Responses to reviewers: "Cyanobacterial species richness and *Nostoc* highly correlated to seasonal N enrichment in the northern Australian savannah"

Williams et al.

Overall

We thank all referees for providing their time and recognising the importance of this research that focuses on the critical role of cyanobacteria within biocrust communities and their contribution to soil nutrients, especially N-fixation.

We appreciate the constructive comments for improvements to the manuscript and have addressed each one below.

Referee 1

"There are some flaws in the methodology that are critical to the central findings of this article. These need to be explained more thoroughly or additional experiments conducted."

Specific areas:

R1: 'Identification of species "richness". The method by Anagnostidis is a very classical method of classifying cyanos based on morphology, but this is quite outdated and not a very reliable metric of species 'richness'. From this approach, how exactly is abundance and richness determined? Can you really differentiate the different species (of, for example, Microcoleus) using this visual approach? If samples are still available, the authors should consider 16S sequencing. At the very least, they should extract the pigment Scytonemin as another proxy for the abundance of N-fixers present in their sample.'

Response: The authors acknowledge that advances in molecular techniques have shown the limitation of morphology-based metrics due to cryptic diversity, however in this case we are interested in relative differences between treatments, not absolute measures of richness. Therefore, in this application, the use of morphology-based measures (by two cyanobacterial specialists as described below) of species richness is adequate. 16S sequencing might be explored in future works to fully understand the diversity of these mat communities.

Both B. Büdel (for 43 years!) and W. Williams (for 10 years) are specialists in taxonomic identification of cyanobacteria using high resolution DIC microscopy (examples of scientific publications include: Aboal et al., 2016; Anagnostidis and Komarek, 2005; Büdel et al., 2009; Komárek, 2013; Ullmann and Büdel, 2001; Williams et al., 2014; Williams and Büdel, 2012). *Microcoleus paludosus*, M. *lacustris* are first described in Australia by Williams and Büdel, (2012) together with M. *vaginatus* and others. We recognise that there are differences when morphological and sequenced data is studied. For example: Almost

non-detectable visual differences between M. *vaginatus* and M. *steenstruppii* however there is evidence that M. *steenstruppii* (often described in literature as M. *vaginatus*) may represent several different cryptic species (Boyer et al., 2002); or in the case of *Nostoc commune*, ecomorphs such as N. *flagelliforme* that clearly have different morphological features, yet cannot be separated from N. *commune* through sequencing (Aboal et al., 2016). On the other hand, species which are not necessarily actively found in the soil but found with 16s sequencing might just be present with their DNA. In a recent publication, we compared the use of both methods and found, expert knowledge presupposed, that reliable results can be achieved with both methods.

Here is an extract of the Abstract from the manuscript submitted to Polar Biology now ready for resubmission after minor revision (Rippin et al., Algal biodiversity of Arctic and Antarctic biological soil crusts - Morphological vs. molecular approaches):

Morphological identification using light microscopy and the annotation of ribosomal sequences taken from metatranscriptomes. The analyzed samples were collected at Ny-Ålesund, Svalbard, Norway, and the Juan Carlos I Antarctic Base, Livingston Island, Antarctica. This study is focused on the following taxonomic groups: Klebsormidiophyceae, Chlorophyceae, Trebouxiophyceae, Xanthophyceae and Cyanobacteria. In total, 143 and 103 genera were identified in the Arctic and Antarctic sample, respectively, via both approaches, while 15 and 7 taxa were determined concordantly. Hence, some genera were identified only by one of the two techniques. In general, the molecular analysis indicated a higher degree of microalgal and cyanobacterial diversity (about 11 times higher). In terms of eukaryotic algae, the two sampling sites displayed comparable genus counts while the cyanobacterial diversity was much higher in the BSC from Ny-Ålesund. Furthermore, the Arctic and the Antarctic BSC shared a total of 63 microalgal species. For the first time, the presence of the genera Chloroidium, Ankistrodesmus and Dunaliella in Polar Regions was determined. Overall, these findings illustrate that only the combination of morphological and molecular techniques, in contrast to one single approach, reveals a higher degree of biodiversity for complex communities such as polar BSCs.

The description of species richness in this manuscript has been derived from the separation of different cyanobacterial species through morphological features and identified to a species level where possible (see p5, Section 2.3.3). Please note the reference list used for identification has been updated (see p5, Section 2.3.3) to include more recent taxonomic literature. We respectfully disagree that identifying cyanobacteria morphologically is outdated, inasmuch that many species from the Australian continent are undescribed both in literature and sequences not available in current gene libraries (e.g. (Chilton et al., 2017 and Williams, Chilton and Alchin, unpublished data). Samples have been preserved for future sequencing analysis (when funds are available) that will be part of a larger biogeographical study.

If scytonemin has been used as a proxy for N-fixers it assumes that only diazotrophic cyanobacteria produce scytonemin which is not the case. The position of filaments within the mat profile (and their relative exposure to UV light) is a large determinant of scytonemin production. The authors respectfully disagree that additional data relating to scytonemin would add to the records presented here of known N-fixing species.

