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Wet season cyanobacterial N enrichment highly correlated with species richness and *Nostoc* in the northern Australian savannah

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Abstract. The Boodjamulla National Park research station is situated in the north-western Queensland dry savannah, where the climate is dominated by summer monsoons and virtually dry winters. Under shrub canopies and in between the tussock grasses cyanobacterial crusts almost entirely cover the flood plain soil surfaces. Seasonality drives N fixation, and in the savannah this has a large impact on both plant and soil function. Many cyanobacteria fix dinitrogen that is liberated into the soil in both inorganic and organic N forms. We examined cyanobacterial species richness and bioavailable N spanning 7 months of a typical wet season. Over the wet season cyanobacterial richness ranged from 6 to 19 species. N-fixing *Scytonema* accounted for seasonal averages between 51 and 93 % of the biocrust. Cyanobacterial richness was highly correlated with N fixation and bioavailable N in 0–1 cm. Key N-fixing species such as *Nostoc*, *Symploca* and *Gloeocapsa* significantly enriched soil N although *Nostoc* was the most influential. Total seasonal N fixation by cyanobacteria demonstrated the variability in productivity according to the number of wet days as well as the follow-on days where the soil retained adequate moisture. Based on total active days per month we estimated that N soil enrichment via cyanobacteria would be $\sim 5.2\,\mathrm{kg\,ha^{-1}}$ annually which is comparable to global averages. This is a substantial contribution to the nutrient-deficient savannah soils that are almost entirely reliant on the wet season for microbial turnover of organic matter. Such well-defined seasonal trends and synchronisation in cyanobacterial species richness, N fixation, bioavailable N and C fixation (Büdel et al., 2018) provide important contributions to multifunctional microprocesses and soil fertility.

1 Introduction

The northern Australian savannah is one of the largest natural savannahs remaining on Earth with grasslands and shrublands that cover more than 1.5 million $\mathrm{km^2}$ (Nix et al., 2013). Over the past century there have been several major degradation episodes, leaving about half of these important ecosystems in a degenerated state (Smith et al., 2007). It is a harsh environment where climate shapes the ecology, distribution and abundance of resources, affecting plant and animal species (Nix et al., 2013). There is a pronounced dry season, often lasting around 6 months, followed by violent storms and flooding rains. Across the savannah landscapes broadscale livestock grazing is the primary land use; however, managing these extensive perennial grasslands and woodlands demands an approach on several different scales. On a continental scale, empirical evidence clearly demonstrates the negative impact grazing has exerted on ecosystem structure including key aspects of soil function (Eldridge et al., 2016). Thus, to understand the scope and variability of the northern Australian savannah, soil function is important in the context of a holistic approach to land management (Vanderduys et al., 2012), and more importantly the preservation of soil microprocesses.

In the northern Australian savannah the interspaces between grass plants are inhabited by microorganisms that form an almost continuous cover dominated by cyanobacteria and liverworts, occasionally lichens and mosses, bacteria, algae and fungi (Williams et al., 2014). Cyanobacterial crust communities exist where there are only small fractions of organic nutrients, where diazotrophs (bacteria that fix dinitrogen into a more useable form) fuel soil food webs through photosynthesis and N fixation (Elbert et al., 2012). In these savannah

landscapes, cyanobacteria seasonally re-establish and facilitate soil surface stabilisation.

Cyanobacterial diversity characteristically provides a range of biochemical and physical attributes that promote resilience to microhabitat variability and climatic extremes (Rossi and De Philippis, 2015). As the biophysical structural form of the community develops, diversity of macro- and microorganisms increases (Büdel et al., 2009). As phototrophic organisms, cyanobacteria are, in mass, valuable as ecosystem engineers facilitating soil fertility on several levels (Jones et al., 1994; Eldridge et al., 2010). Newly developed cyanobacterial colonies exude slimy extracellular polysaccharides (EPS) that form organic bridges, tightly binding soil aggregates and particles (Rossi et al., 2017). Cyanobacterial EPS also form a cohesive and protective layer at the soil surface, minimising the effects of wind erosion (Eldridge and Leys, 2003). EPS provide cyanobacteria the capacity to maintain fitness and sustain growth for other cohabiting species (Rossi et al., 2017; Rossi and De Philippis, 2015).

Cyanobacterial mediated N fixation results in N liberated into the soil in both inorganic and organic forms that in turn leads to elevated soil inorganic N pools in the soil surfaces (Barger et al., 2016). Bioavailable N fixed as atmospheric N_2 by cyanobacteria delivers a direct source for plant uptake (Mayland and Mcintosh, 1966; Belnap, 2003). This reinforces the value of the relationship between plants and cyanobacterial crusts in arid, semi-arid and savannah landscapes. In N-depleted environments nostocalean cyanobacteria develop specialised thick-walled heterocyte cells as dedicated N-fixing sites to exclude oxygen that inhibits the N-fixing enzyme (Helm and Potts, 2012). These biophysical traits for N uptake are critical catalysts for cyanobacterial productivity and growth. To initiate rapid growth when conditions are favourable, storage of cyanophycin (N-storage granules) and carbohydrates are essential (Schneegurt et al., 1994). The relative abundance of cyanophycin within the cells and in the storage of polysaccharides, proteins, cell remnants and secondary metabolites in the extracellular matrix (ECM) provides cyanobacteria the capacity to withstand natural environmental stresses (Helm and Potts, 2012; Whitton and Potts, 2012). Communication between the cells and the environment occurs within the EPS (Rossi et al., 2017). With an increase in humidity the EPS alter their rheological properties and become hydrophilic, permitting water absorption (Helm and Potts, 2012). When it rains, up to 70 % of stored N is flushed out of the cyanobacterial outer matrix into to the surrounding substrate (Elbert et al., 2012; Magee and Burris, 1954; Rascher et al., 2003), where the release of N can increase if conditions are suboptimal following desiccation (Jeanfils and Tack, 1992), thus increasing inorganic N pools in the upper few millimetres of the soil (Barger et al., 2016).

In the northern Queensland savannah we had previously established that cyanobacteria detect the onset of the wet season, rehydrating and resurrecting cellular functions within 24 h of the first rains (Williams et al., 2014). It was apparent from the earlier studies that even when artificially rehydrated over several days cyanobacteria would not reactivate during the dry season (Williams et al., 2014). Yet, following the first rains, the cyanobacterial crust system appeared to disintegrate and regrow. It has been shown that there is a strong effect of precipitation variability on N cycling within the crust (Aranibar et al., 2004). Thus, the potential for pulses of bioavailable N over the course of the wet season was thought to be most likely connected to rainfall events. The observed rapid growth of the cyanobacterial crusts at the height of the wet season followed by EPS hyperproduction (Williams et al., 2014) led us to believe that bioavailable N would peak during this time. Based on the premise that community species richness directly impacts nutrient cycling (Maestre et al., 2012) and EPS secretions maintain fitness and store crucial resources (Helm and Potts, 2012; Rossi and De Philippis, 2015), we examined the cyanobacterial species richness and bioavailable N spanning the 7 months of a typical wet season. We hypothesised that cyanobacterial richness and bioavailable N would peak at the time of the heaviest rains and gradually decline in the latter stages of the wet season. We also expected that N fixation and N enrichment of the surface soils would be correlated to the abundance of N-fixing cyanobacteria.

