is lower and no recovery is observed. Interestingly, the initial $QY_{(Fv/Fm)}$ and its capacity to recover during the LIC-measurement slightly improve after a post-exposure regeneration period of 240 h. Such regeneration is consistent with prior results on the regeneration of $QY_{(Fv/Fm)}$ by about 10% for most irradiation doses ≥5.2 J cm^{-2} (Fig. 1(c) and (d)).

QY of the C. gyrosa-PB

In general, the *C. gyrosa*-PB reveals comparable effects of UVC on PS II as described for the *B. frigida*-PB. Measurements directly after UVC-irradiation with 1.7 J cm^{-2} show a less severe $QY_{(Fv/Fm)}$ reduction to about 64% and a recovery close to initial values after the end of the light exposure period (Fig. 2(c)). Additionally, a slight regeneration of $QY_{(Fv/Fm)}$ is observed after 240 h of cultivation. After the UVC-dose of 41.7 J cm^{-2}, the post-irradiation $QY_{(Fv/Fm)}$ drops to c. 19% of the initial control and does not show neither a significant recovery of photosynthetic capacity during each LIC measurement nor during the 240 h regeneration period (Fig. 2(d)). Restorative processes in the course of each individual LIC measurement and in the course of the 10-day regeneration period are occasionally observed, but insignificant.

Non-photochemical quenching

The data on NPQ of both photobionts (*B. frigida* and *C. gyrosa*) and after both $UVC_{254\,nm}$-doses (1.7 and 41.7 J cm^{-2}) are largely similar. In all cases neither the post-exposure recovery period leads to a significant recovery of the NPQ values nor is there a trend of time-dependent recovery observed. Therefore the result section will provide a general description of the NPQ plots. Particular observations are briefly presented below. Compared to the NPQ plots of the non-exposed control (black plots in Fig. 2(e)–(h)), irradiated samples show clearly reduced curves of NPQ. During the light acclimation phase, the increase of NPQ is less pronounced while the overall course is comparable. During the 7 min light acclimation phase the maximum NPQ values of the *B. frigida*-PB reach 10–45% of the control after 1.7 J cm^{-2} (Fig. 2(e)) and 20–60% after 41.7 J cm^{-2} (Fig. 2(f)). The *C. gyrosa*-PB exhibits correspondent NPQ values of 30–50 and 25–40% after 1.7 and 41.7 J cm^{-2}, respectively. With the end of light acclimation, the controls show a short rise of c. 10% before the dark relaxation begins. The dark relaxation phase is characterized in all controls by a exponentially declining curve with a half-life ($t_{1/2}$, a characteristic of NPQ relaxation) of 3–4 min and a residual NPQ value of about 10% after the full period of 18 min (●, Fig. 2(e)–(h)). In the *B. frigida*-PB that was irradiated with 1.7 J cm^{-2} (Fig. 2(e)) the $t_{1/2}$ is >18 min directly after irradiation (■) while it is c. 17, 10 and 14 min after the recovery periods of 24 (□), 120 (◆) and 240 h (◊), respectively. After an UVC-dose of 41.7 J cm^{-2} (Fig. 2(f)) $t_{1/2}$ is >18 min directly after irradiation (■) and after 240 h of recovery (◊), while intermediate $t_{1/2}$ are 11 (□) and 7 min (◆). With the *C. gyrosa*-PB, the half-lives are less impaired. After 1.7 J cm^{-2} they range between 4 and 7 min irrespectible of the period of post-irradiation cultivation (Fig. 2(g)). After 41.7 J cm^{-2} $t_{1/2}$

