Desiccation stress in two intertidal beachrock biofilms

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Running header: Desiccation in beachrock biofilms

Abstract

Chlorophyll *a* fluorescence was used to look at the effect of desiccation on the photophysiology in two beachrock microbial biofilms from the intertidal rock platform of Heron Island, Australia. The photophysiological response to desiccation differed between the beachrock microbial communities. The black biofilm from the upper shoreline, dominated by *Calothrix* sp., showed a response typical of desiccation-tolerant cyanobacteria, where photosynthesis closed down during air-exposure with a rapid and complete recovery upon rehydration. In contrast, the pink biofilm from the mid-intertidal zone, dominated by *Blennothrix* sp., showed no distinct response to desiccation stress, and instead maintained reduced photosynthesis throughout drying and rewetting cycles. Spatial differences in photosynthetic activity within the black biofilm were evident with a faster recovery rate of photosynthesis in the surface cyanobacteria than in the deeper layers of the biofilm. There was no variation with depth in the pink biofilm. The photophysiological differences in desiccation responses between the beachrock biofilms exemplify the ecological niche specialisation of these complex microbial communities, where the functional differences help to explain their vertical distribution on the intertidal shoreline.

Keywords: Desiccation; beachrock; microbial biofilms; photophysiology

Introduction

Beachrock is formed through the carbonate cementation of sand and gravel and is a typical feature of many tropical and sub-tropical coastlines leading to the formation of intertidal rock platforms. Like all intertidal environments, the beachrock habitat is an environment of extreme conditions, being exposed to strong insolation, extreme temperatures, periodic desiccation and concurrent hypersalinity stress in ponded regions of the rock platform during low tide and air exposure. Such intertidal habitats are extremely diverse, as they provide a steep environmental gradient over a small spatial scale (Davison and Pearson 1996), exhibiting distinct transitions of diverse epilithic and endolithic microbial communities (Diez et al. 2007).

On Heron Island, the steep intertidal gradients across the beachrock results in three distinctly coloured microbial biofilm communities (Cribb 1966). The conspicuous pigmentation of each biofilm community provides a strong contrast between the apparent beachrock zones that lie parallel to the shore. The zones can be described according to their dominant cyanobacterial species: (1) the pale green-white *Entophysalis duesta* zone, which occupies the lowest intertidal area (Davies and Kinsey 1973), (2) the intermediate pale pink zone dominated by the unicellular, non-heterocystous *Blennothrix* sp. (Diez et al. 2007), and (III) the dark brown-black uppermost zone dominated by the filamentous, heterocystous, cyanobacteria *Calothrix* sp. (Diez et al. 2007). There are a broad range of morphotypes and phylotypes within these differently pigmented zones, some of which are shared across biofilms and others which are associated strongly with only one zone (Diez et al. 2007). While all three zones consist predominately of cyanobacteria, they also house a complex mixture of other bacteria, microalgae and fungi.

The diversity of the microbial communities of the Heron Island beachrock and their distribution patterns along the shoreline can be largely attributed to the heterogeneity in the substrate (porosity and chemical composition) and external environmental factors (differences in wet/dry cycles driven by tidal heights). However, the physiological plasticity of the microbial communities also plays a role in the vertical distribution (from lower to upper shore) of these microbial mats across the beachrock and is most likely driven by the microbial community's tolerance of desiccation (Dring and Brown 1982).

Desiccation is one of the most extreme physical conditions that organisms may endure, with damage being evident in growth, development and metabolism (Smirnoff 1993). Desiccation can result in damage to cell membranes, proteins and nucleic acids and is lethal to most organisms, with only a few able to withstand complete dehydration (Potts 1999). Cyanobacterial mats are generally poikilohydric, that is, they are able to

withstand desiccation by entering a dormant state (suspended metabolism) when dehydrated and resuming metabolic function almost immediately when water becomes available, absorbing water directly and quickly through their cell surface (Billi and Potts 2002). Sugars, such as trehalose and sucrose, protect membrane integrity during dehydration, keeping lipids in a fluid phase (Potts 2001; Singh et al 2002). In photosynthetic organisms, one of the primary impact sites from desiccation stress is the photosystem II (PSII) complex (Govindjee et al. 1981; Genty et al. 1989), where photosynthesis becomes inhibited by a lack of electron donors to PSII, i. e., water (Nabe et al 2007). Photosynthesis can also be inhibited by the increased viscosity and concentration of ions in the cytosol, as well as increased rigidity in the thylakoid membrane (Nabe et al 2007). To avoid photodamage, cells must match energy transfer, electron transport and carbon fixation rates during desiccating and wetting events. Tidal patterns are cyclic and when low tide corresponds with midday peak insolation (solar noon), this represents the period of greatest desiccation and therefore maximum photosynthetic stress. Photosynthesis can often continue during air-exposure in intertidal organisms, but this is highly dependent on the level of desiccation; the longer the duration of air exposure, the greater the proportion of photoinhibition relative to photosynthetic carbon fixation. Under more severe conditions, photosynthesis during aerial exposure is strongly inhibited or completely closed down (Nabe et al. 2007). The level of photosynthetic activity and photoprotection are therefore likely to vary between the different beachrock biofilms depending on their vertical distribution along the rock platform and thus duration of cyclic desiccation events.