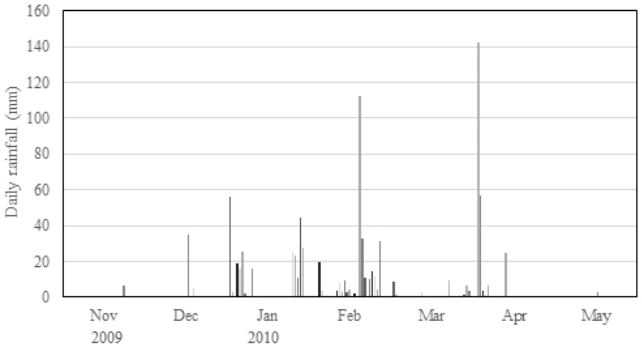

Figure 1. Daily rainfall events for November–May over the 2009–2010 wet season at the Boodjamulla National Park research site (source: www.bom.gov.au).

2 Methods

2.1 Site description

The Boodjamulla National Park research station (18.7395° S, 138.3190° E) is situated in the north-western Queensland dry tropics where the climate is heavily influenced by the summer monsoon season and described in detail in Williams et al. (2014). The lead-in 6 months to the 2009–2010 wet season at the research site was completely dry. In late November as humidity increased the early rainfall was typically a few small rain events (0.2, 6.8 and 0.2 mm) followed by heavy

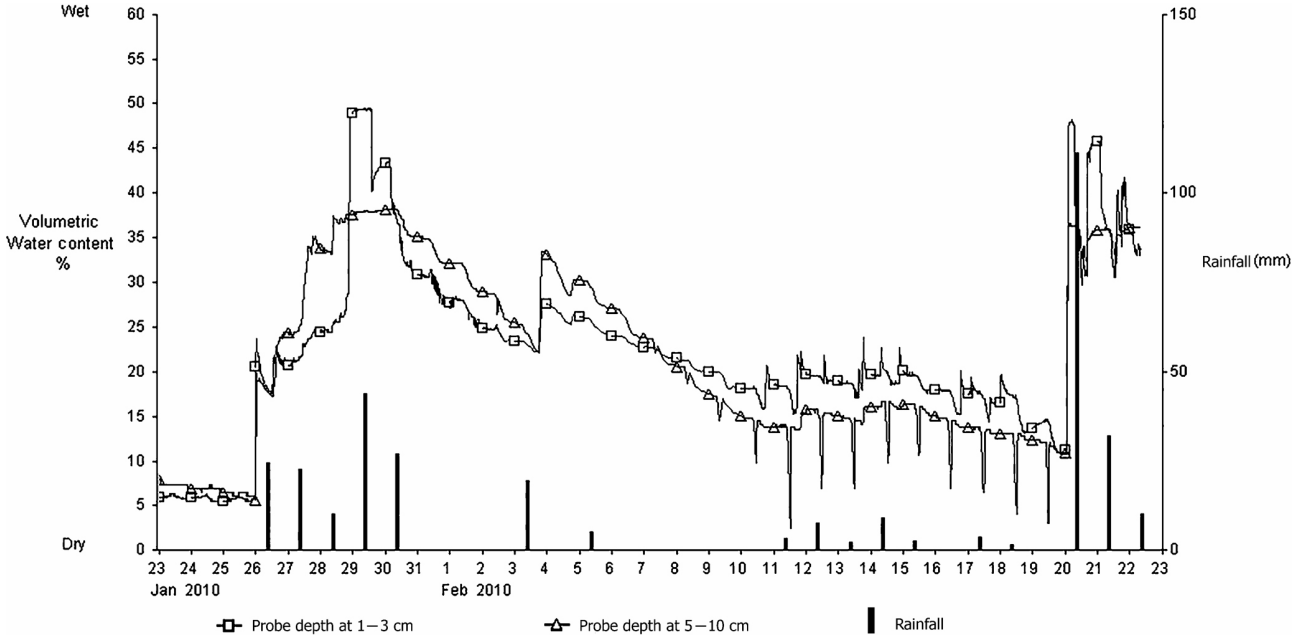

Figure 2. Example of soil moisture (volumetric water content %) patterns plotted against rainfall (mm) for 23 January to 23 February 2010 illustrating the retention of soil moisture after rain. A drying trend following significant rain was intermittently supplemented by smaller rain events, and then after 45 mm of rain on the 20 February the soil profile was recharged from 15 to 50 %. Note: downward spikes for 5–10 cm depth after 11 February are sensor artefacts and should be ignored. Moisture was measured with MEA ThetaProbe and GP1 data logger probes (mea.com.au).

rains throughout January (279 mm) and February to April (555 mm) (Fig. 1).

2.2 Field sampling

To determine seasonal patterns of cyanobacterial mediated bioavailable N, multiple sample sets were taken from November 2009 (pre-wet season rains) to May 2010 (end of wet season). Sampling was timed before, during and after major rain events to provide a snapshot in time (also see Williams and Eldridge, 2011), and incorporated cyanobacterial surface crusts (0–1 cm) and immediately below the crust (1–3 cm) ($n = 5$ each depth). Cyanobacterial surface crusts and subsoils were removed using a 10 cm metal spatula to extract exactly 0–1 cm vertical depth with care to include the complete sample, followed by 1–3 cm depth directly below the crust. For analysis we allocated the samples into nine time frames: one sample set for November, December, March, April and May, and two sample sets from early and late January and February. Each time (monthly and twice-monthly) represented at least two separate sample periods, before and after rain. For pigment content (chlorophyll *a*), rates of N fixation (acetylene reduction assays, ARA) and identification (for species richness and abundance studies), an additional four petri dishes of 0–1 cm were collected at the same time (total $n = 100$).

2.3 Laboratory analysis

2.3.1 Seasonal trends in N

Cyanobacterial crusts (0–1 cm) and subsurface soils (1–3 cm) were analysed for bioavailable N (NH_4^+ and NO_3^-) according to Method 4, Gianello and Bremner (1986) and Williams and Eldridge (2011). The samples had been immediately dried in the field (> 40 °C) and returned to the laboratory where they were stored dry and analysed after the end of the 2010 wet season. Duplicates for each of the nine time periods and each depth (minimum four reps) were sieved (1.86 mm) and weighed (20 mg) for both hot and cold analysis (total across all time periods for each depth, 0–1 cm $n = 125$, 1–3 cm $n = 99$). This method determines ammonium (NH_4^+) produced from organic soil N when the soil is heated with 2 M KCl in a stoppered tube at 95 °C for 16 h. NH_4^+ is determined by the difference between the NH_4^+ liberation during the hot distillation of 20 mg soil and the NH_4^+ present prior to heating (Gianello and Bremner, 1986). This analysis provides an index for the fraction of N that would become available to plants in a growing season and at the time of sampling (also see Tongway and Ludwig, 1996; Williams and Eldridge, 2011).

Seasonal N fixation was determined through acetylene reduction based on the Hawkes (2003) method for acetylene reduction assays and Stewart et al. (1968). To complete the monthly estimates of rates of N fixation, $\delta^{15}N$ of the crust

(see Sect. 2.3.2) was calculated for each sample and used as a conversion factor for each month's results. Petri dish samples of cyanobacterial crusts (reserved for ARA) for each month (November–May) were reactivated in the glasshouse for approximately 2 weeks. Full resurrection during the wet season (when humidity increases) was critical due to the inability of these cyanobacteria to reactivate during the dry season (see Williams et al., 2014). This was carried out by daily wetting to field capacity but not saturated, then allowing them to dry naturally. An effort was made to ensure the surface crust was unbroken. Following reactivation, 18 mm diameter plugs (six reps per month, $n = 36$) representative of 10 % airspace were carefully removed and inserted into 40 mL glass vials with two-way septa lids. Dry weight was calculated prior to a light rewetting (~ 1 mL liquid) with care not to oversaturate. The vials were then placed in natural light conditions in the glasshouse for a further 2 days to acclimatise prior to ARA. The incubation was carried out from time zero (T0) and measured at 24 h (T2) and 48 h (T3). In between measurements the samples were maintained in the glasshouse at 28 °C (previously determined as an optimum temperature for these crusts) and in natural light conditions (Büdel et al., unpublished data).