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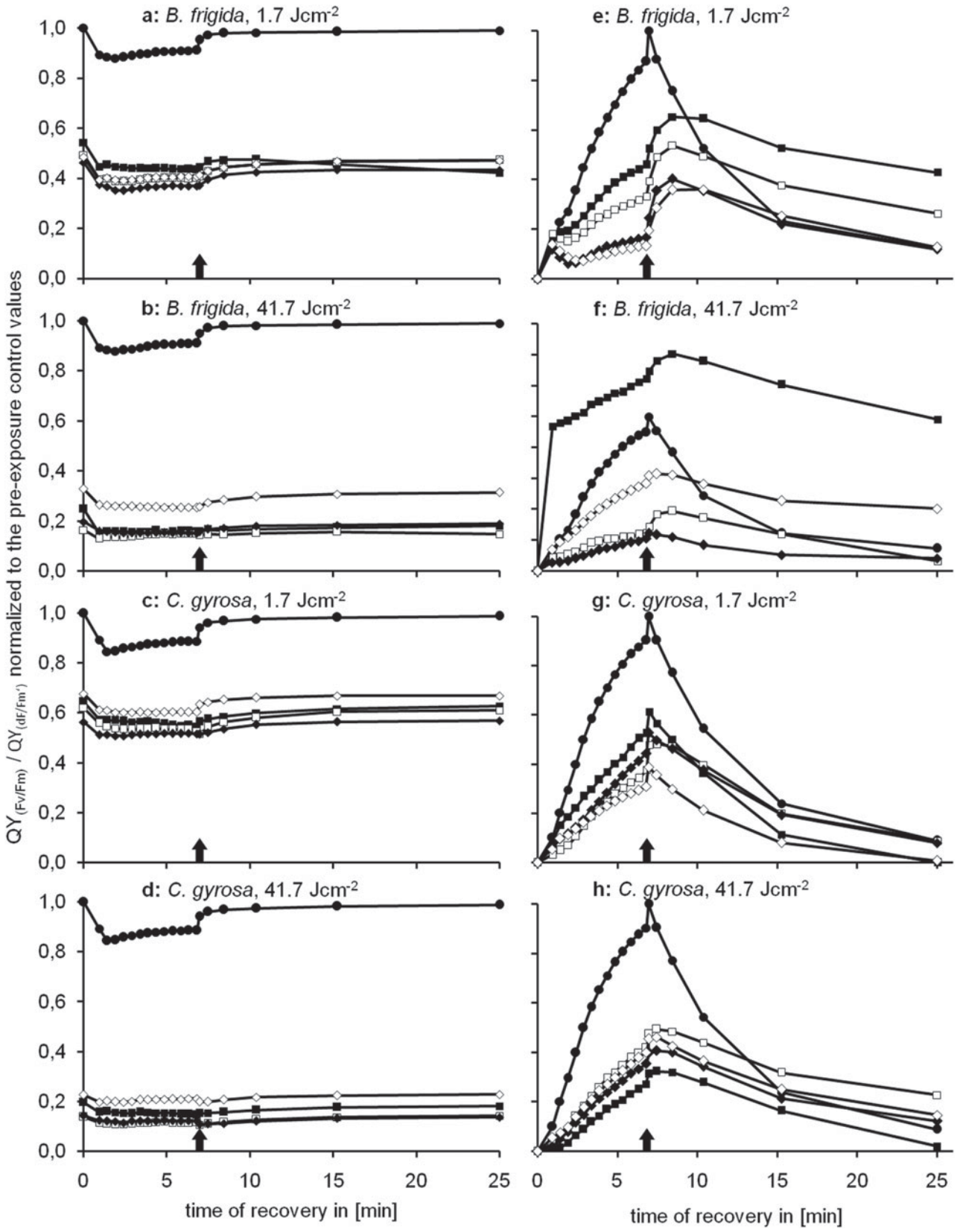

Fig. 2 Quantum yield (as $QY_{(Fv/Fm)}$ and $QY_{(dF/Fm')}$ of PS II of the *B. frigida*-PB (Fig. 2(a), (b), (e) and (f)) and the *C. gyrosa*-PB (Fig. 2(c), (d), (g) and (h)) after irradiation with two $UVC_{254\,nm}$-doses of 1.7 J cm^{-2} (1 h) and 41.7 J cm^{-2} (24 h). QY of the applied algal suspension volumes of 0.33 ml are measured directly before (●), directly after (■) and after recovery periods of 24 (□), 120 (◆) and 240 h (◊). Measurements of effective quantum yield $QY_{(dF/Fm')}$ on the left, measurement of NPQ normalized on the right, both normalized to pre-irradiation values, $n = 3$ replicates, SD not shown, Fig. 2(f): control peak (●) normalized to 1, arrows indicate the start of the dark relaxation phase after 7 min.