Variable chlorophyll fluorescence is a non-invasive tool that has been used previously to monitor PSII activity in desiccated organisms (Huppertz et al. 1990; Schreiber et al. 2002). When a dark-adapted sample is illuminated, the fluorescence yield shows a characteristic induction of fluorescence emission, known as the "Kautsky" curve. The curve has two phases: first there is a rise to a maximum (F_m) over a period of hundreds of ms, followed by a relaxation of fluorescence yield over the next seconds or minutes, to a steady state light level (F_t). Fast induction curves (FICs) measure the fast kinetic rise to $F_{m,}$ which has a number of phases: first a rise from the origin ($O \cong F_o$) to an intermediate step (J) and then a slower rise involving a second intermediate (I) to a peak ($P \cong F_m$). Detailed analysis of the polyphasic induction curves allows for the identification of the impact that desiccation has on the various components of the photosynthetic apparatus. In the case of desiccation, as the thylakoid membrane becomes more rigid, the curve becomes flatter, indicating a reduced size of the operational plastoquinone (PQ) pool for supporting electron transport and thus slower electron transfer from the PQ pool to photosystem I (PSI; Bewley 1979).

To date, very little is known about the ecophysiology of epilithic beachrock communities in response to desiccation stress. Particularly, there is a paucity of information on the physiological strategies these communities use to deal with desiccation and high irradiance on a daily basis, and whether these strategies differ between different beachrock biofilms. In this study, we used a combination of powerful tools to monitor the optical properties and fluorometric estimates of electron transport to provide insight into the photosynthetic responses of two beachrock biofilm communities (representing the pink and black zones of the rock platform) to desiccation. Specifically, spatial and temporal changes in photosynthetic efficiency and shifts in the polyphasic fluorescence rise of the two ecotypes were investigated during desiccation and subsequent rehydration.

Materials and Methods

Beachrock sample collection and environmental condition

Beachrock was collected from the intertidal rock platform on the southern shore of Heron Island, in the Great Barrier Reef (152° 6′ E, 20° 29′ S). Sections of beachrock covered by a thick (1.5 – 3.0 mm) microbial biofilm were collected from the uppermost black zone (*Calothrix* sp.) and the intermediate pink zone (*Blennothrix* sp). The upper biofilm layer was removed from the underlying rock by cutting the rock into approximately 40 x 40 x 30 mm replicate samples using a water-cooled circular saw. The samples were maintained outside under natural light conditions. They were submerged in a flow through seawater bath for 3-4 h each day and air-exposed for the remainder of the day to simulate natural conditions. Variable chlorophyll fluorescence, spectral reflectance and moisture content were measured during the drying and wetting of the two beachrock ecotypes. To determine photosynthetic responses to desiccation, fluorescence measurements were performed on samples that had been submerged for 4 h with measurements taken every hour for 3 h, while being left to dry in full sunlight (from 11:00 – 14:00). To measure photosynthetic recovery upon re-wetting, beachrock samples were re-submerged and measured within one minute, then after 10, 20, 30 and 120 min of submersion, respectively.

In order to establish ecological context of the environmental extremes experienced in the tropical intertidal zone, temperature of the Heron Island rock platform was measured in triplicate across an air – rock – water gradient using small temperature sensors (iButtons; Elco Express Thermo, USA) attached to the substrate with silicon glue, logging temperature at 5 min intervals over 72 h. Simultaneous measurements of ambient downwelling photosynthetically active radiation (PAR; over the 72 h period at 5 min integration time) was

recorded using a quantum irradiance PAR sensor attached to a logging light meter (Licor 1400, Nebraska, USA). Tidal information was downloaded from the island weather station (http://www.mobilegeographics.com) to establish times of emersion and exposure.

Spectral reflectance and moisture content

Spectral reflectance was determined on the beachrock surface every hour using a cosine corrected glass fibre optic connected to a spectrometer (Red Tide USB 650, Ocean Optics, USA). Measurements were made over the 350-750 nm bandwidth, using an integration time of 5 ms. Samples were measured under full solar irradiance and reflectance was normalised to the reflectance of a white standard (TOP, WS-2 Spectralon Reference Standard, Ocean Optics, USA). The relative position of the fibre optic used to collect the reflected spectral signature was maintained at a 30 mm distance between the beachrock surface and the fibre optic, with any small adjustments necessary to maintain the exact distance made using a micromanipulator (MM33, Märzhäuser, Wetzlar, GmbH, Germany). Moisture content of biofilms was measured with a moisture meter (MO250, Extech instruments, USA) in conjunction with the fluorescence measurements to record the percentage of water loss in the biofilm.