R1: The methods overall are a bit difficult to follow:

Response – areas of concern are addressed below.

R1: 'Why were the samples reactivated in the glasshouse' and 'which samples were reactivated (just for ARA)?'

Response – we have added a sentence into the methods and clarified for each section the treatment of each sample set for the different analyses.

Added into methods Section 2.3.1, p5, L10

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).

R1: "Please summarise method used to measure bioavailable N

Response: Added into methods p4 Section 2.3.1

Cyanobacterial crusts (0–1 cm) and sub-surface soils (1–3 cm) were analysed for bioavailable N ($NH_4^+ + NO_3^-$) 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. Duplicates for each of 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, 0–3 cm n=99). This method determines ammonium-N ($NH4^+$) produced from organic soil N when the soil is heated with 2M KCl in a stoppered tube at 95°C for 16 hours. $NH4^+$ is determined by the difference between the $NH4^+$ liberation during the hot distillation of 20 mg soil and the $NH4^+$ present prior to heating (Gianello and Bremner, 1986) and provides an index for bioavailable (mineralizable) N at the time of sampling (also see Tongway and Ludwig, 1996; Williams and Eldridge, 2011).

R1: "How were the samples collected (were they cored) and how were the samples stored after collection? Were they stored at all or were they analysed immediately?

Response: Added to P4, Section 2.2 L16-18,: 'Cyanobacterial surface crusts and sub-soils were removed using a 10 cm metal spatula to extract exactly 0–1 cm vertical depth with care to include the complete sample, followed up by 1–3 cm depth directly below the crust.' In Section 2.3.1 we have added the dry storage and time frame for when the samples were analysed. '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 wet season (2010).'

R1: "More details are needed in the statistics section (section 2.3.4) especially pertaining to section 3.2."

Response: The following detail has been added to improve the description of the statistics and provides an explanation of the analysis of the results presented in section 3.2

2.3.4 Statistics

'We used linear regression models to examine potential relationships between bioavailable N and cyanobacterial richness separately, for N-fixing and non-N-fixing cyanobacteria (see Table 1). We examined differences in bioavailable N between the two depths across time with mixed-models 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.'

R1: "In the first sentence of the discussion it says that isotopic signatures for 15N2 clearly demonstrate that cyanobacteria were the primary source of bioavailable N. The results that are presented do not "clearly" demonstrate that. It says that nitrogen fixation is correlated with cyanobacterial richness (and with certain species) but it cannot be ruled out that other N-fixers are present. This is especially true for other biocrusts where cyanobacteria are not the primary N-fixers."

Response: In the first sentence of the discussion (Section 4) we have changed the start of the first paragraph to read: 'In this study, isotopic signatures for δ 15N across seven months of active N-fixation demonstrated cyanobacteria were likely to be the primary source of bioavailable N (Evans and Ehleringer, 1994), although it is now understood that microbial resource partitioning on a microscale takes place within the biocrust strata (Baran et al., 2015). Given the rapid wetting following the dry season, potential cell lysis (Williams et al., 2014; Williams and Eldridge, 2011) and the presence of other N-fixers (Hawkes, 2003), it is difficult to separate the exact source of bioavailable N (e.g. Baran et al., 2015; Dojani et al., 2011; Johnson et al., 2007)…..'

R1: Technical corrections:

R1: P1, L12: **Response**: Added 'Many'

R1: P1, L18: **Response**: Changed sentence to read: 'Over the wet season cyanobacterial richness ranged from 6–19 species. N-fixing *Scytonema* accounted for seasonal averages between 51–93% of the biocrust.'

R1: P1, L20: **Response**: deleted 'It was established…'

R1: P2, L3: **Response**: changed to 'the northern Australian savannah…'

R1: P2: **Response**: Rossi et al. 2017 added as reference

R1: P4, L5: **Response**: added the word 'fixation'

R1: P5, L17: **Response**: Formatted heading to bold

R1: P6, L5/6: **Response**: revised sentence to '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).'

R1: P6, L14: **Response**: changed to '…before changing to more than half across January to February….'

R1: P8, L1/2: **Response**: Sentence changed to: 'It had been previously shown that the biocrust had high enzymatic relative to the underlying soil (Chen et al., 2014).'

R1: P8, L9: **Response**: changed 'reabsorbed' to 'consumed'

R1: P8, L21: **Response**: added the word 'biocrust'

Referee 2

R2: The manuscript can be improved by shortening the abstract and conclusion.

Response: The abstract has been reduced from 330 words to 255 words:

'Abstract

Boodjamulla National Park research station is situated in north-west Queensland dry savannah where the climate is dominated by summer monsoons and virtually dry winters. Cyanobacterial crusts almost entirely cover the flood plain soil surfaces in between the tussock grasses. 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 seven months of a typical wet season. Over the wet season cyanobacterial richness ranged from 6–19 species. N-fixing *Scytonema* accounted for seasonal averages between 51–93% 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 ~ 5.2 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 (this journal) provide important contributions to multi-functional microprocesses and soil fertility.'