Calculation of ethylene production (C_2H_4 µm mL^{-1}) was carried out using the following standard formula (see Hawkes, 2003; Weaver and Danso, 1994):

$$V_{hs} = V_t - V_w - V_s,$$

where V is the volume, V_{hs} is the head space (volume of air in vial), V_t is the tube (volume of tube), V_w is the volume of water (mL added to sample) and V_s is the volume of solids (V_s is the weight of sample/soil bulk density for these soils of 1.6). Daily rates were calculated by T3–T2 (48–24 h) and then converted to grams per square metre. Monthly averages for $\delta^{15}N$ (derived from ARA samples, see Sect. 2.3.2) were then applied as conversion values and care was taken to ensure units were equivalent prior to final calculations. To estimate seasonal N fixation, the mean values of N fixation were calculated for each month and multiplied by photosynthetically active days. As we have clear data that indicate the periods of activity for these biocrusts (see Williams et al., 2014; Büdel et al., 2018), the number of photosynthetically productive days were calculated by the number of rain days plus the number of days that soil moisture was available following rain. This was based on moisture meter data from the site (example shown in Fig. 2) and in situ photosynthetic yield tests previously carried out by Williams et al. (2014).

2.3.2 Carbon, nitrogen and pigment content

Total C and total N, C : N ratio, and $\delta^{15}N$ and ^{13}C were determined with high temperature digestion using a vario MACRO elemental analyser (Elementar) and the Sercon Hydra 20–22 mass spectrometer (Griffith University laboratories). For each month for ARA analysis the samples were amalgamated, dried and sieved to provide three samples for each time period. Data were averaged to provide the conversion factors used in rates of N fixation; however, there was only one replicate available for Nov–Dec as there was insufficient sample. For pigment content, the chlorophyll *a* extractions were carried out on the cyanobacterial soil crusts (Barnes et al., 1992) and calculated using Wellburn's (1994) equations.

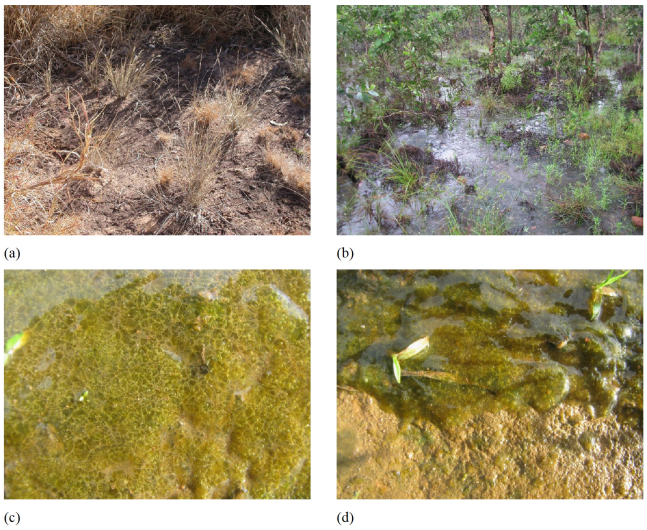

Figure 3. Seasonal cyanobacterial crust functions: **(a)** dry cyanobacterial crust, **(b)** flooded crust at the commencement of heavy rains in January, **(c)** rapid regrowth with extracellular polysaccharide (EPS) hyperproduction from *Nostoc* sp. and **(d)** gelatinous EPS during hyperproduction phase compared with bare area being recolonised.

2.3.3 Cyanobacterial richness and abundance

Morphological features and measurements were carried out from wet mounts prepared from each sample set for nine time periods (total $n = 625$). Abundances were determined from five subsamples of the five samples for each time ($n = 25$). The samples for the nine time periods were rehydrated for 24 h and examined using bright-field, phase contrast and differential interference contrast illumination systems with a Jena Zeiss and an Olympus BX51 compound microscope to a maximum magnification of 1000x. Photomicrographs were obtained using an Olympus DP12 digital microscope camera. Identification was performed to a species level (wherever possible) in the laboratory using the closest available keys (Anagnostidis and Komarek, 2005; Komárek, 2013; Komarek and Anagnostidis, 1999).

2.3.4 Statistics

We used linear regression models to examine potential relationships between bioavailable N (BioN) and cyanobacterial richness separately for N-fixing (Nrich) and non-N-

Table 1. Cyanobacterial species richness and abundance over 7 months (nine time periods) expressed as a percentage. N-fixing species (bold) were shown only if they produced heterocytes although we also determined (see main text) several non-fixing species were associated with N enrichment.

Cyanobacteria	Nov	Dec	Jan early	Jan late	Feb early	Feb late	Mar	Apr	May
***Scytonema* sp. 1**	**50.6**	**93**	**78.4**	**84.4**	**64**	**52.8**	**46.2**	**32.2**	**27.2**
***Scytonema* sp. 2**			**5.8**	**8.4**	**20.6**	**1.8**	**14.8**	**21**	**9.6**
***Scytonema* sp. 3**						**11.2**	**5.8**	**14.6**	**18**
Nostoc commune	**12.2**	**1.4**	**5.9**	**4**	**10.2**	**10.6**	**10.4**	**11.6**	**17**
***Nostoc* sp. 2**						**0.8**			
***Symploca* sp.**	**13**	**1.4**	**3.9**	**0.6**	**4.6**	**0.4**			**3.6**
***Gloeocapsa* sp.**							**10**	**0.8**	
Symplocastrum sp.	6.4	1.2	2			1.2	0.2	8	7
Schizothrix sp.	4	0.8	3.7	0.2		3.8	2.6	6	
Porphyrosiphon sp. 1	13.8	2.2		1.6	0.4	4.8		1.2	4
Porphyrosiphon sp. 2			0.1	0.2			0.6		0.2
Porphyrosiphon sp. 3								1.4	1.8
Porphyrosiphon sp. 4							1		
Microcoleus vaginatus			0.1	0.6	0.2			0.2	
Microcoleus paludosus						4.8	1.8		1.8
Microcoleus lacustris						7.8	4	0.6	4
Oscillatoria sp.									0.2
Phormidium sp.							2.6	2.4	5.2
Chroococcus sp.			0.1						0.4
N fix	**75.8**	**95.8**	**94**	**97.4**	**99.4**	**77.6**	**87.2**	**80.2**	**75.4**
Non-N fixers	**24.2**	**4.2**	**6**	**2.6**	**0.6**	**22.4**	**12.8**	**19.8**	**24.6**

fixing cyanobacteria (see Table 1). We examined differences in bioavailable N between the two depths across time with mixed-model ANOVA. Our model had two strata: one that accounted for the differences among the nine time periods and a second stratum accounting for depth and its interaction with time. All of these analyses were run in Minitab version 16.1.0 (2010). Least significant difference (LSD) testing was used to examine differences in means among the nine time periods. Tests for homogeneity of variance, independence and normality in the data, using Levene's test and other diagnostic tools in the Minitab (2010) statistical package, indicated that no transformations were necessary.

Table 2. Pigment content (Chlorophyll $a = \mathrm{g\,m^{-2}}$), total carbon (%C), nitrogen (%N), C : N ratio, and δ^{13}C and δ^{15}N isotopes for the time period from November to May (2009–2010).