Variable chlorophyll fluorescence

Fluorescence measurements were performed during a wetting and drying cycle on both black and pink beachrock biofilms in conjunction with moisture content and reflectivity. Fast induction curves (FICs) were measured using a double-modulation fluorometer (Photon System Instruments, FL-3000, Brno, Czech Republic) with a specialized flat measuring head and a 5 s multiple turnover flash at >3000 µmol photons m^{-2} s^{-1} light intensity. Fluorescence measurements were recorded every 10 µs for the first 2 ms, every 1 ms until 1 s, then every 500 ms up to 5 s. All O-J-I-P fast induction curves were normalised to F_O, where all values were divided by the initial O step (at 50 µs) for comparison. Fast induction curves (FICs) were then measured on dry samples and samples 1, 10, 20, 30 and 120 min after re-emersion in seawater, respectively.

For investigating the vertical heterogeneity of photosynthetic activity within the beachrock biofilm consortia, thin (3 mm) vertical cross-sections of the black and pink microbial biofilms were sliced with a

razorblade and carefully mounted onto microscope slides. Variable chlorophyll fluorescence measurements were made using a pulse amplitude modulated (Imaging PAM –Max/K, Walz GmbH, Effeltrich, Germany) fluorometer mounted on a compound microscope (Axiostar plus, Zeiss, Germany) (Trampe et al. 2011). Measurements were made using the red excitation light (625 nm) at 10x magnification, and collected using the Imaging Win (V2.32 FW Multi RGB; Walz GmbH, Effeltrich, Germany) software. After 10 min dark-adaptation, minimum fluorescence (F_O) was recorded before application of a saturating pulse of light (saturating pulse width = 0.8 s; saturating pulse intensity > 3000 µmol photons m^{-2} s^{-1}), where maximum fluorescence (F_M) was determined. From these two parameters the quantum yield of PSII was calculated as $F_V/F_M = (F_M-F_O)/F_M$ (Schreiber 2004). This measurement was performed on sections that were completely dry and repeated on the same sections after re-wetting at 0, 10, 30 and 60 min, while maintained under low irradiance (<50 µmol photons m^{-2} s^{-1}). Rehydration of the samples was done in the presence of light, as it has been shown to assist recovery of photosynthesis in dehydrated bacterial mats (Schreiber et al. 2002; Fleming et al. 2007). However, given that the deeper layers of the microbial mat would rarely be exposed to high irradiances (found only on the surface), only low light was applied, thus avoiding photodamage to the species embedded deeper within the biofilm.

Results

The beachrock intertidal environment

The beachrock on Heron Island is subject to a semi-diurnal tidal cycle with two high and two low tides each day and an average spring tidal range of about 2 m. The tidal data overlaid with the PAR data show the receding tide occurred during peak midday irradiance on all three days (Fig. 1a, b), completely exposing the upper (black) and intermediate (pink) beachrock during the afternoon. Temperatures on the rock platform far exceeded the maximum temperatures measured in the water and air (Fig. 1c) and were greatest during the afternoon, when rock pools were exposed. In the black and pink zones of the rock platform, temperatures reached well in excess of 40°C on each of the three days, reaching a maximum of 59°C in the black zone on the first day (Fig. 1c). Over all three days, midday temperatures on the rock platform nearly twice those measured in the lagoon water (28°C).

190 *Spectral reflectance and moisture content*

191 There was a significant decline in moisture content in both biofilms ($P < 0.01$) when exposed to full sunlight
192 over 3 h (Fig. 2a). However, the moisture content in the black biofilm declined by 60% over 3 h, while in the
193 pink biofilm, moisture levels only declined by around 20% (Fig. 2a). The spectral reflectance data is consistent
194 with the changes in moisture content, showing a clear increase in reflectivity with increased desiccation in the
195 both biofilms (Fig. 2b, c). There was, however, a difference in the pattern of the two spectral signatures, such as
196 an increase in reflectance around 400 nm with increased desiccation in black biofilm (Fig 2b) and much higher
197 reflectance in the 700-750 nm range in the pink biofilm (Fig. 2c).

198

199 *Variable chlorophyll fluorescence*

200 Fast induction curves (FICs) revealed a strong decline in amplitude and flattening of the OJIP curve with
201 desiccation in the black biofilm (Fig. 3a) consistent with a loss of electron transport and complete closure of
202 PSII reaction centres. A decline in amplitude and flattening on the fluorescence curve was also seen in the pink
203 beachrock after the first hour of desiccation; however, over the following 3 h no further decline was observed
204 (Fig. 3b). This would suggest some impact on electron transport, but complete cessation of photosynthesis was
205 avoided in the pink biofilm. The re-wetting of the black biofilm showed an immediate recovery in
206 photosynthetic activity and after 2 h, with fluorescence yields much higher than the initial values measured
207 when wet (Fig. 3c). Although a small increase in fluorescence signal was observed in the pink biofilm (Fig. 3d),
208 it was minor when compared with the strong increase measured in the black biofilm.

