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ORIGINAL PAPER

Cyanobacteria in the Australian northern savannah detect the difference between intermittent dry season and wet season rain

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Abstract This research was conducted in the northern Australian savannah at Boodjamulla National Park where cyanobacterial crusts dominate the soil and rock surfaces in between tussock grasses. It is widely accepted that terrestrial cyanobacteria are drought tolerant and rapidly recommence photosynthesis once moisture is available. Initial tests at the research site indicated that cyanobacteria did not respond to rehydration during the dry season, even after several days. We hypothesised that resurrection had not taken place and new growth from survival cells had to take place during the follow-up wet season. To further understand the desiccation–resurrection processes we tested photosystem II (PSII) responses both during the dry and wet seasons. In the 2009 dry season after 125 days without rain, crust samples were regularly rehydrated. Over the 10 day trial cyanobacteria did not recover PSII activity or CO_2-uptake. Although new colonies of *Nostoc* grew other cyanobacteria remained inactive, even though liverworts and lichens in the same crusts had responded within 24 h. Dry season cyanobacterial crusts were collected in 2010 then reintroduced into their natural environment and exposed to rainfall during the 2011 wet season. Within 24 h PSII in cyanobacteria from a range of crust types had resurrected and CO_2-uptake was verified, although different crust types responded at significantly different rates. These are the first studies that have demonstrated that PSII does not respond to rainfall during the dry season and cyanobacterial

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function appears controlled by other environmental conditions. It is likely that mass extracellular polysaccharide (EPS) production during the wet season, once dry, protects cyanobacteria from premature resurrection in the dry season. We propose that EPS regulates moisture penetration, thus the resurrection of PSII at the onset of the wet season, at which time moisture and humidity alters the rheological properties of EPS permitting rehydration.

Keywords Cyanobacteria · Biological crusts · EPS · ECM · Drought · Desiccation · Resurrection · Australian savannah

Introduction

Throughout Australia's tropical and sub-tropical grasslands, savannahs and shrublands ecoregion, cyanobacterial soil crusts are abundant, often entirely covering the soil surfaces in between grass plants. These biological soil crusts are variably comprised of cyanobacteria and liverworts, and to a lesser extent mosses and lichens (Williams and Büdel 2012). In dryland and savannah landscapes cyanobacteria make an important contribution to nutrient cycling, soil structure, hydrological properties and erosion control (Eldridge and Greene 1994; Belnap and Lange 2003; Mager and Thomas 2011; Elbert et al. 2012). These environments are subject to extreme temperatures and ultraviolet (UV) exposure, seasonal droughts and floods. For survival, crust organisms rely on specialist adaptations that provide them with the capacity to detect and respond to environmental conditions (Stal 1995; Ullmann and Büdel 2003).

Cyanobacteria produce extra-cellular polysaccharides (EPS) that encapsulate cells, binds filaments and colonies together in a common crustal structure described as the extracellular matrix (ECM) (Helm and Potts 2012). Seasonal environmental stresses result in changes to both the composition and organization of the ECM, as cyanobacterial cells adjust to environmental perturbations (Helm and Potts 2012). Changes can include the intertwining and tangling of filaments, where rope-like formations are cemented together with EPS excretions and combine to establish the overall structure of the ECM. This ability of "rope"-formation evolved in several discrete filamentous cyanobacterial clades and has been reported to provide fitness advantage in habitats with highly erodible substrates (Garcia-Pichel and Wojciechowski 2009).

It is believed that adaptation to desiccation may include a common set of structural, physiological and molecular mechanisms that constitute desiccation tolerance (Potts et al. 2005). In situ resurrection of *Microcoleus vaginatus* (Vaucher) Gomont showed that regulatory genes signalled the rehydration event accompanied by the up-regulation of anabolic pathways (Rajeev et al. 2013). Understanding the mechanisms that underpin the tolerance of cells to intracellular water loss (desiccation) for extended periods of time (drought) is crucial in the interpretation of crust function and resilience. Stress responses as a result of dehydration are characterised by the activation of genes controlling oxidative and photo-oxidative responses, osmotic stress and the storage of C and N responses (Rajeev et al. 2013). Adaptation of cyanobacterial cells to physiological extremes of pH, temperature or cell stress (due to dehydration) are assumed to be driven toward optimum function rather than maximum stability, even though cyanobacteria do not grow while in a desiccated state (Potts et al. 2005).

Cyanobacterial mechanisms of desiccation tolerance

Cyanobacteria require functional characteristics that allow them to dry to equilibrium when humidity is extremely low and then recover normal function following rehydration (Potts et al. 2005). In times of osmotic stress, cyanobacteria compensate for moisture loss by producing cytoplasmic solutes and scytonemin (pigmentation) that protects against damage to its' photosystem II (PSII) apparatus (Potts 1994). PSII reaction centres containing chlorophyll form a key component of photosynthesis. In order for CO_2 uptake via cyanobacterial crusts to occur, PSII must be activated. Once moisture is available, cyanobacteria have the capacity to rehydrate within seconds and commence photosynthesis within minutes (e.g. Rascher et al. 2003). This desiccation–resurrection process is a mechanism of cell based drought endurance that allows cyanobacterial crusts as a whole to tolerate prolonged dry periods and to reactivate photosynthesis upon rehydration (Alpert 2005).

On an international level, researchers concur that resurrection of PSII in cyanobacteria takes place within approximately 1 h following rewetting. In cyanobacteria from a range of tropical locations it was shown that short term desiccation led to the suppression of variable fluorescence yield and inhibition of gas exchange (Lüttge et al. 1995). Nevertheless, these cyanobacteria all recovered within 1 h following rewetting. In the Negev Desert, cyanobacteria were shown to recover 50 % of PSII activity within about 5 min of rehydration (Harel et al. 2004). Satoh et al. (2002) demonstrated that recovery time of PSII for *Nostoc commune* Vaucher took place approximately 10 min after rewetting with 50 % of photosynthetic activity reached within 1 h of rewetting. Activation of PSII in desiccated cyanobacteria from the Guiana Highlands was typically 30 min following watering (Rascher et al. 2003).