	C*a*	C	N	C : N	δ^{13}C	δ^{15}N
Nov	112.1	0.95	0.06	17	−19.1	2.1
Dec	146.7	0.9	0.06	14.9	−19.1	1.0
Jan	120.7	1.61	0.12	13.9	−17.6	0.9
Feb	171.9	1.8	0.1	17.2	−16.6	0.9
Mar	150.6	1.58	0.09	18.1	−15.5	0.7
Apr	159.5	1.05	0.07	14.1	−18.6	0.3
May	153.8	1.07	0.08	13	−22.2	0.7

3 Results

Three species of the nostocalean N-fixing *Scytonema* accounted for 74 % of the biocrust in varying proportions (range 55–93 %) throughout the season (Table 1). Microscopic examination showed *Scytonema* was also the dominant structural component of the biocrust, and this cyanobacterium was found to be the major contributor to the breakdown of the crust and its re-establishment. This took place through the disintegration of EPS and sheath material (November–January); resurrection of a portion of desiccated filaments, followed by mass release of hormogonia (asexual reproductive cells) across January–February; and then vigorous growth of new material (Figs. 3–4). Average cyanobacterial chlorophyll *a* (pigment content) increased from 112.1 ± 21.3 SE µg C *a* g m^{-2} soil (November) throughout the wet season, peaked in February (171.9 ± 2.4 SE µg C *a* g m^{-2} soil) and declined towards the end of the wet season (153.8 ± 19.9SE µg C *a* g m^{-2}) (Table 2).

3.1 Seasonal trends in bioavailable N

Bioavailable N was elevated in November 2009 (~ 6 mg NH_4^+ kg^{-1} soil), before falling by almost 50 % across January to February, followed by an exponential increase between March and May when it peaked at > 13 mg NH_4^+ kg^{-1} soil (Fig. 5). There were significant differences in depth (with

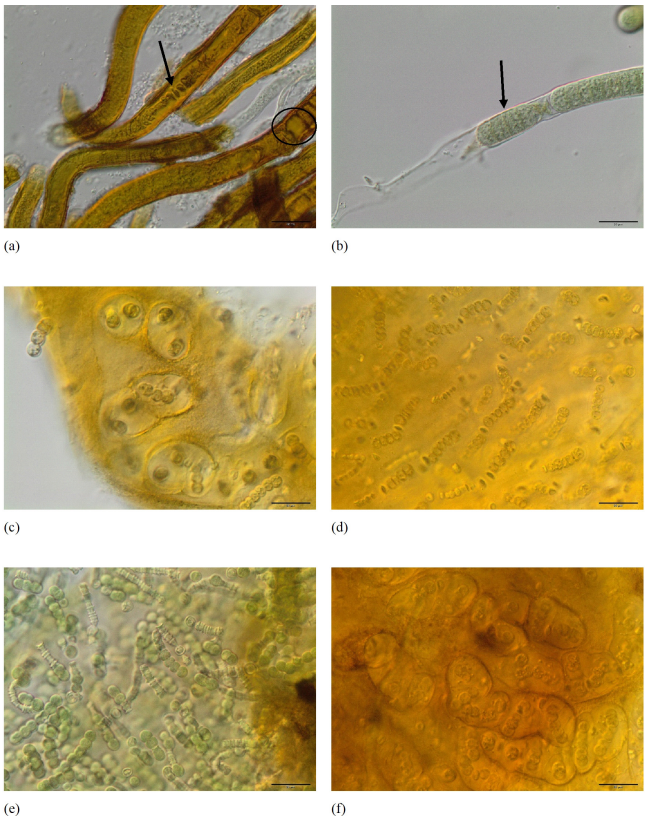

Figure 4. Micrographs of cyanobacterial growth and reproduction, scale bars 20 µm: **(a)** *Scytonema* sp. with desiccated cells and filaments encased in outer sheath containing a high level of pigmentation (arrows), heterocytes (circled) and heavy cyanophycin granulation; **(b)** *Scytonema* sp. new growth filaments illustrating hormogonia release (arrow); **(c, d)** new colonies of *Nostoc* sp. illustrating extracellular polysaccharide (EPS) capsules and surrounding EPS that delivers a microenvironment for other cyanobacteria species cohabitation; **(e)** mature colonies of *Nostoc* sp. with heterocytes; **(f)** example of distinct EPS encapsulating *Nostoc* filaments within the overall colonial structure also bound together by EPS.

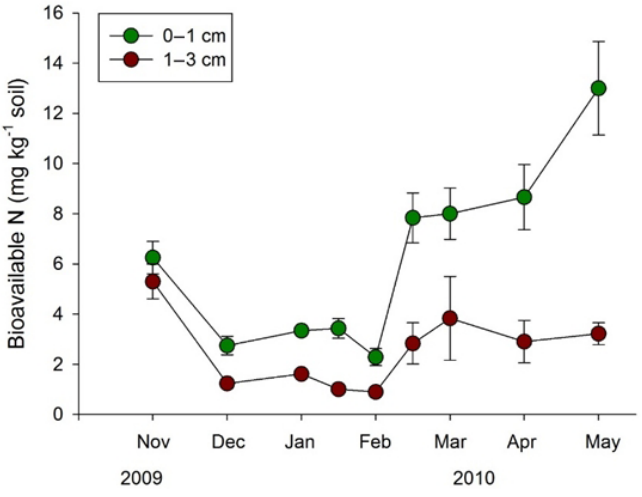

Figure 5. Seasonal trends to bioavailable N over 7 months (November–May 2009–2010) representing nine time periods (early and late January–February) for 0–1 cm and 1–3 cm depths; error bars represent standard error of the mean (SEM).

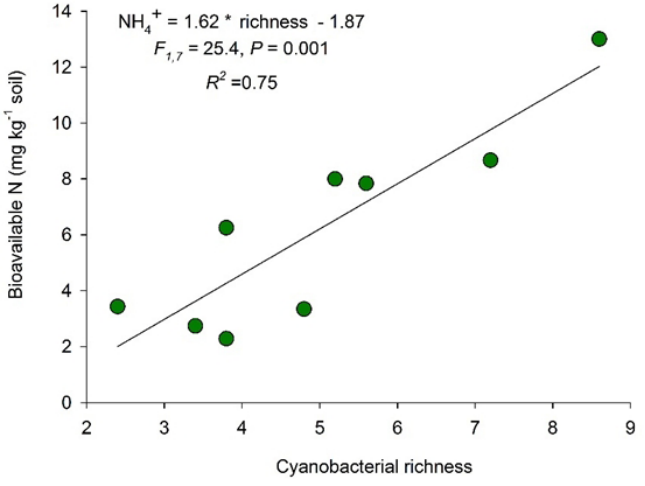

Figure 6. Relationship between bioavailable N and cyanobacterial richness over nine time periods.

more in the 0–1 cm layer) and times (all except November); however, the effect of depth was consistent across time but not significant (depth × time interaction: $P = 0.63$).

3.2 N productivity driven by cyanobacterial richness

Between November 2009 and May 2010 cyanobacterial richness ranged from 6 to 19 species, seven of which were known N fixers (Table 1). Bioavailable N where BioN $= 1.616 + 0.2072$ Nrich4 ($P = 0.001$) was significantly correlated with the presence of four key N-fixing cyanobacteria (where Nrich4 = *Nostoc commune*, *Nostoc* sp. 2, *Symploca* and *Gloeocapsa*). Of these four cyanobacteria, *Nostoc* was the most influential where BioN $= 0.89 + 0.475$ *Nostoc* ($P = 0.004$). There was a strong positive relationship between total cyanobacterial richness and bioavailable N in the top cm ($F_{1,7} = 39.3$, $P < 0.001$, $R^2 = 0.83$) (Fig. 6). There was no relationship between richness and bioavailable N deeper in the profile (1–3 cm; $P = 0.38$). For both N fixers and non-N fixers, increasing richness was associated with an increase in N fixation at a consistent rate (Fig. 7).