209 Maximum quantum yield of PSII (F_V/F_M) measured on the vertical cross section of the dry biofilms and
210 then over a time series from re-submersion with seawater, showed clear differences between the black and pink
211 beachrock (Fig. 4). When dry, the black biofilm showed no variable fluorescence, with an F_V/F_M of zero across
212 the entire cross section of biofilm (3 mm). There was a time-dependent response, with an immediate re-
213 activation of photosynthesis upon re-wetting, which steadily increased with increased submersion time (Fig. 4a).
214 There was also a greater response in the biofilm surface layer compared with the deeper microbial communities,
215 evident from the higher F_V/F_M values at the top of the vertical profile (Fig. 4a). These data correspond well with
216 the patterns seen in the FICs measured on the surface of the biofilm (Fig. 3c). In comparison, there was no
217 significant change in the photosynthetic activity of the pink ecotype over time, with the F_V/F_M staying constant

around 0.210, irrespective of moisture content (Fig. 4b). There was also no heterogeneity in photosynthetic response across the vertical profile of the pink biofilm, suggesting that all species within the biofilm responded similarly in space and time (Fig. 4b). These results closely match the FICs of the pink biofilm, showing relatively small changes in O-J-I-P steps during re-wetting (Fig. 3d) and only a small change in moisture content and high reflectivity (Fig. 2).

Discussion

The impact of desiccation on photosynthesis varied strongly between the black and pink beachrock biofilms. The black biofilm, which inhabits the uppermost reaches of the beachrock platform where it is susceptible to the highest temperatures and the greatest period of air-exposure, showed the greatest photosynthetic response to desiccation and re-wetting. There was a complete cessation of photosynthetic activity when moisture content of the microbial mat dropped below 50% (Fig. 3a). The shutting down of photosynthesis during air-exposure is a photoprotective strategy commonly observed in intertidal macroalgal species and crust forming cyanobacterial mats (Schreiber et al. 2002; Nabe et al. 2007), as it allows the photosynthetic machinery to remain intact for rapid re-activation when conditions become favourable again. The black biofilm showed immediate re-activation of electron transport upon re-wetting, which continued with increased submersion time (Fig. 3c and 4a), a response seen previously in the black beachrock biofilm (Schreiber et al. 2002). The rapid recovery observed in the black biofilm (Fig. 3c and 4a) is a typical response of desiccation-tolerant plants (Proctor and Smirnoff 2000) and consistent with the findings of Schreiber et al. (2002), who obtained fluorescence yields of around 0.3 within 15 minutes of re-wetting in the presence of light. Ecologically, it also fits with the study by Dring and Brown (1982), who showed that the recovery from desiccation correlated to the plants vertical position on the shore, where low shore plants suffered irreversible photoinhibition, while high-shore plants recovered rapidly. The ecological advantage to having such a strategy is that it would allow the microbial community to maximise photosynthesis during submersion and minimise damage during emersion.

The minimal change in photosynthetic activity seen in the pink beachrock biofilm is atypical of desiccation-tolerant species (Figs. 3b, d), but is indicative of its location on the lower reaches of the rock platform, where conditions are less extreme and complete desiccation less frequent. It is possible that the consistent F_V/F_M (Fig. 4b) is the result of permanent photoinhibition, a trait previously observed with lower intertidal species upon air-exposure (Dring and Brown 1982). However, it seems more likely that the consistent

247 and relatively low photosynthetic activity serves as a strategy to avoid the need to regulate the photosynthetic
248 and photoprotective activity with changing conditions. Instead, the cells remain in a suppressed photosynthetic
249 state, just active enough to maintain positive carbon fixation, but not active enough to expose the cells to
250 irreversible damage. Although clear differences in photosynthetic activity were detected between the two
251 biofilms after three hours of desiccation, it could be argued that this difference was the result of the difference in
252 the amount of water loss between the two biofilms (50% loss in the black biofilm and only 20% in the pink
253 biofilm; Fig. 2a). However, the variable fluorescence measured in the cross sections of biofilm (which were
254 dehydrated onto slides) supports the measured low level photosynthetic activity of this community under
255 desiccation (Fig. 4b).

256 The decline in maximum fluorescence (F_M) seen here as a decline in amplitude of the P-step in the O-J-
257 I-P curve, with increased desiccation measured in the black biofilm (Fig. 3a) has been seen previously in other
258 photosynthetic organisms (Bjorkman and Powles 1984; Chen and Hsu 1995; Skotnika et al. 2000), where it was
259 postulated to be due to damage to the oxygen evolving complex (OEC) and invariably cause a slowing of
260 electron transport from PSII to PSI. In the case of the black biofilm, there was a clear drop in the F_M (P-step)
261 and a shift in the kinetics of the J-step toward a faster, albeit lower, rise to J resulting in the formation of a K-
262 step (Strasser 1997) in the desiccated sample, which are both indicators of damage to the OEC (Chen and Hsu
263 1995; Skotnika et al. 2000). However, while there was a slowing of electron transport with a complete loss in
264 variable fluorescence during desiccation (Fig. 3a), there was also a rapid recovery in fluorescence upon
265 rehydration (Fig. 3c), suggesting no long-term damage to the OEC. It is also possible that the black biofilm,
266 being dominated by nitrogen fixing cyanobacteria, could have rapidly switched off the OEC so as not to impact
267 any nitrogenase activity, which has been shown to be the first metabolic process to stop when dry and re-
268 commencing after re-wetting (Jones 1992).