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ranges between 8 and 16 min (Fig. 2(h)). These data may indicate that the relaxation of NPQ in the *C. gyrosa*-PB is less affected compared to the *B. frigida*-PB. Additionally, the *B. frigida*-PB reveals an unusual effect: directly after the end of the light acclimation phase the NPQ values continue to increase for 1–2 min, even when the stress-imposing light stopped. This effect is visible after low and high UVC-doses. In contrast, it is only observed in the *C. gyrosa*-PB after the highest dose of 41.7 J cm^{-2}. As a result, both factors (longer relaxation half-lives and delayed peaking of NPQ) indicate that photoinhibition, as a set of mechanisms to protect the photosynthetic apparatus and face environmental stresses, is impaired to a species-specific extent by UVC-exposure. Two exceptions should be mentioned: After 1.7 J cm^{-2} the *B. frigida*-PB revealed a short drop of NPQ values (1–2 min) at the beginning of the light acclimation phase (Fig. 2(e)) and directly after UVC-irradiation with 41.7 J cm^{-2} the normalized NPQ values were unexpectedly high, peaking at 140% of the control (■, Fig. 2(f)).

To summarize the results, $UVC_{254\,nm}$-irradiation of the isolated *B. frigida*-PB and *C. gyrosa*-PB elicited multiple effects on the photobionts' photosynthetic apparatus: (a) dose-dependent reduction of $QY_{(Fv/Fm)}$ for doses <5.2 J cm^{-2}, (b) no (*B. frigida*) or insignificant (*C. gyrosa*) dose-dependent reduction of $QY_{(Fv/Fm)}$ for doses ≥5.2 J cm^{-2}, (c) additional time-delayed reduction of $QY_{(Fv/Fm)}$ after 24 or 48 h by about 20–50% with low doses, (d) recovery of $QY_{(Fv/Fm)}$ by 10–30% occurs even after the highest dose during post-irradiation cultivation, (e) the thicker the algal layer the less pronounced the impairment of $QY_{(Fv/Fm)}$, and (f) both photobionts basically show the same effects on UVC, but the capacity to recover its $QY_{(Fv/Fm)}$ is more pronounced in the *C. gyrosa*-PB. In respect to the LICs, several results should be highlighted: (g) no (*B. frigida*) or insignificant (*C. gyrosa*) restoration of QY during dark relaxation, (h) reduced $QY_{(Fv/Fm)}$ after UVC-irradiation at the beginning of the LIC, (i) incomplete recovery of $QY_{(Fv/Fm)}$ during post-irradiation recovery, (j) prolonged relaxation half-lives during dark relaxation, (k) impairment of $t_{1/2}$ is dose-dependent, (l) $t_{1/2}$ is more impaired in the *B. frigida*-PB, and (m) both photobionts show unusual, retarded peaking of NPQ values 1–2 min after the end of the light acclimation phase. Besides such quantitative results, photo-documentation of the samples indicates a dose-dependent loss of algal chlorophyll in both photobionts (Fig. 3).

Discussion

Effects of UVC on photosynthesis

Chlorophyll *a* fluorescence is often used in astrobiological studies to assess the viability of photosynthetic organisms after (simulated) space and Mars exposure experiments (de la Torre *et al.* 2007, 2010; de Vera *et al.* 2008, 2010; de Vera & Ott 2010; Raggio *et al.* 2011; Onofri *et al.* 2012; Sánchez *et al.* 2012, 2014; Brandt *et al.* 2014). Consequently, it is necessary to test the effects of non-terrestrial radiation on the photosynthetic apparatus in detail and to extend the knowledge on deleterious UVC-effects. Besides damaging biological macromolecules themselves, as described in the introduction, UVR induces multiple damages on cell physiology. UVB is the most harmful type of UVR experienced under terrestrial conditions (Takeuchi *et al.* 1996) since wavelengths below 290 nm (mostly UVC) do not penetrate the Earth's atmosphere (Jansen *et al.* 1998) and UVA is less hazardous due to lower photon energy. UVR-effects on photosynthesis are predominantly investigated by UVB, while detailed information on the effect of UVC is scarce (Jansen *et al.* 1998). Consequently, the present study chose $UVC_{254\,nm}$ to exemplify the effect of space-typical radiation on photosynthesis, to characterize its specific effects and to compare these effects on two photobionts of astrobiologically relevant lichen species. In order to prevent any other stress to the photobionts except UVC-irradiation, the conditions of post-exposure cultivation (temperature, no limitation of water and nutrient availability) were chosen to be favourable for isolated photobionts of the genus *Trebouxia*.