The northern Australian savannah environment

The tropical and subtropical savannah ecoregion covers an expanse of approximately 1.9 million km^2 which equates to about a quarter of the Australian continent that spans across Western Australia, Northern Territory and Queensland (Online Resource 1), and covers 19 broad-scale geographic regions (bioregions), (www.environment.gov.au/topics/land/nrs/science-maps-and-data/australias-bioregions-ibra/australias-ecoregions). Cyanobacterial crusts are an important component of terrestrial ecosystem structure and function throughout this region (Ullmann and Büdel 2003; Tracy et al. 2010; Williams and Büdel 2012). In northern Australia, south easterly trade winds typify the dry season, with low humidity and minimal dewfalls. The wet season (October to April) is characterised by north-westerly monsoon troughs that frequently produce heavy rain and flooding. During the wet season humidity is consistently high with regular dewfall between rain events. Annually, there is very little rainfall during the winter-dry season months. Storms precede the wet season onset; days are hot with high ambient (>40 °C) and soil surface temperatures (>60 °C).

Cyanobacteria in these environments encounter droughts on an annual basis. This is normally followed by optimum moisture where the soil profile remains saturated for several weeks and the soil micro-surface habitat rarely dries. We had previously observed that cyanobacteria from these crusts had not reactivated during the dry season, even after rainfall. This was verified by CO_2 exchange measurements taken in the 2009 dry season at which time no carbon uptake or photosynthesis was recorded (unpublished data).

This contrasted with current knowledge regarding the resurrection process of PSII in cyanobacteria where it had been shown that cyanobacteria can recommence photosynthesis within seconds, minutes or hours, depending on the time they remained in a desiccated state (Satoh et al. 2002; Lange 2003; Rascher et al. 2003). We hypothesised that resurrection did not take place before new cyanobacterial growth during the next wet season occurred. Accordingly, the objective of this research project was to further the understanding of desiccation–resurrection processes of cyanobacteria that are subject to annual droughts typical of the northern Australian savannah.

Methods

Boodjamulla National Park (18.39°S, 138.62°E) is situated in the Gulf Savannah of northern Australia covering an area of 2,820 km^2 (Online Resource 1). Mean annual rainfall is 641 mm falling mostly between December and February, although it can be highly variable with up to 1121 mm (95th percentile) falling in the wet years (www.bom.gov.au). Boodjamulla is mainly situated on sandstone, limestone, calcium carbonate or tufa formations which sustain *Eucalyptus* and *Melaleuca* woodlands, floodplains, grasslands and riparian vegetation. Soil crusts dominated by cyanobacteria and liverworts are prolific across the floodplains and grasslands.

Desiccation–resurrection tests

An IMAGING-PAM *M-Series*, (Heinz Walz GmbH, Effeltrich, Germany) was set up at our field laboratory for the purpose of recording the fluorescence responses and effective PSII quantum yield of the crust samples following rehydration. The saturation pulse determined the photosynthetic performance of cyanobacterial soil crusts and an assessment of the quantum yield of energy conversion at PSII reaction centres. A colour spectrum depicted intensity of PSII activity. Chlorophyll fluorescence and PSII yield was established with ImagingWin software v2.41a (Heinz Walz GmbH, Effeltrich, Germany). Effective PSII quantum yield (YII) was calculated according to Genty et al. (1989). YII is based on a scale of 0–1 that is applied to all photosynthesising organisms including plants where:

$YII = (Fm' - F)/Fm'$ - where Fm' is maximum fluorescence yield and F is fluorescence yield.

For the duration of the experimental time period rainfall measurements from 2009–2011 were derived from Boodjamulla National Parks official rain gauge, which are recorded at 9 am on a daily basis and transferred to the Australian Bureau of Meteorology (www.bom.gov.au). Relative humidity (RH) was recorded (through infrared) at 30 min intervals in the open gas exchange system (Klapp Kuvette, Heinz Walz GmbH, Effeltrich, Germany). This machine was installed outside under natural field conditions for the purpose of calculating net productivity of cyanobacterial crusts for an associated study. Long-term mean RH (109 years) was derived from Australian Bureau of Meteorology records for Burketown (1890–2009).

Cyanobacterial soil crust sampling

Cyanobacterial crusts were sampled at Boodjamulla over two time periods: (1) August, 2009 dry season, after 125 days without rain and; (2) October, 2010 late dry season, preceding the onset of the wet season. The first stage of the trial was carried out in August,

2009 and, the second stage in January, 2011 (Table 1). For both sampling periods, three uniform crust types dominated by cyanobacteria were chosen as representative of those most commonly found in the region. For the sample set that was put aside for the wet season tests the Petri dishes were tightly packed with padding to restrict air movement, sealed and stored in the research site laboratory at approximately 40 °C. During the wet season (January, 2011) these samples were reintroduced into their natural environment at a time of high humidity and, saturated through natural rain events.

Dry season desiccation tests (2009)

For the dry season desiccation tests, six cyanobacterial–liverwort crust samples (~5 cm diam. × 0.5 cm depth), were removed from Petri dish samples and fixed into 5 × 5 cm aluminium mesh baskets. Rehydration of the crust samples was carried out by the addition of rain water with care taken not to supersaturate the crust. The samples were kept outside in shady conditions (~28 °C daytime maximum) throughout the experiment. PAM images were recorded 1 h after a wetting event each day. Each image was examined and ten points were randomly selected (using the ImagingWin program) to obtain YII values for each time period and each day. Data for all six crusts was combined and used to calculate mean YII for each daily time period.

Wet season resurrection tests (2011)

Due to the time it takes to record images, three main crust types (C1–3) were selected for the daily measurements (Table 1). On Day 1 prior to being exposed to natural rainfall, the crust samples were examined with the Imaging PAM to ensure there was no PSII activity. The first rains occurred on the afternoon of Day 1 (January 12, 2011) and measurements were taken immediately after the samples were wet (2:00 pm), as well as 1 h later (3:00 pm). It rained overnight and the crusts were super-saturated by the next morning (Day 2). Measurement intervals for Days 2 and 3 are described in Table 1. Crust data were analysed using a mixed-models ANOVA with two error terms (time nested within crust type). Statistical analysis was carried out using Minitab (2007). PAM measurements were also taken for all crusts (C1–C6), following rain on Day 7 and Day 14 (Table 1).