The second paragraph from the conclusion has been removed (also see comments from R3)

R2: "Minor comments 2.3.4 Statistics: Please indicate what method you used for linear regression."

Response: Statistics section now has this information included: 'We used linear regression models to examine potential relationships between bioavailable N and cyanobacterial richness separately, for N-fixing and non-N-fixing cyanobacteria (see Table 1). We examined differences in bioavailable N between the two depths across time with mixed-models 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.'

R2: P7, L8: "Here the bioavailable N consisted NH4+ and NO3-, so how about dissolved organic N in soils?"

Response: We did not fractionate the forms of N therefore did not measure DON however with the 2M KCL hot extraction it is possible that some DON could be converted and in this case the measurement would include that. The primary focus for this project was to understand bioavailable N that may have entered the system via cyanobacteria.

Referee 3

R3: "….in the introduction there are some too general sentences leaving the reader what direction the paper will be…"

Response: In the first three paragraphs of the introduction we have deleted some ambiguous and general sentences and rearranged certain sections so the concepts flow from the landscape to the microbe better. We appreciate the referee's comments here as it assists greatly in refining the manuscript.

R3: P3, L1: "I don't think this is a good citation….as it deals with lichens…"

Response: This sentence and citation has been removed in the revision of the introduction.

R3: P3: "I suggest rewriting the last two paragraphs for clarity and merging them."

Response: As suggested these paragraphs have been revised with a couple of sentences removed to improve flow and merged into one.

R3: P4, "…report coordinate of study site."

Response: Coordinates have now been included.

R3: P4, L19: "Chlorophyll may or may not be a good indicator of cyanobacterial biomass, but is certainly not well suited to dry environments where scytonemin is typically the most abundant pigment in soil. Therefore, I suggest avoiding the unnecessary use of the term 'biomass' and just refer to pigment content, which may be a better reflection of photosynthetically active cyanobacteria."

Response: As suggested the term biomass has been used sparingly and replaced with pigment content defined as chlorophyll a (see Section 2.3.2 and others throughout).

Response: Inserted into Section 2.3.1, L2: **'**To estimate seasonal N-fixation the mean values of N-fixation were calculated for each month and multiplied by active days. As we have clear data that indicates the periods of activity for these biocrusts (see Williams et al., 2014 and Büdel et al. this journal) the number of active days was based on the number of rain days and soil moisture availability measurements for key months using moisture meter data from the site, an example shown in Figure 2.'

R3: P4, L24: "What is method 4? Please describe briefly. And the same goes for ARA"

Response: Method 4 descriptions have been addressed with the response to R1 with a more detailed description in methods Section 2.3.1 (shown in this document P3). ARA description has been improved in the second paragraph.

R3: "Please explain more clearly how you estimated your conversion factor"

Response: The conversion factor was derived from isotopic analysis. We clarified the origin of the delta 15N conversion factor in the methods (P5, section 2.3.1): 'Seasonal N-fixation was determined through acetylene reduction based on Hawkes (2003) method for acetylene reduction assays (ARA) (Stewart et al., 1968). To complete the monthly estimates of rates of N-fixation, delta ^{15}N of the crust was calculated for each sample, and used as a conversion factor for each month's sample set (also see section 2.3.2). Petri-dish samples of cyanobacterial crusts (reserved for AR) for each month (Nov–May) were reactivated in the glasshouse for approximately two 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).'

'In Section 2.3.1 additional information has been provided to clarify seasonal estimations: 'Monthly averages for $\delta\ ^{15}N$ (derived from ARA samples, see Section 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 active days. As we have clear data that indicates the periods of activity for these biocrusts (see Williams et al., 2014 and Büdel et al. this journal) the number of active days was based on the number of rain days and soil moisture availability measurements for key months using moisture meter data from the site, an example shown in Figure 2.'

R3: "…I think it is going too far to estimate your N-fixation rates based on ARA rates measured in the lab..." and "P8, L19 5 kg of N per ha is a reasonable estimate but I would suggest stressing the need for considering the number with caution as this is only based on a few measurements in controlled conditions…."

Response: We appreciate that it is difficult to be certain that the estimation of 5 kg N per ha is accurate given the changing nature of field conditions. The authors stand by this estimation as a reasonable attempt to calculate the contribution of cyanobacterial crusts to N for this season at this site. However, at R3's suggestion we have added a statement to reinforce the fact that this is an estimation and there are many variables that could alter this figure.

Section 4.1 has an additional sentence and some edits to read: 'Based on total active days per month we estimated that N-soil enrichment via cyanobacteria would be ~ 5.2 kg ha^{-1} seasonally. 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 a substantial 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 kg N ha-1 year-1 (Elbert et al. 2012).'

And further down in the same paragraph we had already stated: 'Other limitations of N-fixation estimates lie in the variability of cyanobacterial cover, species richness and in this study conditions conducive to *Nostoc commune* productivity and growth.'

R3: P6, L1-2 "This is a very poor description of statistics…. non-significant interaction not described"

Response: The statistics section has been rewritten and includes aforementioned descriptions (see R1 insertion P4, this document).