269 In the pink biofilm, an increase in the J-step relative to the P-step (or flattening of the O-J-I-P) was
270 observed (Fig. 3b). This pattern has previously been attributed to the formation of Q_B non-reducing centres
271 (where PSII electron acceptor and donor Q_B becomes slower at accepting electrons, preventing the complete re-
272 oxidation of the electron transport chain) as a result of inhibition of the acceptor side of PSII i.e., from a lack of
273 water (Skotnika et al. 2000) or due to nitrogen limitation (Petrou et al. 2012). In Skotnika et al's (2000) case
274 however, the change in the J:P ratio was again observed with a concomitant shift in the J-step towards much
275 faster kinetics and also with the appearance of a K-step (Strasser 1997; Lazar 1999), both of which have been
276 attributed to damage of the donor side of PSII and neither of which were observed here. There is of course the

277 possibility that the decline in fluorescence measured in the pink biofilm as it dried, is simply the result of
278 increased reflectivity, thereby causing a decline in overall fluorescence intensity (Skotnika et al. 2000).
279 The notable increase in the fluorescence yields of the O-J-I-P curves in the re-wetted samples
280 compared with those measured prior to drying (Fig. 3) could be due to the changes in irradiance, as re-wetting
281 measurements were carried out in the afternoon when solar irradiance was lower. Biofilms exposed to higher
282 irradiances during the drying measurements would increase fluorescence quenching and result in a lower overall
283 fluorescence signal (lower P). Alternatively, the difference in maximum fluorescence could be associated with
284 other cellular processes such as nitrogen fixation. However, this was not measured in this study.
285 The variable fluorescence measured in the vertical cross-section of the black biofilm showed
286 differences in maximum quantum yield of PSII (F_V/F_M) between community layers, with the surface filamentous
287 cyanobacteria reactivating more rapidly and reaching higher F_V/F_M values than the deeper microbes (Fig. 4a).
288 This would suggest that the dominant photosynthetic activity occurs in the surface layers of the biofilm that is
289 exposed to the greatest irradiances, ensuring maximum production when conditions are optimal. In the pink
290 biofilm, no differences were detected across the vertical profile (Fig. 4b), suggesting that the desiccation
291 response and photosynthetic strategy was similar in all the species within the biofilm. The relatively low
292 fluorescence yields measured in this study (at excitation 625 nm) are typical of cyanobacteria (Schreiber et al.
293 1995), which have accessory pigments (phycocyanin and allophycocyanin) that absorb strongly in 620-640 nm
294 range. Previous work by Schreiber et al. (2002) showed differential responses to various wavelengths, but they
295 were able to select for cyanobacteria using red (640nm) excitation light, with variable fluorescence yields
296 reaching a maximum of around 0.3, similar to those measured here.

297 The morphology of the two different beachrock biofilms needs to be considered, as it likely plays a role
298 in the rate and extent of desiccation. The black biofilm was much less reflective across all wavelengths,
299 absorbing much more of the down-welling irradiance than the pink biofilm (Fig. 2b, c), resulting in a faster rate
300 of desiccation (Fig. 2a). Additionally, the black biofilm is dominated by a layer of filamentous cyanobacteria
301 (Diez et al. 2007). These long filaments provide a greater surface area and therefore greater potential for air
302 exchange, enhancing the rate with which desiccation and similarly, rehydration could occur. In contrast, the pink
303 biofilm was highly reflective, especially at the higher wavelengths (Fig. 2c). This reflectivity, which increased
304 with exposure time, combined with the smooth, non-filamentous surface morphology, could help the biofilm to
305 minimise water loss via evaporation. By forming a highly reflective crust, total desiccation deeper within the
306 biofilm may be limited and thus greater insulation for the inner communities and less impact on photosynthetic

processes. This could explain the minimal loss in water content (Fig. 2a) and the uninterrupted, albeit moderate, photosynthetic rates within the deeper layers throughout drying and rewetting (Fig. 4b).

In addition to morphological and physiological differences, stress-tolerance is no doubt also influenced by the complexity and diversity of the biofilm communities. Despite having species common to both biofilms, DGGE based 16S rRNA analyses of microbial diversity revealed that the pink and the black biofilms were the most genetically distinct of all the beachrock communities on Heron Island (Diez et al. 2007). Of particular interest, the black biofilm is dominated by heterocystous diazotrophs, whereas non-heterocystous cyanobacteria dominate the pink biofilm (Diez et al. 2007). The potential difference in nitrogenase activity between the two biofilms could help to explain the differences in the photosynthetic strategies they employ during desiccation. Nitrogen fixation relies on the carbon and ATP derived from photosynthesis and oxidative metabolism, but the enzyme nitrogenase is extremely sensitive to oxygen and needs to be isolated (either in space or time) to protect it from the high oxygen environment of photosynthesis (Gallon 1981). Heterocystous cyanobacteria use spatial separation of nitrogenase activity and oxygen evolving photosynthesis (heterocysts). In this way they can photosynthesise and fix nitrogen simultaneously. For the black biofilm, this means these processes can occur in the day when submerged, however, upon air exposure, both processes cease allowing cells to preserve energy (ATP) for rapid reactivation of photosynthesis and nitrogen fixation upon rehydration (Jones 1992; Harel et al. 2004). In contrast, high nocturnal nitrogen fixation rates have been measured in the pink biofilms (Diez et al. 2007), suggesting that the phylotypes that dominate the pink biofilm community use temporal separation (non heterocystsous) to protect the nitrogenase enzyme. Thus, it follows that they would benefit from continued photosynthesis throughout the day, avoiding complete shut down during emersion, in order to have sufficient substrate (carbon and ATP) for nitrogen fixation to occur throughout the night, when cellular oxygen concentrations are low.