Across the same time frame (January 12–17, 2011), a series of in situ tests were undertaken to record the photosynthetic yield of wild crusts (Table 1). These were carried out with a portable MINI–PAM (Walz, Germany) at 11 am over a 5 day period. A 25 cm square light weight mesh grid (with 25 mm internal squares) was constructed and ten squares were selected using randomly generated computer numbers. The mesh grid was arbitrarily thrown out five times at the selected site ($n = 50$ replicates per site, see Table 1). Zero point was established with the MINI–PAM then YII was recorded in each of the predetermined squares. This process was repeated for crust types C1–4 representative of sample collection sites for the associated resurrection trials (Table 1).

Laboratory identification of cyanobacteria

Initial identification of cyanobacteria and crust types had been carried out as part of the Queensland–wide survey (e.g. Williams and Büdel 2012). Samples used in this experiment were also identified in terms of cyanobacterial composition based on the dominant taxa. Initial inspection of the soil crust was made using an Olympus SZH10 microscope at

Table 1 Descriptions of dry season and wet season desiccation and resurrection sampling periods and trials. Field samples were collected using a 100 mm metal spatula to carefully excavate the crust into Petri dishes (90 × 25 mm); at the time of sampling all dry season samples contained <1 % moisture

Collection time period & season	Experiment time & season	Sample collection	Photosynthetic Yield tests with Imaging PAM	Rehydration treatment
August 2009 Dry season	August 2009 Dry season	6* crust replicates (n = 6) *(C1–C6)	Days 1–10 3× daily (18 daily images) 8:00 am, 10:00 am & 2:00 pm	Rewet 2–3 ml rainwater three times daily, measurements 1 h later
October 2010 Late dry season	January 2011 Wet season	3* crust types × 5 reps (n = 15) *(C1–C3)	Days 1–3 4× daily (60 daily images) 8:00 am, 10:00 am, 12:00 pm & 2:00 pm	Natural rainfall Day 1–5 mm Day 2–15.6 mm
October 2010 Late dry season	January 2011 Wet season	6* crust types × 5 reps (n = 30) *(C1–C6)	Days 7 and 14 2× daily (60 daily images) 10:00 am & 2:00 pm	Natural rainfall and rewet Exposed as above Day 7 & Day 14
January 2011 Wet season	January 2011 Wet season	In situ measurements 4* crust types × 5 reps at 10 points (n = 20 × 10) *(C1–C4)	Days 1, 3 & 5 1× daily (200 daily records) 11:00 am	Natural rainfall As above (days 1–2)

* C1–C6 are described in Fig. 1

70 × magnification. Live material was examined by Nomarski differential interference contrast (DIC) microscopy with a Jenaval (Zeiss Jena) and an Olympus BX51 compound microscope (magnifications 400–1000×). Morphological features and measurements were carried out from multiple wet mounts that were prepared from each sample. Photomicrographs were taken using an Olympus SC100 digital microscope camera, and measurements of live material with Olympus cellSens® digital imaging software. Identification was performed to a species level (wherever possible) in the laboratory using the following taxonomic references: Geitler 1932; Komárek and Anagnostidis 1999; Skinner and Entwisle 2001; Komárek and Anagnostidis 2005. It was often necessary to record the closest named genus or species as attributes varied somewhat to temperate climate and aquatic specimens described in literature.

For electron microscopy a low temperature scanning electron microscope (Supra 55VP, Carl Zeiss, Oberkochen, Germany) was used to study fully hydrated cyanobacterial crust. The crust samples were frozen in liquid nitrogen slush (K1250X Cryogenic preparation system, Quorum technologies; Ashford, UK) and mounted on special brass trays. After sublimation at −80 °C for 30 min, samples were sputter-coated with gold–palladium and viewed at a temperature of −130 °C and 5 kV accelerator voltage.

Statistical analyses for the dry season and in situ tests were carried out in MS Excel and XLSTAT (www.xlstat.com). For the wet season analysis, a mixed-models ANOVA (Minitab 2007) with two error terms was used with time nested within crust type.

Results

Northern Australian 2009–2011 season

Dry season sample collections were taken in August (2009) after 125 days without rain. Preceding the 2010 sample collection there had been only 11.4 mm of rain scattered across five events over the months of May to September (174 days), with minimal rainfall for a period of 88 days. This pattern of a seasonal drought with lower temperatures and low humidity throughout the cooler months is typical of the northern Australian savannah (www.bom.gov.au/climate/about/). RH between August, 2010 and March, 2011 exhibited mean daily values of <50 % throughout the dry season to >90 % during the wet season. In April, 2011, RH declined as rainfall decreased towards the end of the wet season. Our data compared well with long-term mean RH (9 am and 3 pm) over 109 years for nearest available weather station at Burketown (Online Resource 2) where RH peaks at the height of the wet season (February), compared with low humidity (<50 %) experienced throughout the dry season (May–September).

Habitat and diversity of cyanobacterial crusts

Around the research site the alluvial floodplains, wetland areas, clay flats and shallow stony loam areas were host to a combination of early successional and well established crusts. The crusts collected during the dry seasons of 2009 and 2010 ranged from early to mid-successional (after Büdel et al. 2009), and are described in Fig. 1. The flats and loams were mostly mid-successional crusts dominated by *Scytonema* and/or *Symplocastrum*, often together with *N. commune* and other less abundant cyanobacteria. The mid-successional crusts were recognisable due to a large representation of liverworts (*Riccia* spp.).