R3: "Report isotopic values as delta 15N"

Response: This has been altered throughout the manuscript.

R3: "Citation about N-fixation on Mars does not make sense"

Response: This citation was merely an example of anaerobic N-fixation but the words "such as Mars" have been removed so as not to detract from general point.

R3: P9, L28-30 "….these conclusions come out of the blue…"

Response: In line with R2 and R3's comments this paragraph has been removed to make the conclusion more concise and to the point.

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

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Abstract

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 seven months of a typical wet season. Over the wet season cyanobacterial richness ranged from 6–19 species. N-fixing *Scytonema* accounted for seasonal averages between 51–93% 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 ~5.2 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 (this journal) provide important contributions to multi-functional microprocesses and soil fertility.

1.0 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 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 six months, followed by violent storms and flooding rains. Across the savannah landscapes broad-scale livestock grazing is the primary land use, however managing these extensive perennial grasslands and woodlands demands an approach at 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 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, 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 micro-organisms increase (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 forms a cohesive and protective layer at the soil surface, minimising the effects of wind erosion (Eldridge and Leys, 2003). EPS provides cyanobacteria the capacity to maintain fitness and sustain growth of other cohabiting species (Rossi and De Philippis, 2015; Rossi et al. 2017).

Cyanobacterial mediated N fixation results in N liberated into the soil in both inorganic and organic N 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 extra-cellular 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 alters its rheological properties and becomes 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 sub-optimal following desiccation (Jeanfils and Tack, 1992), thus increasing soil 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 hours 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 seven 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.

2.0 Methods

2.1 Site description

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 six months to the 2009–2010 wet season at the research site was completely dry. In late November as the humidity

increased the early rainfall was typically a couple of small rain events (0.2, 6.8 and 0.2 mm) followed by heavy rains throughout Jan (279 mm) and Feb to Apr (555 mm) (Fig 1).

2.2 Field sampling

To determine seasonal patterns of cyanobacterial 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 sub-soils were removed using a 10 cm metal spatula to extract exactly 0–1 cm vertical depth with care to include the complete sample, followed up by 1–3 cm depth directly below the crust. For analysis we allocated the samples into nine x 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 bi-monthly) represented at least two separate sample periods, before and after rain. For pigment content (chlorophyll *a*), rates of N-fixation (ARA), 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 sub-surface 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 wet season (2010). Duplicates for each of 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-N (NH_4^+) produced from organic soil N when the soil is heated with 2M KCl in a stoppered tube at 95°C for 16 hours. 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 Hawkes (2003) method for acetylene reduction assays (ARA) and Stewart et al., (1968). To complete the monthly estimates of rates of N-fixation, $\delta^{15}N$ of the crust (see section 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 (Nov–May) were reactivated in the glasshouse for approximately two 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 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 two days to acclimatise prior to ARA. The incubation was carried out from time zero (T0) and measured at 24 hours (T2) and 48 hours (T3). In between measurements the samples were maintained in the glasshouse at 28°C (previously determined as an optimum temperature for these crusts) and natural light conditions (Büdel, Williams and Reichenberger, unpublished data).

Calculation of ethylene production (C_2H_4 µm/mL) was carried out using the standard formula (see Hawkes, 2003; Weaver and Danso, 1994):

$$Vhs=Vt-Vw-Vs$$

where V= volume, Vhs = head space (volume of air in vial), Vt = tube (volume of tube), Vw = volume of water (ml added to sample), Vs = volume of solids (Vs = weight of sample/soil bulk density for these soils of 1.6). Daily rates were calculated by T3-T2 (48 hours – 24 hours) then converted to grams per square metre. Monthly averages for $\delta^{15}N$ (derived from ARA samples, see Section 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 active days. As we have clear data that indicates the periods of activity for these biocrusts (see Williams et al., 2014 and Büdel et al. this journal) the number of active days were based on the number of rain days and soil moisture availability measurements for key months using moisture meter data from the site, an example shown in Figure 2.

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 Mass Spec: Sercon Hydra 20-22 (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 was 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 with Wellburn's (1994) equations.

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 hours 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 ×1000. 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-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.

3.0 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 reestablishment. This took place through the disintegration of EPS and sheath material (Nov–Jan), resurrection of a portion of desiccated filaments, followed by mass release of hormogonia (asexual reproductive cells) across Jan–Feb, then vigorous growth of new material (Figures 3-4). Average cyanobacterial pigment content (chlorophyll *a*) increased from 112.1 ± 21.3SE µg C*a* g^{-1} soil (Nov) throughout the wet season; peaked in Feb (171.9 ± 2.4SE µg C*a* g^{-1} soil) and declined towards the end of the wet season (153.8 ± 19.9SE µg C*a* g^{-1} soil) (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 more in the 0-1 cm layer) and times (all except Nov), however the effect of depth was consistent across time but not significant (depth x 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) were 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 season N-fixation are reported in Figures 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). Total C and N both doubled across the course of the wet season (Table 2).