This study has shown that photophysiological plasticity can reflect the ecological niche specialisation of beachrock-associated biofilms. Functional differences in the photosynthetic response of the two biofilms correspond well with their distribution on the rock platform. The response of the black ecotype was typical of a desiccation-tolerant species, with complete inactivation of photosynthesis followed by a rapid and complete recovery upon rehydration (Bewley 1979). This strategy allows for greater efficiency, where the rate of photosynthesis and recovery are optimised to ensure productivity during the photoperiod is maximal when submerged. In contrast, the pink biofilm, which differed in community compositition, morphology and

physiology, showed minimal response to desiccation and instead maintained a relatively consistent rate of electron transport and photosynthetic quantum efficiency.

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458 **Figures**

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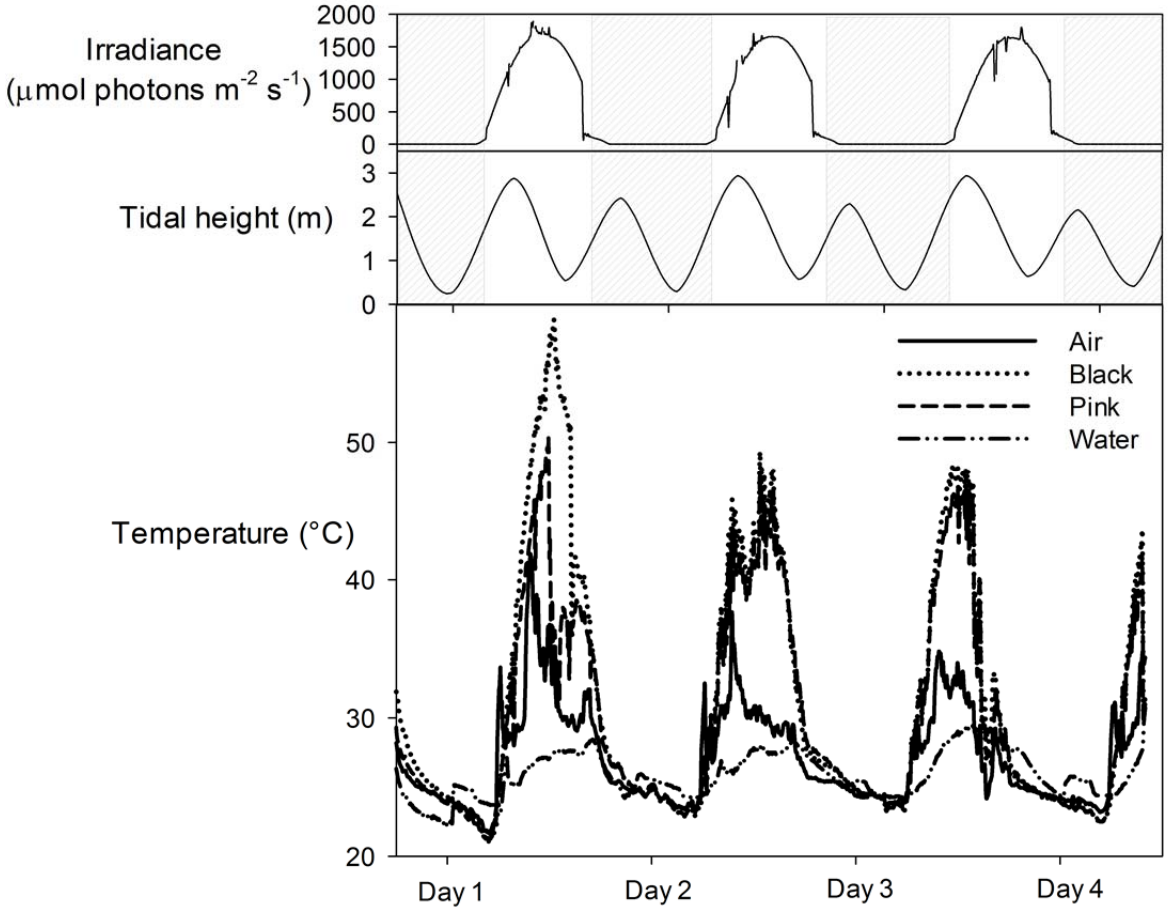