Both isolated photobionts are clearly impaired by $UVC_{254\,nm}$-irradiation as shown by dose-dependent and time-delayed reduction of $QY_{(Fv/Fm)}$ (Fig. 1), by incomplete recovery of $QY_{(Fv/Fm)}$ (Fig. 2(a)–(d)), as well as by affected NPQ-relaxation and QY recovery during dark relaxation period after actinic light exposure (Fig. 2(e)–(h)). The destructive and lasting effect of UVC on chlorophyll (Rozema *et al.* 1997; Joshi *et al.* 2007; Rahimzadeh *et al.* 2011) is illustrated by the dose-dependent algal bleaching even after 240 h of recovery (Fig. 3). These damages can be explained by the hazardous effect of UVC in general on cell physiology, especially on photosynthesis.

UVB and UVC have different action sites on photosynthesis (Jenkins *et al.* 1995; Takeuchi *et al.* 1996) but both types induce the formation of ROS, destroy photosynthetically essential proteins, chlorophylls, carotenoids and plastoquinones leading to a concomitant loss of photosynthetic activity (Strid *et al.* 1994; Nogués & Baker 1995; Rao *et al.* 1996; Rozema *et al.* 1997; Jansen *et al.* 1998; Hollósy 2002; Nasibi & M'Kalantari 2005; Joshi *et al.* 2007; Rahimzadeh *et al.* 2011). Additional effects are exemplified by UVB: it reduces the activity of crucial enzymes as Rubisco and ATP-synthase and impairs PS II as well as PS I (Aro *et al.* 1993; Vass *et al.* 1996, 2005; Rozema *et al.* 1997; Sass *et al.* 1997). As a result CO_2 fixation and O_2 evolution are significantly diminished (Teramura & Sullivan 1994; Rozema *et al.* 1997; Joshi *et al.* 2007). Regardless of the type of UVR, the photosynthetic apparatus is found to be a prime site of UVR-damage and the PS II-complex is its most sensitive part (Aro *et al.* 1993; Teramura & Sullivan 1994; Rozema *et al.* 1997; Jansen *et al.* 1998; Lytvyn *et al.* 2010). The PS I and the cytochrome $b_{6/f}$ complex are less affected (Strid *et al.* 1994; Hollósy 2002). The special targets of UVB in PS II are the central D1 protein, the quinone electron acceptors, the redox-active tyrosines and the water-oxidizing complex (Teramura & Sullivan 1994; Vass *et al.* 2005). The D1-protein is much more affected by UVR than the D2-protein (Nogués & Baker 1995; Vass *et al.* 1996; Jansen *et al.* 1998).

The belated decrease of photosynthetic activity in samples that experienced <5.2 J cm^{-2} and its slow recovery in samples that experienced ≥5.2 J cm^{-2} can be explained by the severity