3.3 N fixation and chemistry

Rates of N fixation and total seasonal N fixation are reported in Figs. 8 and 9. Rates of N fixation over seven months were significantly correlated with cyanobacterial richness for all species ($P = 0.002$). Average δ^{15}N isotope across the season was 0.9 (range −0.2–2.1) and δ^{13}C isotope was −19.1 to −22.0 with C : N ratios reasonably stable (average 19.1).

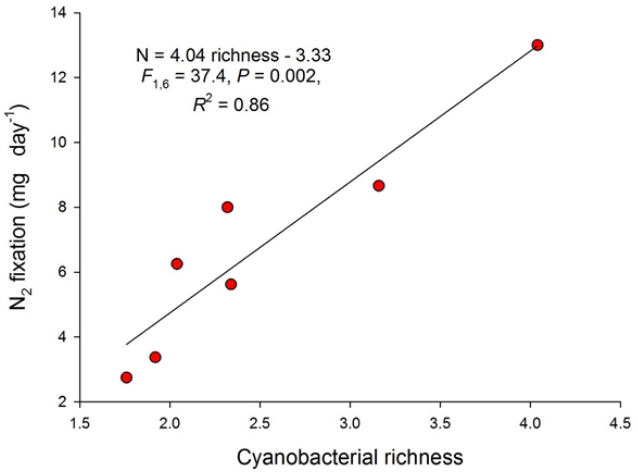

Figure 7. Relationship between cyanobacterial richness and N-fixation rates. As shown, N fixation increases significantly with increases in cyanobacterial richness.

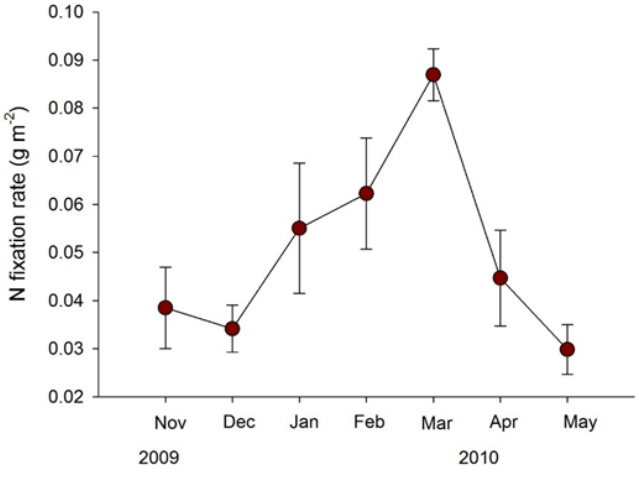

Figure 8. Daily rates of N fixation based on ARA over 7 months; error bars represent standard error of the mean (SEM).

Total C and N both doubled across the course of the wet season (Table 2).

4 Discussion

Atmospheric N_2 transformed to organic N through biological fixation by cyanobacteria has $\delta^{15}N$ values close to zero (Aranibar et al., 2004; Nadelhoffer and Fry, 1994). Across 7 months of active N fixation, isotopic N values averaged 0.9 ‰, indicating cyanobacteria were likely the primary source of bioavailable N (Evans and Ehleringer, 1994). Yet, given the rapid wetting following the dry season, potential cell lysis (Williams et al., 2014; Williams and Eldridge, 2011), the presence of other N fixers (Hawkes, 2003) and resource partitioning (e.g. Baran et al., 2015), it is difficult to separate the exact source of bioavailable N (also see Dojani et al., 2011; Johnson et al., 2007). At the Boodjamulla research sites, bioavailable N was highly correlated with species richness (Fig. 6). This was further underpinned by the analysis of cyanobacterial richness that established key N-fixing species such as *Nostoc commune*, *Symploca* and *Gloeocapsa* significantly enriched soil N. We had hypothesised that N fixation and bioavailable N would pulse at times of high rainfall and gradually decline in the latter stages of the wet season as the rainfall events decreased. For seasonal N fixation the hypothesis was true as it peaked at the height of the wet season. On the other hand, bioavailable N pulsed after the first rains at the beginning of the wet season then declined but was followed up by incremental increases and an exponential rise at the end of the wet season (Fig. 5).

Dark cyanobacterial crusts dominated by species such as *Nostoc*, *Scytonema* and *Microcoleus* that were all influential in the northern savannah crusts are known for their association with high rates of N fixation and absorption (Barger et al., 2016). At the commencement of the wet season, *Scytonema*, due to its macroscopic size and colonial form, was dominant (Table 1). After the first rains in November the crust structure broke down (Williams et al., 2014). Subsequently, bioavailable N was elevated in November, most likely due to the disintegration of the EPS and some cell lysis (Williams et al., 2014) as EPS is known to store N (Otero and Vincenzini, 2003). This was followed by a reduction in bioavailable N after rain in December. We suggest this reflected the favoured investment in C fixation by cyanobacteria (Helm and Potts, 2012) to rebuild their colonies. This was demonstrated later, when in December 2010 to January 2011 there was a net loss in productivity coinciding with rainfall and growth (Büdel et al., 2018). On the other hand, the significant increase in bioavailable N in May appeared to be related to late season rains (in April) indicative of the investment in the storage of N in cyanophycin (granules) and EPS. Other records of seasonal influence on both C and N fixation have been previously demonstrated; however, the synchrony between these events on a monthly and bimonthly basis shows how well balanced the cyanobacterial biophysical and chemical functions were, dictated by rainfall and consequently soil moisture (Büdel et al., 2009; Castillo-Monroy et al., 2010).

We had anticipated that N fixation and bioavailable N enrichment of the surface soils would be correlated to the abundance of N-fixing cyanobacteria. This prediction was true with a significant relationship to both N fixation and bioavailable N at 0–1 cm depth but not for 1–3 cm depth (Fig. 7). This observation points to the importance of biocrusts in the maintenance of N in the soil, thus giving access to this nutrient for the microbial communities in the crust. It had been previously shown that the biocrust had high enzymatic activity relative to the underlying soil (Chen et al., 2014). In this study there was also a significant relationship between non-N-fixing species and N fixation which may be explained from other research: for example, the mutualistic beneficial rela-