4.0 Discussion

Atmospheric N_2 transformed to organic N through biological fixation by cyanobacteria has δ^{15}N values close to $0^0/_{00}$ (Aranibar et al., 2004; Nadelhoffer and Fry, 1994). Across seven months of active N-fixation isotopic N values averaged $0.9^0/_{00}$ 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. this journal). 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 bi-monthly 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 relationship 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 identified first in December but were more prominent between Feb-May (Table 2), 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* (Tiwari 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 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 this journal) we estimated that seasonal N-soil enrichment via cyanobacteria would be ~5.2 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 kg N ha-1 year-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 kg ha^{-1} $year^{-1}$ (Zhao et al., 2014), or in situ results from the Negev of 10-41 kg ha^{-1} $year^{-1}$ (Russow et al., 2005). Yet, many studies do not take into account a range of mitigating factors or fail to determine the $\delta^{15}N$ conversion factor (Aranibar et al., 2004; Barger et al., 2016). The $\delta^{15}N$ values in this study ranged between 0.3 and 2.1 (Table 2), pointing towards cyanobacterial fixed N_2 whereas theoretical $\delta^{15}N$ 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 created uncertainty although these values are comparable to other studies that have tested for $\delta^{15}N$ (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 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, EPS excretion that in turn trigger a range of metabolic processes (Chen et al., 2014; Rossi and De Philippis, 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. in this journal). 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 Jan–Feb 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. The role of EPS is to create a microenvironment for the cyanobacterial community that has low oxygen concentrations for carrying out N_2 fixation under anaerobic conditions (Rossi and De Philippis, 2015).

5.0 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 was also present in C-fixation results from parallel research for cyanobacterial crusts at the same research site (Büdel et al. in this journal). Well-defined seasonal trends and synchronisation in cyanobacterial species richness, N-fixation, bioavailable N and C fixation clearly provide significant contributions to multi-functional microprocesses and soil fertility. Further research in the northern Australian savannah is needed to support these findings. Variability in northern Australian wet season rainfall patterns could result in significant fluctuations in soil N-enrichment that are vital in the conservation of soil microprocesses and cyanobacterial diversity.

Data and sample availability

Research data and primary sample material (duplicates where available) for this project are filed with The University of Queensland's School of Agriculture and Food Science (Gatton Campus) according to the University's policy for the use of other researchers and interested persons for future research.

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

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-fixers	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

Table 2: Pigment content (Chlorophyll *a* = µg C*a* g^{-1} soil), total carbon (%C), nitrogen (%N), C:N ratio and $\delta^{13}C$ and $\delta^{15}N$ isotopes for the time period from Nov-May (2009–2010).

	C*a*	C	N	C:N	$\delta^{13}C$	$\delta^{15}N$
NOV	112.1	0.95	0.06	17	-19.1	2.1
DEC	146.69	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	0.7

Legends to Figures

Figure 1: Daily rainfall events for 2009-2010 wet season at Boodjamulla National Park (source: bom.gov.au)

Figure 2: Example of ongoing soil moisture even when there is no rain for a period of time at 1-3 cm (■) and 5-10 cm (▲) for January 2010 measured with MEA TBug probes (mea.com.au)

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 EPS hyperproduction from *Nostoc* sp. and; (d) gelatinous EPS during hyperproduction phase compared with bare area being recolonised

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 (arrow) release; (c,d) New colonies of *Nostoc* sp. illustrating 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 seven months (Nov-May, 2009–2010) representing nine-time periods (early and late Jan-Feb) for 0-1 cm and 1-3 cm depths

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

Figure 7: N-fixation increases significantly with increases in cyanobacterial richness

Figure 8: Rates of N-fixation over seven months

Figure 9: Total cyanobacterial N-fixation estimated on a monthly basis for 2009-2010 wet season