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462 **Fig. 1** Photosynthetically active radiation (PAR) on Heron Island is shown (upper panel) for the three days of
463 the experiment (15/11/2009 – 18/11/2009). Tidal data for that period is also shown (middle panel). Temperature
464 of air and water as well as the black and pink beachrock zones collected using temperature loggers recording
465 temperature every 5 min. Temperature data represent the average of three transects from the water to the upper
466 intertidal rock platform. Tidal information was taken from the Heron Island mobile geographics web page for
467 the appropriate dates www.mobilegeographics.com:81/locations/2508.html

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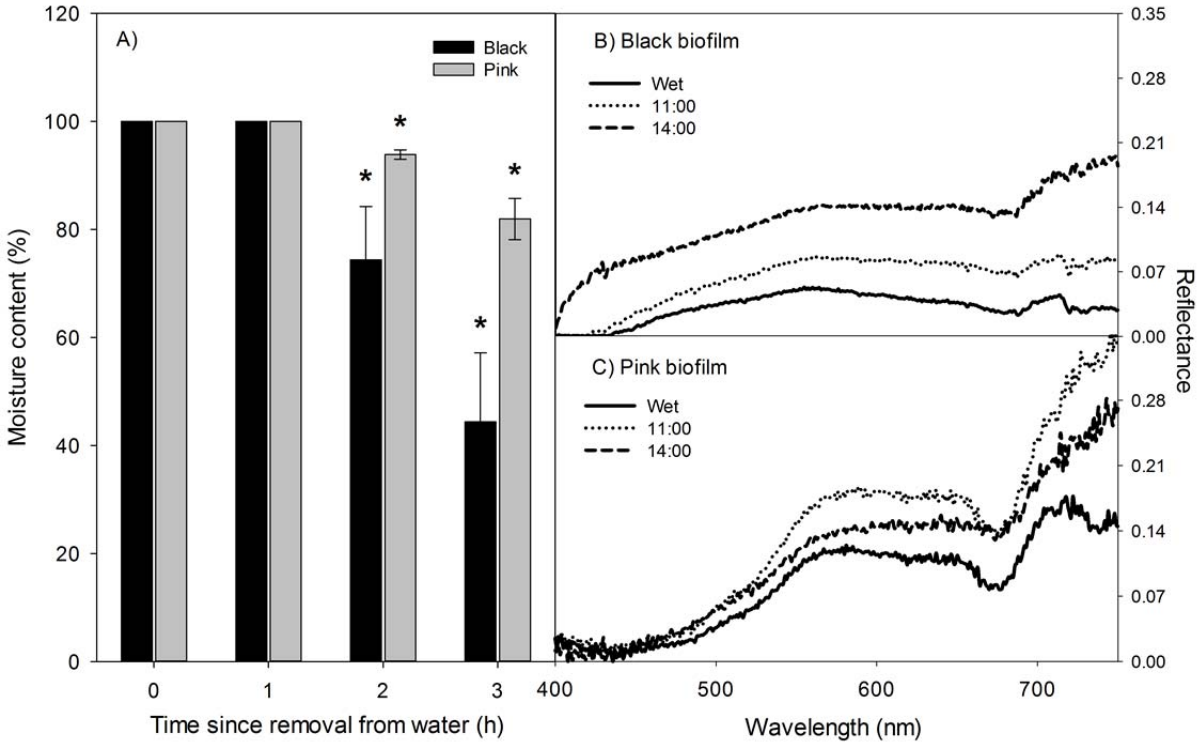

Fig. 2 Beachrock desiccation over time A) measured as a percentage moisture content B) using spectral reflectance in black and C) pink beachrock biofilm. A) Data represent the mean ± SD ($n = 5$), B and C) data represent the average reflectance (400-750 nm) of five individual measurements. *significant decline in moisture content at $\alpha < 0.05$, analysed by rmANOVA.