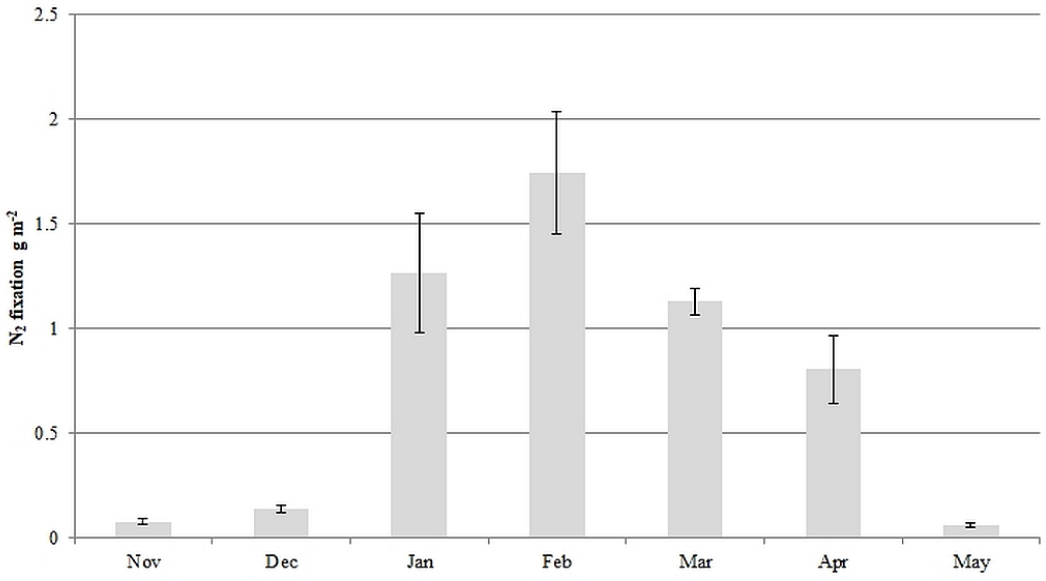

Figure 9. Total cyanobacterial N fixation estimated monthly for 2009–2010 wet season that ranged from 0.08 to 1.74 g m^{-2}, shown with standard error.

tionship with heterotrophic bacteria that fix N that is subsequently taken up by the non-N-fixing cyanobacterium *Microcoleus vaginatus* (Baran et al., 2015), and the role of bacteria and mycorrhizal fungi in rapid N transformation (Hawkes, 2003). In this study three species of *Microcoleus* were first identified in December but were more prominent between February and May (Table 2), an observation that could provide insight into the relationships with N enrichment and non-N-fixing cyanobacteria. It is now understood that there is a broad range of N-rich metabolites that are continually released and consumed by *Microcoleus* (Baran et al., 2015). It has also been demonstrated that N enrichment was associated with *Gloeocapsa* (Wyatt and Silvey, 1969), *Porphyrosiphon* (Prasanna et al., 2000) and *Schizothrix* (Berrendero et al., 2016). Indeed, many cyanobacteria obtain N by scavenging from mutually shared EPS (Rossi et al., 2017) or have multiple mechanisms for N fixation either in the dark (Lüttge, 1997), through O_2 inhibition (Stal, 1995), in anaerobic circumstances (Murukesan et al., 2016) or aquatic cyanobacterial mats when submerged under water (Berrendero et al., 2016; Stewart, 1980).

4.1 Seasonal trends in N fixation

Total seasonal N fixation by cyanobacteria was based on the number of wet days as well as the follow-on days whilst the soil retained adequate moisture (Fig. 2) that extended the period of photosynthetic activity (Williams et al., 2014). Based on the calculated number of photosynthetically active days each month (also supported by Büdel et al., 2018), we estimated that seasonal N soil enrichment via cyanobacteria would be $\sim 5.2\,\mathrm{kg\,ha^{-1}}$ (Fig. 9). This estimation must be treated with caution as in the field there are multiple environmental variables that could result in this figure being higher or lower. Notwithstanding, this indicates an important contribution to the nutrient-deficient savannah soils that are almost entirely reliant on the wet season for microbial turnover of organic matter (Holt and Coventry, 1990). These estimations are comparable to global averages of biocrust N fixation of $6\,\mathrm{kg\ N\,ha^{-1}\,yr^{-1}}$ (Elbert et al., 2012). There are numerous examples with a broad range of values such as those of cyanobacterial crusts in grasslands from the Loess Plateau in China of $4\,\mathrm{kg\,ha^{-1}\,yr^{-1}}$ (Zhao et al., 2014) or in situ results from the Negev of 10–41 $\mathrm{kg\,ha^{-1}\,yr^{-1}}$ (Russow et al., 2005). Yet, many studies do not take into account a range of mitigating factors or fail to determine the δ^{15}N conversion factor (Aranibar et al., 2004; Barger et al., 2016). The δ^{15}N values in this study ranged between 0.3 and 2.1 (Table 2), pointing towards cyanobacterial fixed N_2, whereas theoretical δ^{15}N conversion rates of 3–4 or higher may be overestimating N production (Barger et al., 2016). Isotopic measurements were taken from the cyanobacterial crusts used in ARA. The conversion rate often creates uncertainty although these values are comparable to other studies that have tested for δ^{15}N (e.g. Aranibar et al., 2004; Russow et al., 2005). Other limitations of N-fixation estimates lie in the variability of cyanobacterial cover, species richness, and in this study seasonal conditions conducive to *Nostoc commune* productivity and growth.

At the height of the wet season following supersaturation of the soil profile there were two EPS hyperproduction events attributed to *Nostoc commune* (Williams et al., 2014). There is a tight link between rainfall, soil moisture, bioavailable N and EPS excretion that in turn trigger a range of metabolic processes (Chen et al., 2014; Rossi and De Philip-

pis, 2015). C and N fixation in cyanobacteria are closely interconnected as N fixation is energy demanding and dependent on carbohydrates provided by photosynthesis (Murukesan et al., 2016). It has been reported that diazotrophic growth by cyanobacteria occurs when the N to C balance is 1 to 1.5, and the EPS is used as a sink for excess C when the C : N ratio is unbalanced (Otero and Vincenzini, 2004) with C assimilation and diversion to EPS favoured over N fixation (Murukesan et al., 2016; De Philippis et al., 1996; Rossi and De Philippis, 2015). We were unable to make a direct comparison between C : N ratios (see Table 2) and EPS hyperproduction. Nevertheless, some in situ measurements of C fixation at this time (unpublished data) and the following year showed that during storm events optimal temperatures, humidity, moisture and light intensity resulted in extremely high CO_2 uptake (Büdel et al., 2018). With wet season storms this would potentially result in a high C concentration when N could prove a limiting factor. In other research, authors have reported laboratory and field conditions where optimum conditions lead to EPS hyperproduction (Helm and Potts, 2012; Otero and Vincenzini, 2003; Rossi and De Philippis, 2015). This balancing mechanism (Otero and Vincenzini, 2004) could explain the decline in bioavailable N in January–February 2010 at a time when it would be anticipated that a substantial increase in N would occur. In this study *Nostoc commune*, known for its secretion of large amounts of EPS in optimum conditions, was the key species influencing N enrichment, which suggests that *Nostoc* growth and EPS production is an important sequence in the seasonal trends in N bioavailability. EPS creates a microenvironment for the cyanobacterial community that has low oxygen concentrations to enable N fixation under anaerobic conditions (Rossi and De Philippis, 2015).

5 Conclusions

Considering the limited knowledge of N enrichment by both heterocyte-forming cyanobacteria and cyanobacteria that rely on other strategies under different environmental conditions, we need to better understand their function especially in terms of the importance of species richness. Importantly, we discovered that at this site N enrichment was highly correlated to cyanobacterial richness and especially the presence of *Nostoc*. The seasonal patterns in atmospheric N fixation and transformation to a bioavailable form were also present in C fixation results from parallel research for cyanobacterial crusts at the same research site (Büdel et al., 2018). Well-defined seasonal trends and synchronisation in cyanobacterial species richness, N fixation, bioavailable N and C fixation clearly provide significant contributions to multifunctional microprocesses and soil fertility. Further research in the northern Australian savannah is needed to support these findings. Land management practices and climate variability, especially rainfall timing and frequency, could result in changes to cyanobacterial species richness, which is vital in the sustainability of soil N enrichment, and directly affects ecosystem productivity.

Data availability. Currently data can only be accessed in the form of Excel spreadsheets via the corresponding author.

Sample availability. Limited access to sample material is only available via the corresponding author.

Competing interests. The authors declare that they have no conflict of interest.