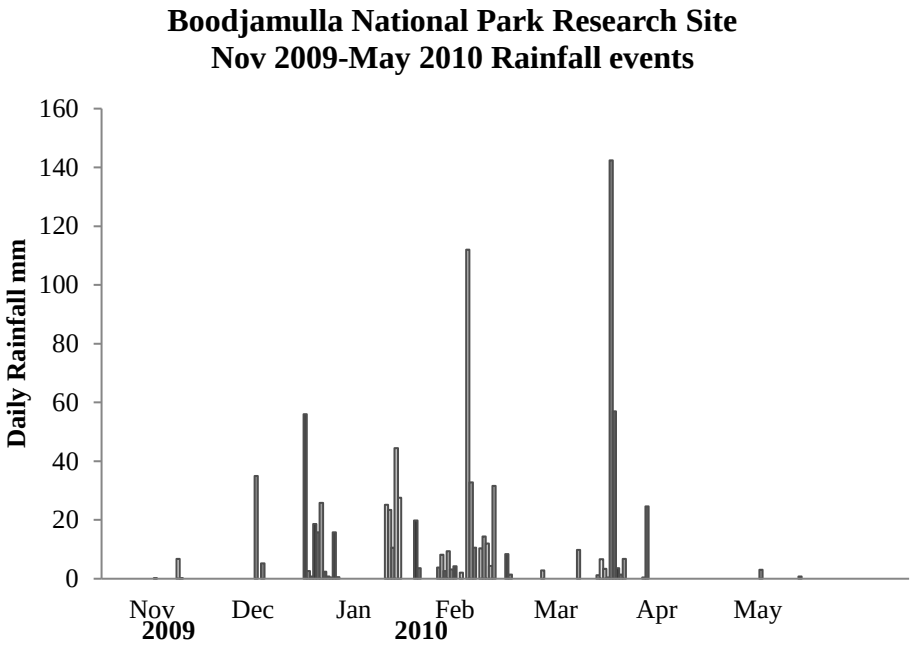

Boodjamulla National Park Research Site
Nov 2009-May 2010 Rainfall events
Daily Rainfall mm
160
140
120
100
80
60
40
20
0
Nov
Dec
Jan
Feb
Mar
Apr
May
2009
2010

Figure 1

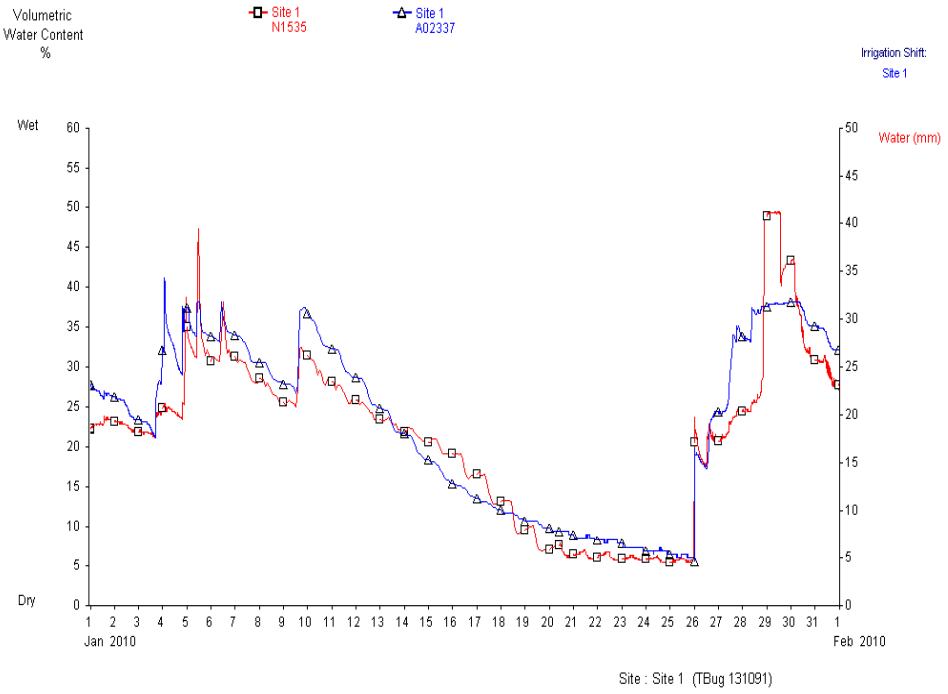
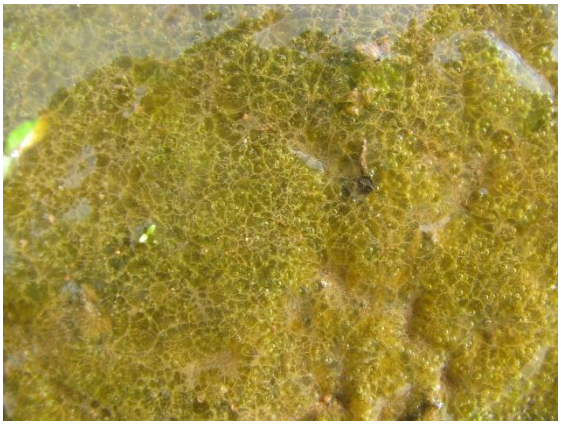

Volumetric
Water Content
%
Site 1
N1535
Site 1
A02337
Irrigation Shift:
Site 1
Wet
Dry
Water (mm)
60
55
50
45
40
35
30
25
20
15
10
5
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 1
Jan 2010
Feb 2010
Site : Site 1 (TBug 131091)

Figure 2

(a)

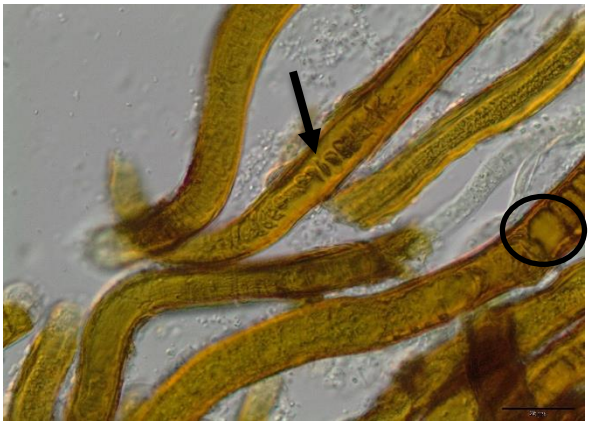
(b)