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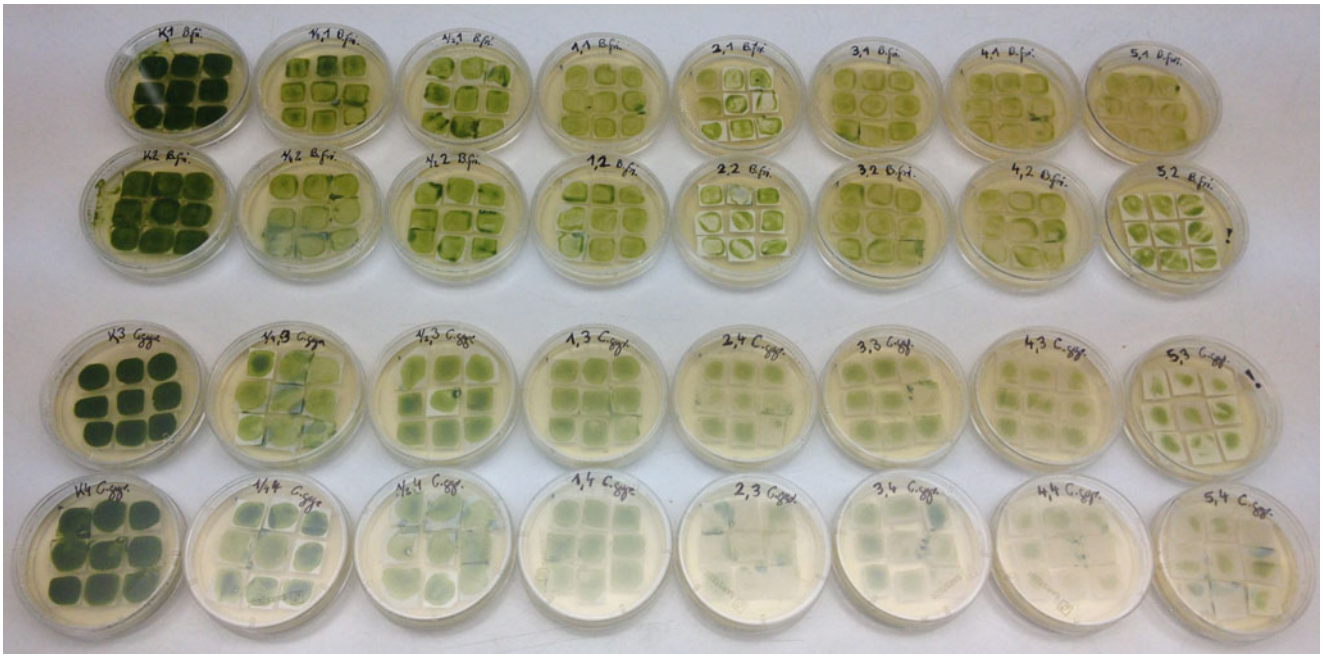

Fig. 3 Bleaching of the *B. frigida*-PB (upper rows) and the *C. gyrosa*-PB (lower rows) after irradiation with various doses of $UVC_{254\,nm}$ and after 240 h of post-irradiation recovery. Control is on the left, followed by UVC-doses of 0.43, 0.87, 1.7, 3.5, 5.2, 6.9 and 8.7 J cm^{-2} which are corresponding to irradiation periods of 0.25, 0.50, 1, 2, 3, 4 and 5 h. The effect of dose-dependent algal bleaching is seen from left to right.

of the UVC-induced damages. In literature, such impairment of PS II is described to be more harmful the shorter the wavelength of applied UVR is (Vass *et al.* 1996; Hollósy 2002). It leads to diminished quantum yields of PS II and delayed or hindered recovery of photosynthetic activity (Kulandaivelu & Noorudeen 1983; Sass *et al.* 1997). In this context, the results are interpreted to display these UVC-induced hazardous processes. Besides the direct damage on photosynthesis, UVC induces DNA- and protein-disruption, subsequently blocking transcription and replication (Strid *et al.* 1994; Jansen *et al.* 1996b; Takeuchi *et al.* 1996) as well as leading to enzyme inactivation (Nasibi & M'Kalantari 2005; Vass *et al.* 2005). In consequence, UVC may affect protein biosynthesis which in turn inhibits the restoration of metabolic performance, including photosynthesis.