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References

Anagnostidis, K. and Komarek, J.: Cyanoprokariota, Teil 2: Oscillatoriales, Gustav Fischer Verlag, Berlin, 2005.

Aranibar, J. N., Otter, L., Macko, S. A., Feral, C. J. W., Epstein, H. E., Dowty, P. R., Eckardt, F., Shugart, H. H., and Swap, R. J.: Nitrogen cycling in the soil–plant system along a precipitation gradient in the Kalahari sands, Glob. Change Biol., 10, 359–373, https://doi.org/10.1111/j.1365-2486.2003.00698.x, 2004.

Baran, R., Brodie, E. L., Mayberry-Lewis, J., Hummel, E., Rocha, U. N. D., Chakraborty, R., Bowen, B. P., Karaoz, U., Cadillo-Quiroz, H., Garcia-Pichel, F., and Northen, T. R.: Exometabolite niche partitioning among sympatric soil bacteria, Nat. Commun., 6, ncomms9289, https://doi.org/10.1038/ncomms9289, 2015.

Barger, N. N., Weber, B., Garcia-Pichel, F., Zaady, E., and Belnap, J.: Patterns and Controls on Nitrogen Cycling of Biological Soil Crusts, in: Biological Soil Crusts: An Organizing Principle in Drylands, Springer, Cham, 257–285, 2016.

Barnes, J. D., Balaguer, L., Manrique, E., Elvira, S., and Davison, A. W.: A reappraisal of the use of DMSO for the extraction and determination of chlorophylls a and b in

lichens and higher plants, Environ. Exp. Bot., 32, 85–100, https://doi.org/10.1016/0098-8472(92)90034-Y, 1992.

Belnap, J.: Factors Influencing Nitrogen Fixation and Nitrogen Release in Biological Soil Crusts, in: Biological Soil Crusts: Structure, Function, and Management, Springer, Berlin, Heidelberg, 241–261, 2003.

Berrendero, E., Valiente, E. F., Perona, E., Gómez, C. L., Loza, V., Muñoz-Martín, M. Á., and Mateo, P.: Nitrogen fixation in a non-heterocystous cyanobacterial mat from a mountain river, Sci. Rep., 6, srep30920, https://doi.org/10.1038/srep30920, 2016.

Büdel, B., Darienko, T., Deutschewitz, K., Dojani, S., Friedl, T., Mohr, K. I., Salisch, M., Reisser, W., and Weber, B.: Southern African biological soil crusts are ubiquitous and highly diverse in drylands, being restricted by rainfall frequency, Microb. Ecol., 57, 229–247, https://doi.org/10.1007/s00248-008-9449-9, 2009.

Büdel, B., Williams, W. J., and Reichenberger, H.: Annual net primary productivity of a cyanobacteria-dominated biological soil crust in the Gulf Savannah, Queensland, Australia, Biogeosciences, 15, 491–505, https://doi.org/10.5194/bg-15-491-2018, 2018.

Castillo-Monroy, A. P., Maestre, F. T., Delgado-Baquerizo, M., and Gallardo, A.: Biological soil crusts modulate nitrogen availability in semi-arid ecosystems: insights from a Mediterranean grassland, Plant Soil, 333, 21–34, https://doi.org/10.1007/s11104-009-0276-7, 2010.

Chen, L., Rossi, F., Deng, S., Liu, Y., Wang, G., Adessi, A., and De Philippis, R.: Macromolecular and chemical features of the excreted extracellular polysaccharides in induced biological soil crusts of different ages, Soil Biol. Biochem., 78, 1–9, https://doi.org/10.1016/j.soilbio.2014.07.004, 2014.

De Philippis, R. D., Sili, C., and Vincenzini, M.: Response of an exopolysaccharide-producing heterocystous cyanobacterium to changes in metabolic carbon flux, J. Appl. Phycol., 8, 275–281, https://doi.org/10.1007/BF02178570, 1996.

Dojani, S., Büdel, B., Deutschewitz, K., and Weber, B.: Rapid succession of Biological Soil Crusts after experimental disturbance in the Succulent Karoo, South Africa, Appl. Soil Ecol., 48, 263–269, https://doi.org/10.1016/j.apsoil.2011.04.013, 2011.

Elbert, W., Weber, B., Burrows, S., Steinkamp, J., Büdel, B., Andreae, M. O., and Pöschl, U.: Contribution of cryptogamic covers to the global cycles of carbon and nitrogen, Nat. Geosci., 5, 459–462, https://doi.org/10.1038/ngeo1486, 2012.

Eldridge, D. J. and Leys, J. F.: Exploring some relationships between biological soil crusts, soil aggregation and wind erosion, J. Arid Environ., 53, 457–466, https://doi.org/10.1006/jare.2002.1068, 2003.

Eldridge, D. J., Bowker, M. A., Maestre, F. T., Alonso, P., Mau, R. L., Papadopoulos, J., and Escudero, A.: Interactive Effects of Three Ecosystem Engineers on Infiltration in a Semi-Arid Mediterranean Grassland, Ecosystems, 13, 499–510, https://doi.org/10.1007/s10021-010-9335-4, 2010.

Eldridge, D. J., Poore, A. G. B., Ruiz-Colmenero, M., Letnic, M., and Soliveres, S.: Ecosystem structure, function, and composition in rangelands are negatively affected by livestock grazing, Ecol. Appl., 26, 1273–1283, https://doi.org/10.1890/15-1234, 2016.

Evans, R. D. and Ehleringer, J. R.: Water and Nitrogen Dynamics in an Arid Woodland, Oecologia, 99, 233–242, 1994.

Gianello, C. and Bremner, J. M.: Comparison of chemical methods of assessing potentially available organic nitrogen in soil, Commun. Soil Sci. Plan., 17, 215–236, https://doi.org/10.1080/00103628609367709, 1986.

Hawkes, C. V.: Nitrogen Cycling Mediated by Biological Soil Crusts and Arbuscular Mycorrhizal Fungi, Ecology, 84, 1553–1562, https://doi.org/10.1890/0012-9658(2003)084[1553:NCMBBS]2.0.CO;2, 2003.

Helm, R. F. and Potts, M.: Extracellular Matrix (ECM), in: Ecology of Cyanobacteria II, Springer, Dordrecht, 461–480, 2012.

Holt, J. A. and Coventry, R. J.: Nutrient Cycling in Australian Savannas, J. Biogeogr., 17, 427–432, https://doi.org/10.2307/2845373, 1990.

Jeanfils, J. and Tack, J. P.: Identification and study of growth and nitrogenase activity of nitrogen fixing cyanobacteria from tropical soil, Vegetatio, 103, 59–66, https://doi.org/10.1007/BF00033417, 1992.

Johnson, S. L., Neuer, S., and Garcia-Pichel, F.: Export of nitrogenous compounds due to incomplete cycling within biological soil crusts of arid lands, Environ. Microbiol., 9, 680–689, https://doi.org/10.1111/j.1462-2920.2006.01187.x, 2007.

Jones, C. G., Lawton, J. H., and Shachak, M.: Organisms as Ecosystem Engineers, in Ecosystem Management, Springer, New York, NY, 130–147, 1994.

Komárek, J.: Süsswasserflora Von Mitteleuropa: Cyanoprokaryota, Heterocytous Genera, Springer, 2013.

Komarek, J. and Anagnostidis, K.: Susswasserflora von Mitteleuropa Band 19/1 Cyanoprokaryota I, Chroococcales Gustav Fisch, Verlag, 1999.

Lüttge, U.: Cyanobacterial Tintenstrich Communities and their Ecology, Naturwissenschaften, 84, 526–534, https://doi.org/10.1007/s001140050439, 1997.