(c)

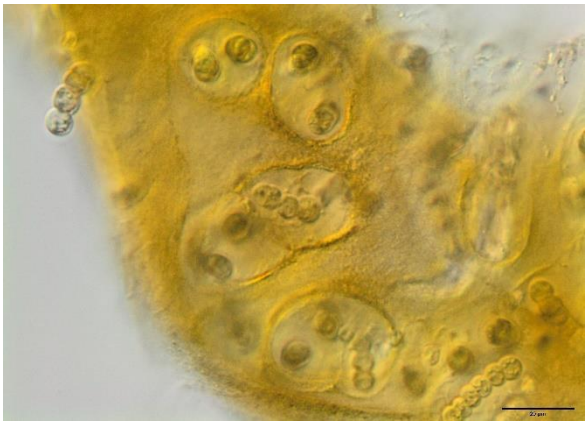
(d)

Figure 3

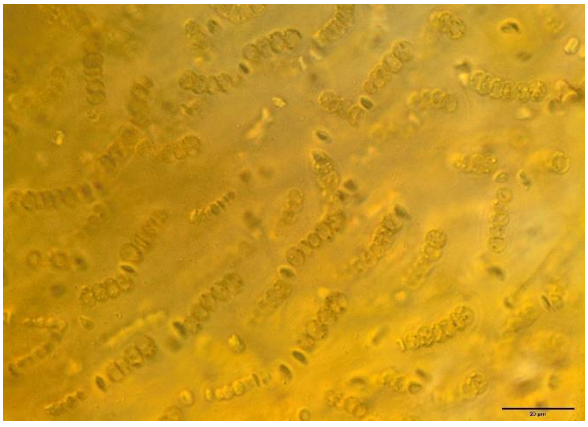


(a)

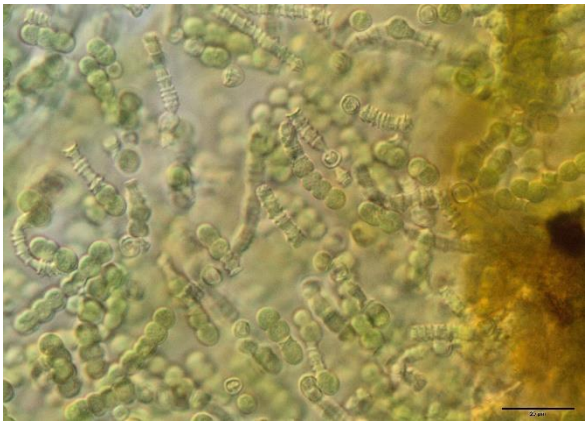
(b)

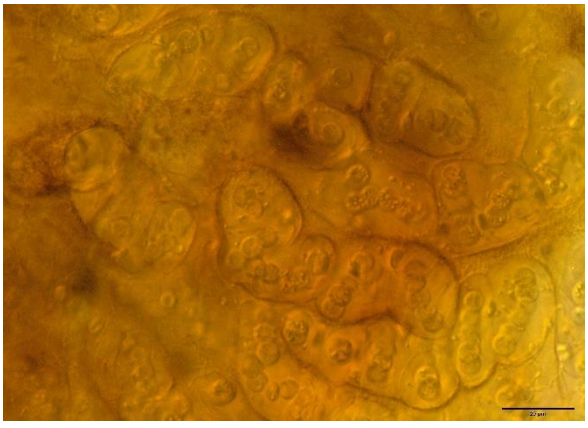


(c)



(d)





(e) (f)