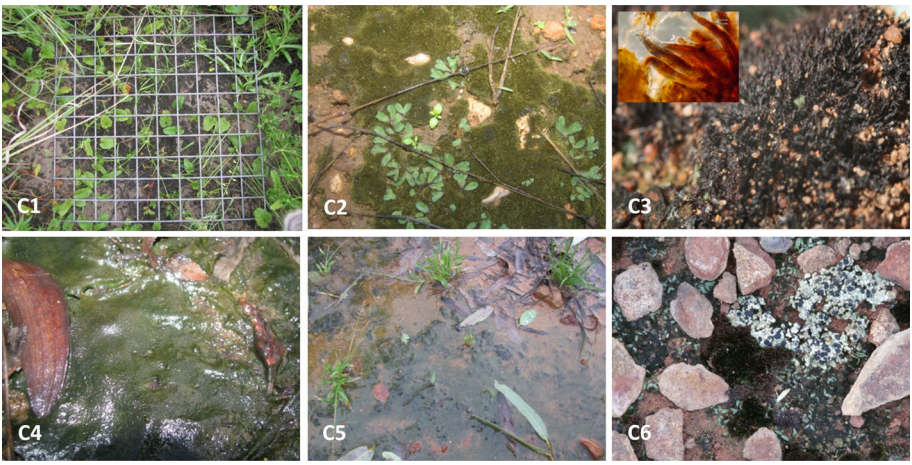

Fig. 1 **C1–C6** crust types and habitat: **C1** Black soil flood plains, ephemeral cyanobacterial crusts dominated by *Nostoc* (25 mm grid overlay); **C2** Mid-successional crusts co-dominated by *Scytonema* with liverworts *Riccia* abundant in the perennial grass and shrubland flood plains; **C3** Mid-successional crusts of *Symplocastrum* formed small colonies or large areas of tufted velvety crusts that looked dark green (new), changing to deep burgundy and black once pigmentation developed, inset *Symplocastrum* magnification × 400 (scale bar 20 μm); **C4** Early successional crusts of *Symploca* formed homogenous leathery mats throughout low-lying ephemeral flood zones, often under shady stands of *Melaleuca*. Once mature (1–3 days) a vibrant red pigmentation (UV protectant) was visible; **C5** *Nostoc* and *Scytonema* were common in highly disturbed areas and preceded C2 (early successional) crust development; **C6** Mid to late successional crusts with small colonies of lichens and liverworts were common in the stony Spinifex ridges and plateaus, dark green/black tufts are new colonies of *Symplocastrum*

Early successional crusts were clearly visible due to their dark blackish or red colour and covered almost the entire soil surface between the trees, shrubs and grasses.

The dry season – desiccation tolerance

In the savannah cyanobacterial crusts remained in a desiccated state for around half of the year. The results showed that upon rehydration cyanobacteria that had remained dry for 125 days without any rain had not reactivated and there was no resurrection of PSII within a 10 day period (Fig. 2). The initial wetting of the samples did not yield any PSII activity. Following rehydration on Day 2, liverworts and lichens had resurrected and over the next 10 days most samples consistently improved in yield (Fig. 3). Days 5 and 8 showed the start of what was later established to be a new colony of *N. commune,* however there was no evidence of resurrection of PSII in other cyanobacteria. Following Day 10 microscopic examination established there were new colonies of *N. commune,* while other cyanobacteria such as *Scytonema, Symplocastrum* and *Stigonema* although apparently viable, still remained in a desiccated state. Whilst for these new colonies of cyanobacteria YII still remained low on Day 10 (0.15 ± 0.02SD, $n = 60$), liverworts and lichens attained about 70 % resurrection on Day 2 and 95 % by Day 3 (0.43 ± 0.13SD, $n = 60$), (Fig. 3).

Wet season resurrection

At the height of the wet season, following 7 months with no significant rainfall, cyanobacteria in crust types C2 and C3 commenced photosynthesis within 12 h of rain–induced