Besides the general results discussed above, the measurements of $QY_{(Fv/Fm)}$ elicited four additional findings that need to be discussed: (1) the less affected $QY_{(Fv/Fm)}$ with higher volumes of applied algal suspension; (2) the belated decrease of $QY_{(Fv/Fm)}$ in photobionts that experienced <5.2 J cm^{-2}; (3) the dose-independency of $QY_{(Fv/Fm)}$ after UVC-doses ≥5.2 J cm^{-2}; and (4) the minor restoration of $QY_{(Fv/Fm)}$ in these samples. The first aspect may be explained by the mutual shielding effect of algal cells to each other by the formation of close clusters. The penetration depth of UVR in organic matter is limited (Kovács & Keresztes 2002). For example, $UVB_{300\,nm}$ was found to penetrate conifer needles down to a depth of 20–160 µm (Day *et al.* 1993). Thus, thickness and density of the applied algal layer diminish the UVC-dose experienced by the lower cells. Concerning photobionts incorporated in astrobiologically relevant lichens, the thickness of the algal layer and the characteristic clustering of the photobiont cells within the algal layer was already identified as a protective strategy in *B. frigida, X. elegans* and *C. gyrosa* (de Vera *et al.* 2004a, b, Meeßen *et al.* 2013). The present results suggest a comparable effect of mutual protection in the algal layers as demonstrated by the lower reduction of $QY_{(Fv/Fm)}$ in samples with the thickest layer. The three subsequent findings of belated $QY_{(Fv/Fm)}$-decrease, dose-damage independencies and poor $QY_{(Fv/Fm)}$-restoration are thought to be related: UVC is a stressor not appearing on the Earth and a UVC-photoreceptor is not yet identified. Consequently, the photobionts are likely unable to perceive UVC directly but by its damaging effect only, at least by a rising ROS-level which is *the* crucial interface in stress response cross-talk (Suzuki *et al.* 2012). Therefore longer irradiation periods may give the organism time to sense elevating ROS-levels – instead of UVC itself – and initiate unspecific stress responses. It can be hypothesized that short UVC-exposure times are insufficient to elicit a protective effect, as there is not enough time to sense accumulating ROS-levels, but anyway confer a lasting hazardous effect, e.g. on DNA. This may explain the belated decrease of $QY_{(Fv/Fm)}$ after doses <5.2 J cm^{-2}, the dose-independent $QY_{(Fv/Fm)}$ with doses ≥5.2 J cm^{-2} and the minor $QY_{(Fv/Fm)}$-restoration of the latter samples. Alternatively, this restoration may be due to proliferation of intact cells, presumably those who were shaded by additional algal cells and algal clusters during the irradiation procedure. As chlorophyll *a* fluorescence measurements are not able to distinguish between algal cells that restored their photosynthetic activity and those which were newly generated by cell proliferation, additional research is necessary to evaluate both hypotheses.

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Measurements of NPQ display a set of protective adaptations (referred to as photoinhibition) that counteract photodamage by excess insolation and prevent electron transfer to singlet oxygen, finally resulting in ROS (Hanelt *et al.* 1997; Hollósy 2002; Kranner *et al.* 2005). The results of slow chlorophyll *a* fluorescence induction – reduced $QY_{(Fv/Fm)}$, incomplete recovery, prolonged relaxation half-lives and retarded peaking of NPQ after the light acclimation phase – indicate that these NPQ-mechanisms are also impaired by UVC. Among others, two mechanisms prevent light-induced ROS formation: the xanthophyll-cycle and the active degradation of the D1 protein (Richter *et al.* 1990; Demming-Adams & Adams 2006). The xanthophyll cycle reduces excess excitation in the antennae of PS II by dissipating light energy as heat (Demming-Adams & Adams 1996; Jahns & Holzwarth 2012). During light stress violaxanthin is converted via antheraxanthin to zeaxanthin by the enzyme violaxanthin de-epoxidase. Under non-hazardous conditions, the xanthophyll contents in the photosynthetic apparatus are mostly re-balanced within minutes when light stress ceases (Jahns & Holzwarth 2012). As an additional protective mechanism towards high light intensities, active D1-degradation stops excess electron flow in PS II and thus reduces ROS-formation (Jansen *et al.* 1996a, 1998; Vass *et al.* 1996, 2005). Therefore, D1 is rapidly degraded under excess light conditions, but not as rapidly restored by *de novo* synthesis (mostly within hours, Jansen *et al.* 1996b). The present results of prolonged QY half-lives during NPQ-relaxation (Fig. 2(e)–(h)) and of reduced and incompletely recovering $QY_{(Fv/Fm)}$ indicate that the xanthophyll cycle as well as the quantity and restoration rate of D1 are affected by UVC-irradiation. This interpretation is reasonable as it was already shown that UVB and UVC bleach carotenoids (Kulandaivelu & Noorudeen 1983), damage the xanthophyll cycle (Kovács & Keresztes 2002) and in particular inhibit the crucial enzyme violaxanthin de-epoxydase (Pfündel *et al.* 1992; Hollósy 2002) as well as the restoration of D1 (Sass *et al.* 1997).