Maestre, F. T., Castillo-Monroy, A. P., Bowker, M. A., and Ochoa-Hueso, R.: Species richness effects on ecosystem multifunctionality depend on evenness, composition and spatial pattern, J. Ecol., 100, 317–330, https://doi.org/10.1111/j.1365-2745.2011.01918.x, 2012.

Magee, W. E. and Burris, R. H.: Fixation of N_2 and Utilization of Combined Nitrogen by Nostoc muscorum, American Journal of Botany, 41, 777–782, https://doi.org/10.2307/2438966, 1954.

Mayland, H. F. and Mcintosh, T. H.: Availability of Biologically Fixed Atmospheric Nitrogen-15 to Higher Plants, Nature, 209, 421–422, https://doi.org/10.1038/209421a0, 1966.

Minitab: MINITAB Reference Manual, Release 16.1, Minitab Inc. 3081 Enterprise Drive, State College PA 16801-3008, 2010.

Murukesan, G., Leino, H., Mäenpää, P., Ståhle, K., Raksajit, W., Lehto, H. J., Allahverdiyeva-Rinne, Y., and Lehto, K.: Pressurized Martian-Like Pure CO_2 Atmosphere Supports Strong Growth of Cyanobacteria, and Causes Significant Changes in their Metabolism, Origins of Life and Evolution of Biospheres, 46, 119–131, https://doi.org/10.1007/s11084-015-9458-x, 2016.

Nadelhoffer, K. J. and Fry, B.: N-isotope studies in forests, Stable Isot. Ecol. Environ. Sci. Oxf. Blackwell, 22–62, 1994.

Nix, H., Mackey, B., Traill, B., and Woinarski, J.: The Nature of Northern Australia: its natural values, ecological processes and future prospects, ANU Press, available from: http://www.oapen.org/search?identifier=459382 (last access: 1 September 2017), 2013.

Otero, A. and Vincenzini, M.: Extracellular polysaccharide synthesis by Nostoc strains as affected by N source and light intensity, J. Biotechnol., 102, 143–152, 2003.

Otero, A. and Vincenzini, M.: Nostoc (cyanophyceae) Goes Nude: Extracellular Polysaccharides Serve as a Sink for Reducing Power Under Unbalanced C/N Metabolism1, J. Phycol., 40, 74–81, https://doi.org/10.1111/j.0022-3646.2003.03-067.x, 2004.

Rascher, U., Lakatos, M., Büdel, B., and Lüttge, U.: Photosynthetic field capacity of cyanobacteria of a tropical inselberg of the Guiana Highlands, Eur. J. Phycol., 38, 247–256, https://doi.org/10.1080/0967026031000121679, 2003.

Rossi, F. and De Philippis, R.: Role of Cyanobacterial Exopolysaccharides in Phototrophic Biofilms and in Complex Microbial Mats, Life, 5, 1218–1238, https://doi.org/10.3390/life5021218, 2015.

Rossi, F., Mugnai, G., and Philippis, R. D.: Complex role of the polymeric matrix in biological soil crusts, Plant Soil, 5, 1–16, https://doi.org/10.1007/s11104-017-3441-4, 2017.

Russow, R., Veste, M., and Böhme, F.: A natural ^{15}N approach to determine the biological fixation of atmospheric nitrogen by biological soil crusts of the Negev Desert, Rapid Commun. Mass Sp., 19, 3451–3456, https://doi.org/10.1002/rcm.2214, 2005.

Schneegurt, M. A., Sherman, D. M., Nayar, S., and Sherman, L. A.: Oscillating behavior of carbohydrate granule formation and dinitrogen fixation in the cyanobacterium *Cyanothece* sp. strain ATCC 51142, J. Bacteriol., 176, 1586–1597, https://doi.org/10.1128/jb.176.6.1586-1597.1994, 1994.

Smith, D. M. S., McKeon, G. M., Watson, I. W., Henry, B. K., Stone, G. S., Hall, W. B., and Howden, S. M.: Learning from episodes of degradation and recovery in variable Australian rangelands, P. Natl. Acad. Sci. USA, 104, 20690–20695, https://doi.org/10.1073/pnas.0704837104, 2007.

Stal, L. J.: Physiological ecology of cyanobacteria in microbial mats and other communities, New Phytol., 131, 1–32, https://doi.org/10.1111/j.1469-8137.1995.tb03051.x, 1995.

Stewart, W. D. P.: Some Aspects of Structure and Function in N Fixing Cyanobacteria, Annu. Rev. Microbiol., 34, 497–536, https://doi.org/10.1146/annurev.mi.34.100180.002433, 1980.

Stewart, W. D. P., Fitzgerald, G. P., and Burris, R. H.: Acetylene reduction by nitrogen-fixing blue-green algae, Arch. Mikrobiol., 62, 336–348, https://doi.org/10.1007/BF00425639, 1968.

Prasanna, R., Tiwari, O. N., Dhar, D. W., Shukla, H. M., Singh, P. K., and Tiwari, G. L.: Growth and nitrogen fixation by nonheterocystous filamentous cyanobacteria of rice fields of Uttar Pradesh, India, Philipp. J. Sci., 129, 101–107, 2000.

Tongway, D. J. and Ludwig, J. A.: Rehabilitation of Semiarid Landscapes in Australia, I. Restoring Productive Soil Patches, Restor. Ecol., 4, 388–397, https://doi.org/10.1111/j.1526-100X.1996.tb00191.x, 1996.

Vanderduys, E. P., Kutt, A. S., and Perkins, G. C.: A significant range extension for the northern Australian gecko Strophurus taeniatus, Aust. J. Zool., 36, 20–21, https://doi.org/10.7882/AZ.2012.003, 2012.

Weaver, R. W. and Danso, S. K. A.: Dinitrogen fixation, in: Methods of Soil Analysis, Part 2, Microbiological and Biochemical PropertiesSoil Science, edited by: Weaver, R. W., Angle, J. S., and Bottomley, P. J., Society of America, Inc. Madison, 1019–1045, 1994.

Wellburn, A. R.: The Spectral Determination of Chlorophylls a and b, as well as Total Carotenoids, Using Various Solvents with Spectrophotometers of Different Resolution, J. Plant Physiol., 144, 307–313, https://doi.org/10.1016/S0176-1617(11)81192-2, 1994.

Whitton, B. A. and Potts, M.: Introduction to the Cyanobacteria, in: Ecology of Cyanobacteria II, Springer, Dordrecht, 1–13, 2012.

Williams, W. J. and Eldridge, D. J.: Deposition of sand over a cyanobacterial soil crust increases nitrogen bioavailability in a semi-arid woodland, Appl. Soil Ecol., 49, 26–31, https://doi.org/10.1016/j.apsoil.2011.07.005, 2011.

Williams, W. J., Büdel, B., Reichenberger, H., and Rose, N.: Cyanobacteria in the Australian northern savannah detect the difference between intermittent dry season and wet season rain, Biodivers. Conserv., 23, 1827–1844, https://doi.org/10.1007/s10531-014-0713-7, 2014.

Wyatt, J. T. and Silvey, J. K. G.: Nitrogen Fixation by Gloeocapsa, Science, 165, 908–909, 1969.

Zhao, Y., Zhu, Q., Li, P., Zhao, L., Wang, L., Zheng, X., and Ma, H.: Effects of artificially cultivated biological soil crusts on soil nutrients and biological activities in the Loess Plateau, J. Arid Land, 6, 742–752, https://doi.org/10.1007/s40333-014-0032-6, 2014.