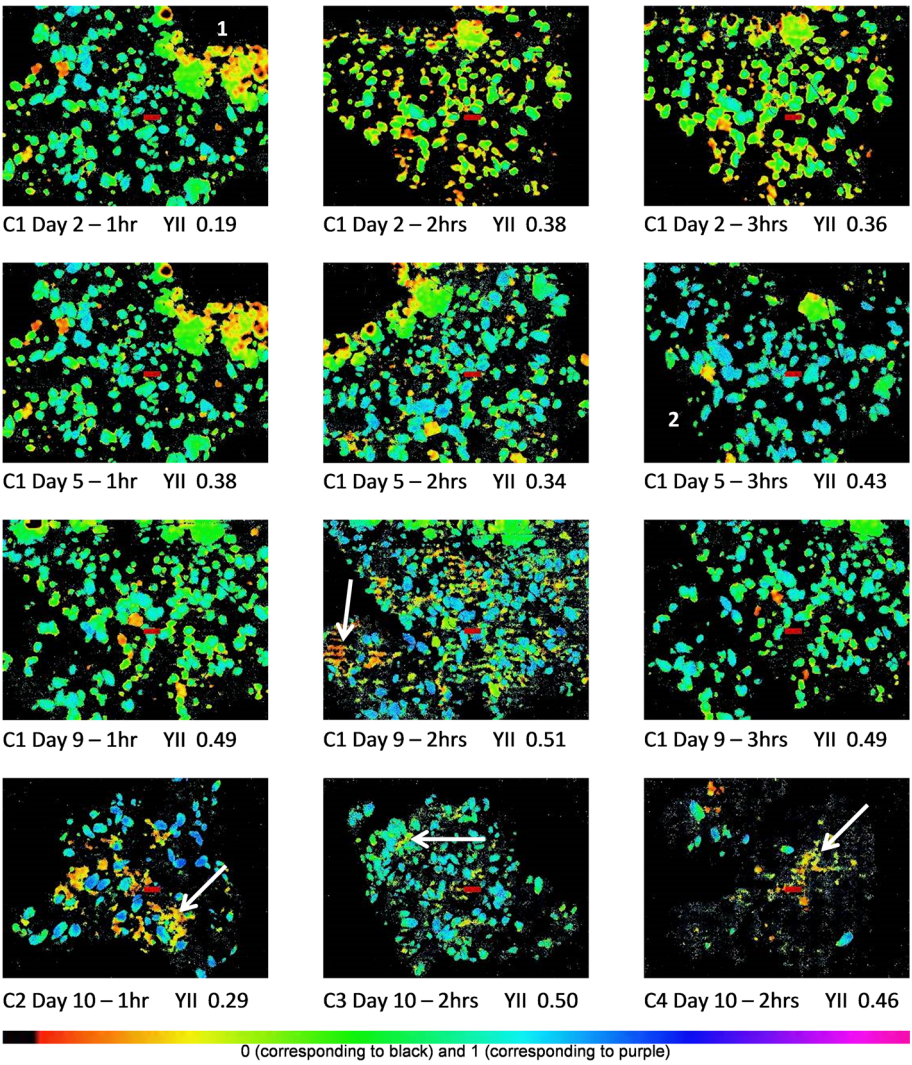

Fig. 2 Dry season resurrection trials: Imaging PAM records for C1 on Days 1, 5 and 10 with overall mean photosynthetic yield (YII) for liverworts and lichens illustrated by a colour spectrum of 0 (black) to 1 (purple) corresponding to an increase in yield intensity: **1** represents the resurrection of lichen colonies (adjacent to number in green/yellow); **2** represents liverworts almost completely resurrected (adjacent to number in blue); *arrows* represent the new growth of *Nostoc commune* in Days 9 and 10

resurrection and, C1 within 24 h (Fig. 4). There were significant time × crust interactions (df 14, $p < 0.05$) during the stages of PSII resurrection in C1–C3 (Fig. 4). C2 incorporated a larger representation of liverworts and sustained a higher level of activity for the majority of the resurrection phase. C3 that contained occasional lichens and a greater overall diversity attained the highest mean yield at the final measurement, although not significantly higher than C1 and C2.

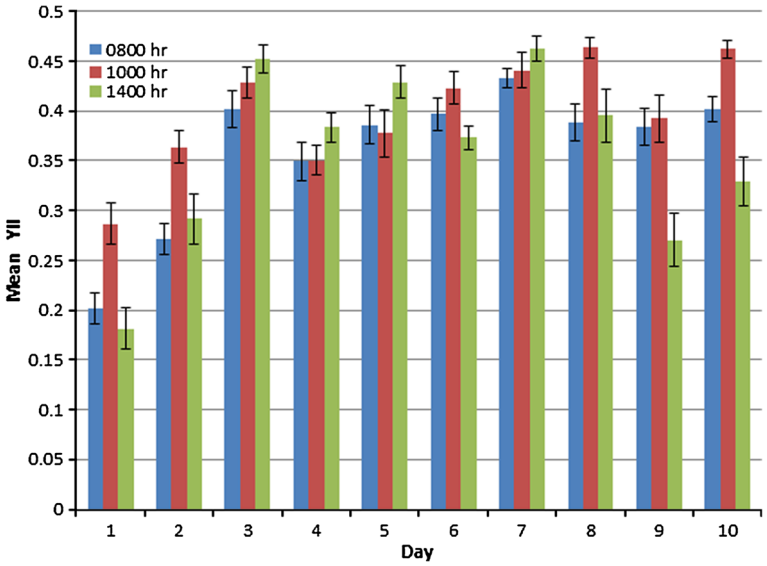

Fig. 3 Daily mean yield (YII) over 10 days, Error bars ± SE, n = 60 for all other than Day 3 0800 n = 58 and Day 6 all times n = 50. Prior to Day 1 the samples were dry hence no yield

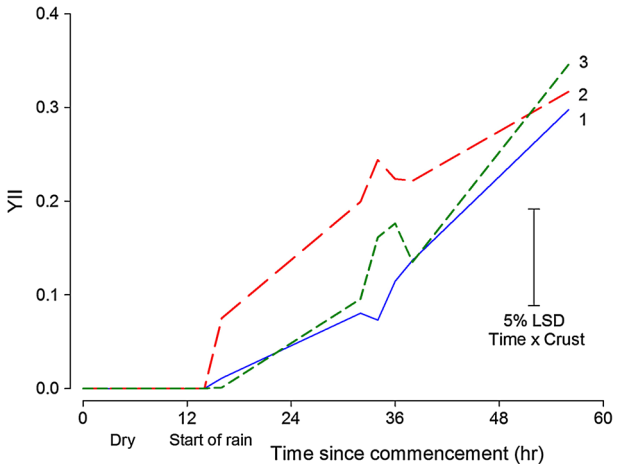

Fig. 4 Photosynthetic yield (YII) in the resurrection of PSII over time showed significant differences between time and crust type, where any times on any crust that are greater in size than the 5 % LSD bar are significantly different. Annotations 1–3 represent sample sets C1–C3

On Day 7 all of the crusts had attained full resurrection of PSII. Since initial exposure to rain (Day 1), these were the first measurements recorded for C4–C6 (Day 7) where YII ranged between 0.44 ± 0.1SD and 0.48 ± 0.01SD (n = 15 per crust type). On Day 14 there was a small rain event (6 mm), 8 h prior to measurements; YII for C1–C6 ranged between 0.28 ± 0.02SD (C1) and 0.34 ± 0.02SD (C5), typically representative of the low moisture levels (see also in situ field results below).

Table 2 In situ wet season PAM measurements of crusts C1–C4 over five days; n = 50 per crust type

Crust	Photosynthetic yield (YII ± SE)		
	Day 1	Day 3	Day 5
C1	0.41 ± 0.11	0.47 ± 0.15	0.20 ± 0.21
C2	0.64 ± 0.14	0.55 ± 0.07	0.40 ± 0.24
C3	0.46 ± 0.08	0.38 ± 0.12	0.16 ± 0.17
C4	0.49 ± 0.81	0.47 ± 0.15	0.22 ± 0.21

During the wet season experiment (Days 1–5), in situ measurements of photosynthetic activity of crusts C1–C4, similar to the resurrection trials, showed there were significant differences in PSII yield (df 49, $p < 0.001$). All cyanobacterial crusts remained activated for 5 days, even though there had been no rain since the first measurements were taken (Table 2). This was due to the ongoing saturation of the soil profile (data not shown). Over the 5 days there was a gradual decline in PSII activity however, like the resurrection tests, C2 yield was significantly higher than the other crusts (df 49, $p < 0.0001$).