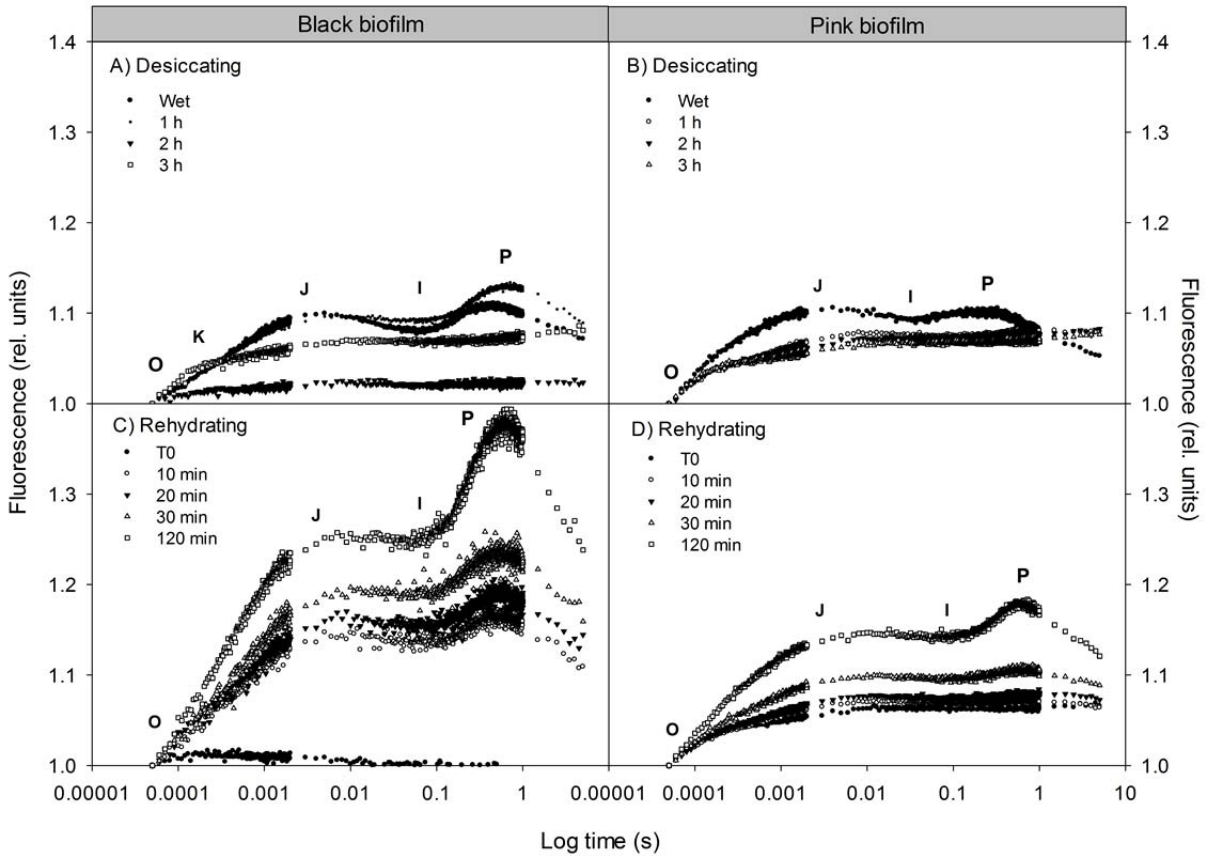

Fig. 3 Fast induction curves during desiccation (A and B) and rehydration (C and D) of black and pink beachrock biofilms. Data are plotted on a semi-log scale and represent the average of individual curves (n = 5). Approximate positions of O-J-I-P steps are given.

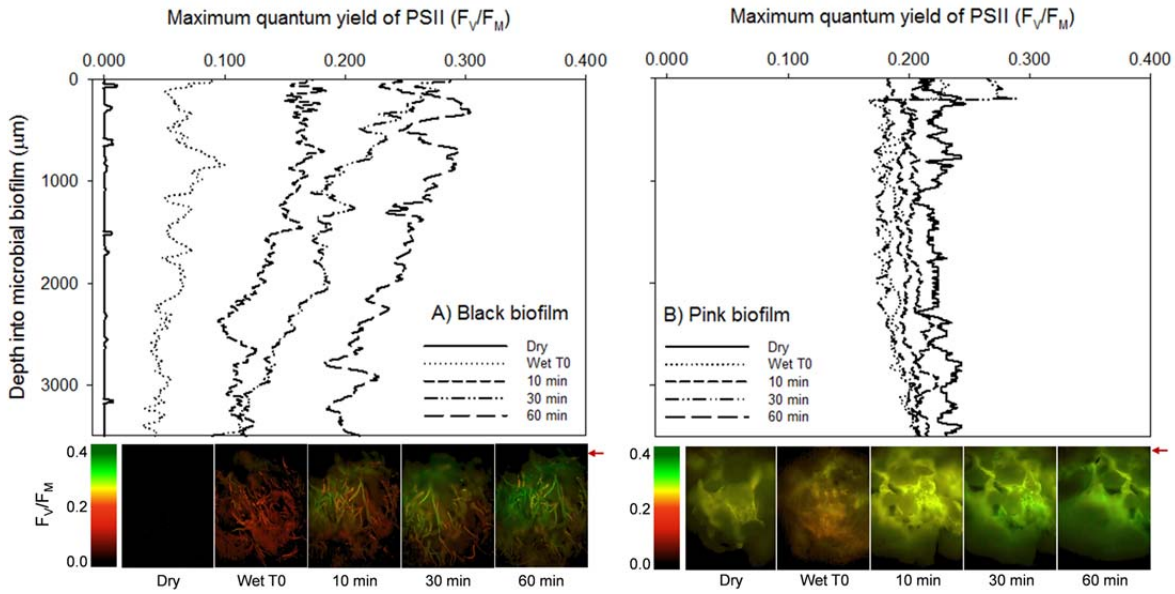

Fig. 4 Sequential measurements of maximum quantum yield of PSII (F_V/F_M) in a vertical cross-section of A) black and B) pink beachrock biofilms from dry up to 1h after rehydration (x10 optical magnification). Data represent averages of independent measurements (n = 8). Representative microscopy PAM images of the temporal and spatial changes in F_V/F_M from the surface to depth (3 mm; surface indicated by red arrow) in both biofilms types are shown below.