Implications for space-exposure experiments

Compared to the high viability and photosynthetic activity rates of photobionts in former simulation, space and Mars exposure experiments (de la Torre *et al.* 2007, 2010; de Vera *et al.* 2008, 2010; Raggio *et al.* 2011; Onofri *et al.* 2012; Sánchez *et al.* 2012, 2014; Brandt *et al.* 2014) the present study reveals a strong decrease of the photosynthetic capacities in both investigated photobionts. To explain this, the differences between former and recent studies should be addressed: while the photobionts of the present studies were exposed to UVC as isolated cultivars under constantly wet (i.e. metabolically active) conditions, the former studies investigated the photobionts' viability and/or photosynthetic activity after exposure in the anhydrobiotic state of the entire lichen thallus (see references above). For example, the quantum yields of the photobionts integrated in the thallus of *C. gyrosa* and *R. geographicum* are not significantly diminished when irradiated in the anhydrobiotic state with polychromatic $UVC_{200-280\,nm}$-doses of up to 7.2×10^7 J m^{-2} (Sánchez *et al.* 2014). Within the thalline structure the photobiont benefits from shielding effects towards excess PAR and UVR (Ertl 1951) as well as from the prevention of oxidative stress, photoinhibition and biological damage (Solhaug & Gauslaa 2004; McEvoy *et al.* 2006) which are provided by the lichens' cortical structures and the SLCs contained therein. In the particular cases of *B. frigida* and *C. gyrosa*, these morphological–anatomical traits as well as their extremotolerance conferring SLCs were recently investigated (Meeßen *et al.* 2013, 2014). In the former studies, experiments have been performed in the anhydrobiotic state which is an additional crucial difference compared to the present study. In lichens, the poikilohydric lifestyle results in a complete shutdown of physiological processes during desiccating conditions making both symbionts less susceptible to other stressors that accompany drought (Kranner *et al.* 2008). Moreover, drought has been discussed to confer UVR-resistance (Nasibi & M'Kalantari 2005; Vass *et al.* 2005) which might be explained by cross-talk between mechanisms that counteract various stressors. For instance, rising ROS levels are produced under osmotic stress, water-stress and stress imposed by excess PAR or UVR (Kranner & Birtić 2005; Suzuki *et al.* 2012; Cruces *et al.* 2013). As an effect, many types of stress lead to the production of antioxidants and the up-regulation of the antioxidant enzyme system which scavenge and detoxify ROS, such as catalase, ascorbate peroxidases, superoxide-dismutase or glutathione reductase (Jansen *et al.* 1996a, 1998; Rao *et al.* 1996; Schmitz-Hoerner & Weissenböck 2003). It might be hypothesized that these mechanisms also assist lichen photobionts to resist high UVR exposure better under desiccating conditions. Besides the direct damage of critical cell targets, ionizing radiation (as hard UVC-, X-, γ- and cosmic radiations) particularly interacts with water and produces free radicals that can damage important cell compounds (Kovács & Keresztes 2002). In the anhydrobiotic state experienced under the extremely desiccating conditions of space, radiolysis of water is effectively prevented in lichens. Therefore anhydrobiosis most likely contributes to the high survival rate of lichens after space exposure. In contrast, the damages on photosynthetic activity which are observed in the present study demonstrate the higher susceptibility of isolated lichen photobionts towards UVC when being metabolically active and again stress the importance of the anhydrobiotic state of the entire thallus to attenuate severe photodamage. The results stress the higher susceptibility of photobionts towards extreme, non-terrestrial conditions (de Vera & Ott 2010) and the great adaptive advantage of the anhydrobiotic state (Ertl 1951; Sadowsky & Ott 2012) and of other lichen-specific protective mechanisms, such as morphological–anatomical traits (Meeßen *et al.* 2013) and SLCs (Meeßen *et al.* 2014).

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