Microscopic cyanobacterial crust structure

The development of the cyanobacterial crusts appeared dependant on both morphological attributes of different cyanobacteria and the crust diversity. The dominant cyanobacterial genera (*Scytonema, Symploca, Symplocastrum, Stigonema* and *Nostoc*) identified microscopically had well developed extra-cellular sheaths (EPS) (Fig. 5a, b). This was accompanied by high levels of pigmentation mostly appearing dark red and black (e.g. Fig. 1 C3). Although it was frequently easy to identify different macroscopic colonies and genera, it was notable that the majority of the crusts contained an abundance of liverworts (*Riccia*) and were 'cemented' together. These crusts could be lifted in large pieces and were well entrenched into the upper soil surface. During the wet season the formation of these crusts was evidenced by mass EPS production (Fig. 5c, d). This mass EPS appeared to be associated with *N. commune* and often coated large tracts of diversified crusts. Nevertheless, *Symploca* also produced jelly-like mucilage (Fig. 5e) that created a leathery mat when dry. Mass EPS production incorporated into the crust structure is referred to as the extra-cellular matrix (ECM) (Helm and Potts 2012). *N. commune* was found to have two distinct outer layers representative of the ECM (Fig. 5a, b). Following dry season desiccation and wet season rehydration early microscopic images revealed many cells still remained in a desiccated state along with cells that were fully rehydrated (e.g. Fig. 5a). During the dry season cyanobacterial crusts dried and, often fractured (Fig. 5f), eventually breaking up after the first heavy rains of the wet season.

Cyanobacterial crust stratification on a seasonal basis was clearly evident in *Symplocastrum* crusts where the current active crust was growing over a decaying crust from the previous year (Fig. 6a). A basal layer constructed from previous seasons' crusts was closely integrated into the soil surface. Detailed SEM images illustrate that tufted filaments intertwined with soil particles at all levels of the crust (Fig. 6b). *Symplocastrum* thallus was felt-like, bound together with EPS to form a tufted surface (Fig. 6c) with trichomes enveloped by a common sheath (Fig. 6d).

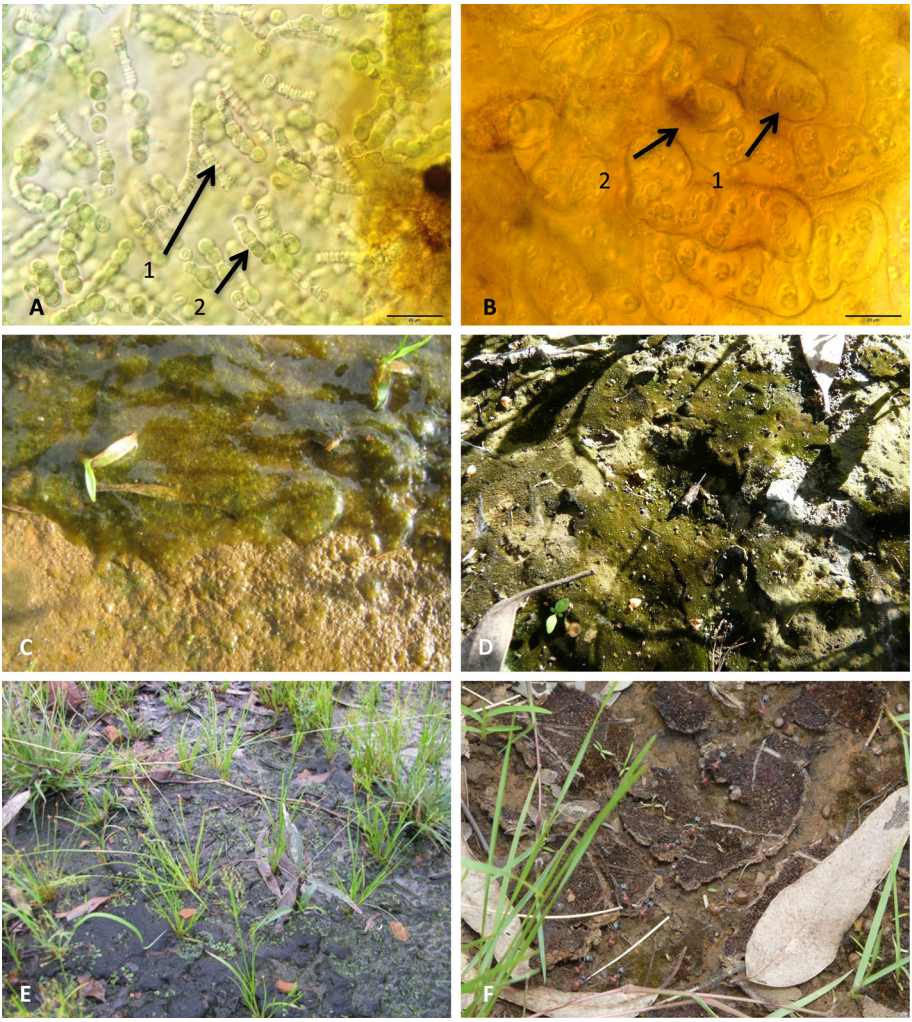

Fig. 5 Cyanobacteria and associated crusts in desiccation–resurrection phases: **a** *Nostoc commune* in a transition state between desiccation (*arrow* 1) and rehydration (*arrow* 2), embedded in EPS (*scale bar* 20 μm); **b** *Nostoc commune* encapsulated in EPS (*arrow* 1) and embedded in ECM (extra-cellular matrix, *arrow* 2), (*scale bar* 20 μm); **c** *Nostoc* in gelatinous mass EPS production state overflowing new *Scytonema* crust; **d** following mass EPS production, consolidation of ECM occurred with defined crust formation; **e** Newly developed crust (still saturated from rain) that incorporated *Symplocastrum, Scytonema, Symploca, Nostoc* and liverworts (*Riccia*); **f** Dried and cracked *Symploca* dominated crust in situ in early stages of disintegration following the first rains early in the wet season (Merluna, Cape York, 2010)

Discussion

Cyanobacterial soil crusts found at Boodjamulla National Park were representative of a suite of cyanobacterial taxa found throughout the Gulf Savannah of northern Australia that produce EPS to create a crust matrix. From this current research we have shown that

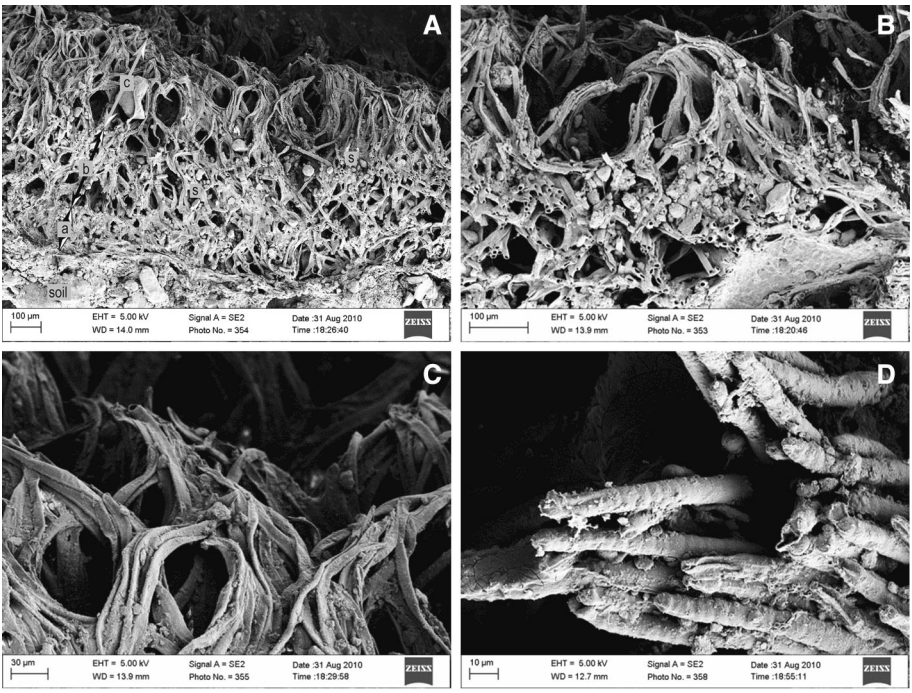

Fig. 6 SEM images of *Symplocastrum* crust: **a** Stratification of cyanobacterial crust seasonal growth (*c*), preceding season (*b*), including basal layer of the remnants of last season's crusts (*a*), s = soil particles included in between the cyanobacterial filaments; **b** transection detail illustrating incorporation of soil particles into filamentous crust structure; **c** tufted thallus; **d** trichome detail depicting cells inside outer sheath

cyanobacteria which survive in these environments do not respond to dry season rainfall. In the northern Australian savannah the ECM appeared to be an important defence against unseasonal rainfall events, yet are not critical in desiccation tolerance. These crusts differed from southern Australian late successional crusts, whose structure is underpinned by an abundance of lichens, mosses and liverworts that bind and reinforce the crust (Eldridge and Greene 1994; Eldridge and Rosentreter 1999). Both the northern and southern Australian crusts are subject to drought and long periods of desiccation. In southern dryland crusts, cyanobacteria are frequently restricted to smaller surface colonies or more mat-like crusts with a greater representation of subsurface cyanobacteria (e.g. Williams and Büdel 2012).

Cyanobacterial soil crusts in the northern Australian savannah are an integral part of the terrestrial ecosystem. Crust communities are dynamic –they are composed of a diversity of cyanobacterial taxa within a common matrix (ECM), suggesting that collectively they possess mechanisms that result in the survival of the crust system under extreme conditions (Helm and Potts 2012). Diversity and crust composition appears varied according to their responses to moisture and subsequent recovery of PSII following rehydration. It is likely that different crust types (including the individual species therein) have physiological attributes that influence the reactivation time periods of PSII. The results of this study have demonstrated that the resurrection of PSII in cyanobacteria was strongly influenced not

only by the length of time that the cyanobacteria remained in a desiccated state, but also by other environmental attributes of the savannah.

Cyanobacterial desiccation tolerance in the dry season

Previously it has been documented that drought resistant cyanobacteria have the capacity to rapidly rehydrate following desiccation, once moisture becomes available (Garcia-Pichel and Belnap 1996; Lange 2003). Although there had been differences in the reported rate of PSII resurrection following rehydration it has generally occurred within a time scale of 1 h (Scherer et al. 1984; Lange et al. 1994; Potts 1994; Dodds et al. 1995; Garcia-Pichel and Belnap 1996; Satoh et al. 2002; Harel et al. 2004; Ohad et al. 2005). During the resurrection of *M. vaginatus* a hydration event was signalled by the induction of DNA repair and regulatory genes with apparent synchronization between the cyanobacterium and its environment (Rajeev et al. 2013).

In the savannah of Boodjamulla National Park, cyanobacterial crusts are abundant despite being exposed on an annual basis to extensive dry periods of about 6 months at which time moisture is a limiting factor. Clearly, the crust organisms are well adapted to the wet–dry seasonal patterns and contain inherent survival mechanisms. The capacity of cyanobacteria to suspend the processes of photosynthesis and then reactivate following rehydration partly explains their survival strategy. Notwithstanding, the results of the dry season experiment showed no resurrection of PSII in cyanobacteria occurred over the 10 day rehydration trial. Likewise, during the dry season, cyanobacterial crusts from a 1,500 km east–west transect across the northern Australia savannah ecoregion could not be resurrected.[1] This led to speculation as to whether certain cyanobacteria from preceding wet seasons may not recover but rather breakdown organically, followed by the development of new colonies of cyanobacteria. A number of environmental and physiological factors may have contributed to this including: (1) the length of time in which cyanobacteria remain dormant (Scherer et al. 1984; Lüttge et al. 1995; Qiu and Gao 1999; Lange 2003); (2) physiological functions (Mazor et al. 1996; Potts 1999); (3) moisture availability (Lange et al. 1994; Dodds et al. 1995; Satoh et al. 2002; Rao et al. 2012); (4) micro-environmental conditions including soil surface temperatures (Schreiber et al. 2002; Rao et al. 2012) and; (5) crust type and species diversity (Büdel et al. 2009).

Even though studies on rehydration and recovery of metabolic activity have shown the time required to reactivate PSII increases with the length of desiccation (Scherer et al. 1984; Potts 1999; Qiu and Gao 1999), it is possible that the time period separating the event of desiccation and resurrection could also compromise viability. This has been demonstrated through studies of the widespread, unicellular rock inhabiting and lichen photobiont forming cyanobacterial genus *Chroococcidiopsis*. In old desiccated colonies most cells die, and the few surviving cells were interpreted as being resting cells from which new growth started again (Caiola et al. 1996). Such survival cells in colonies of *Chroococcidiopsis* sp. were also found under nitrogen limitation and explained by Billi and Caiola (1996) as "functionally comparable to akinetes", the latter being known as resting cells from filamentous cyanobacteria of the Nostocales

[1] In July and September, 2012, cyanobacterial crusts at nine sites across a 1500 km EW transect (through QLD, NT and WA) were tested using a Pocket PAM (Gademann Instruments, Germany) by W. Williams and B. Alchin. Cyanobacteria were soaked with rainwater in situ at hourly intervals (× 3) and the following day (at selected sites) and there was no PSII activity recorded at any of the sites.

order. Nonetheless, there is a difference between cyanobacteria subject to long dry periods, in that desiccation-tolerant cells accrue proteins that serve some structural protective role (Potts 1994).

Resurrection of photosystem II in the wet season

When cell desiccation processes occur, compounds like disaccharides protect against protein breakdown by means of replacing the water-shell around the cyanobacterial molecules with extracellular glycan (Potts 1999). This has been observed in *N. commune*, where in times of low humidity and high UV, glycan acts as a protective polymer-like coating preventing fusion across the cell membrane (Hill et al. 1997; Potts 1999). For reactivation to occur this process must be reversed. Under humid conditions glycan's rheological properties reinstate the qualities of the cell membrane, hence allowing the diffusion of water into the cells (Potts 1999). In the savannah landscapes following several months of low humidity, high UV and high soil temperatures, the glycan processes may account for the apparent cell dormancy observed in this study. In these environments, under most circumstances EPS production is paramount to the formation of a stable crust matrix. We propose that in the northern Australian savannah, ECM is critical to cyanobacterial desiccation-resurrection processes and therefore long-term persistence of cyanobacterial crust communities.

These are the first field studies in the Australian savannah which have demonstrated the influence of environmental conditions that appear to control the function of cyanobacterial ECM and, subsequently the resurrection of PSII. Mass EPS production occurred several times throughout the wet season. As the humidity dropped, temperatures remained high and sunny conditions prevailed, at which time the ECM hardened and dried to form hydrophobic polymeric-like surfaces. As temperatures oscillated, crusts cracked and curled, then after the first wet season rains, the crusts started to disintegrate. Once there had been follow-up rains new crusts started to grow. Under these conditions the ECM apparently provided a key survival mechanism as it remained hydrophobic throughout the dry season. In conjunction with the first rain events of the wet season, the ECM disintegrated and the crust exhibited hydrophilic properties. Annually, it is likely that the breakdown of the ECM and partial cell lysis occurs during the first significant rain events of the wet season. Evidence of the disintegration of the crust structure was observed in the SEM images of *Symplocastrum* where a basal layer of the previous season's crust is still visible (Fig. 5a). The breakdown of the crust structure appeared to commence once humidity levels were maintained at a high level (e.g. October–December).

The EPS forms a structural scaffold with rheological properties that can accommodate the rapid changes that occur upon rehydration following desiccation (Helm and Potts 2012). Following rain, the rheological properties of glycan likely result in a transformation of the ECM to permit the diffusion of water into cells at which point rehydration takes place. The wet season resurrection trials demonstrated responses to rehydration had taken place within 24 h of exposure to the natural environment (Fig. 4), suggesting that cyanobacterial crusts respond to differences in the seasonal conditions. Nevertheless, it was unclear what lead time (days, months) is necessary under conditions of high humidity for resurrection to take place. Wet season resurrection was in direct contrast to the dry season tests where cyanobacteria had not reactivated after 10 days. The dry season experiment was carried out during a time where humidity was low and soil temperatures high. These

environmental conditions may well have been highly influential in the failure of PSII to reactivate in cyanobacteria from this region.

Crust responses to resurrection

In both the wet season trials and the associated in situ measurements it was evident there were significantly higher rates of resurrection and photosynthesis in crusts where liverworts were abundant (Table 2, Fig. 5). Although the reason for this is not immediately clear, we suggest that small–scale alterations to infiltration where there was a profusion of liverworts in the crusts may be an explanation. Structurally, the liverworts may improve water retention within their immediate vicinity (Warren 2003). PAM Images of PSII resurrection indicated that cyanobacteria immediately adjacent to liverwort leaves reactivated first. In the wet season trials PSII yield measurements were restricted to cyanobacteria whereas in the field measurements, the randomness of the points and the seasonal abundance of liverworts meant that PSII yields from liverworts were included in the data. Nevertheless, both tests resulted in similar trends implying that there is an underlying structural difference that enhances cyanobacterial PSII yield. The abundance of liverworts has been recorded in cyanobacterial crusts ranging across central to northern Australia (Hodgins and Rogers 1997; Williams et al. 2008; Williams and Büdel 2012). Field data from a number of locations across the northern savannah indicate that in certain seasons liverworts dominate the crust and conversely cyanobacteria overgrow them at other times (Williams and Büdel, unpublished data). The association of liverworts and cyanobacteria in these environments is not well studied and deserves further investigation.

Concluding remarks

Our findings contrast greatly with current knowledge that has demonstrated rapid resurrection in cyanobacteria following drought. Clearly, mechanisms exist to protect cyanobacteria from cell lysis during the dry season; should cyanobacteria resurrect prematurely extra-cellular energy reserves would be depleted before function was fully restored. In the northern Australian savannah following the dry season drought, the ECM that binds cyanobacteria and liverworts into the crust formation apparently breaks down. Once the wet season commences cyanobacterial resurrection occurred and these ‘perennial crusts’ grow and restructure. We propose that the ECM regulates moisture penetration, thus the resurrection of PSII at the onset of the wet season, at which time moisture and humidity alters the rheological properties of EPS permitting rehydration.

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