Figure 4

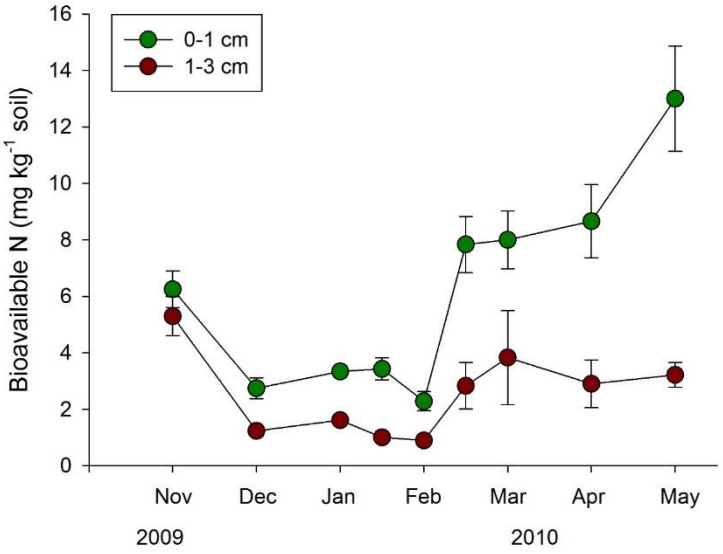


Figure 5

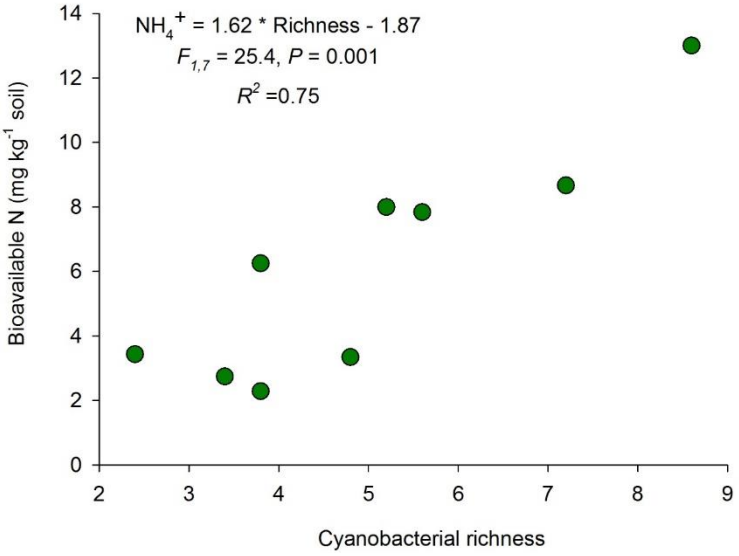

NH4+ = 1.62 * Richness - 1.87
F1,7 = 25.4, P = 0.001
R2 =0.75
Bioavailable N (mg kg-1 soil)
Cyanobacterial richness
0
2
4
6
8
10
12
14
2
3
4
5
6
7
8
9

Figure 6

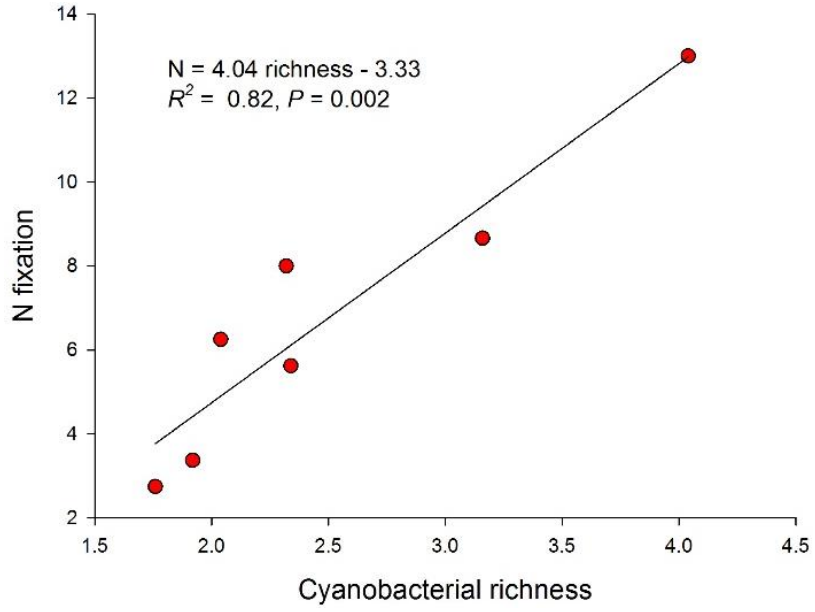

N = 4.04 richness - 3.33
R^2 = 0.82, P = 0.002
N fixation
Cyanobacterial richness
14
12
10
8
6
4
2
1.5
2.0
2.5
3.0
3.5
4.0
4.5

Figure 7

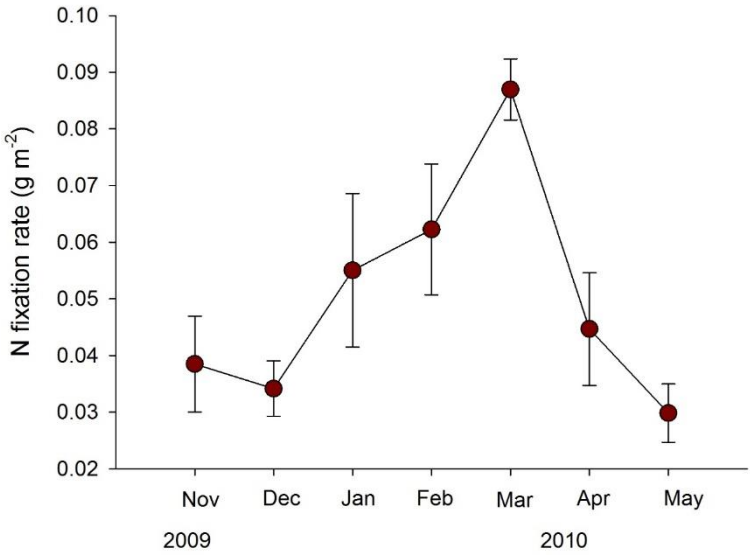

0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
N fixation rate (g m-2)
Nov
Dec
Jan
Feb
Mar
Apr
May
2009
2010

Figure 8

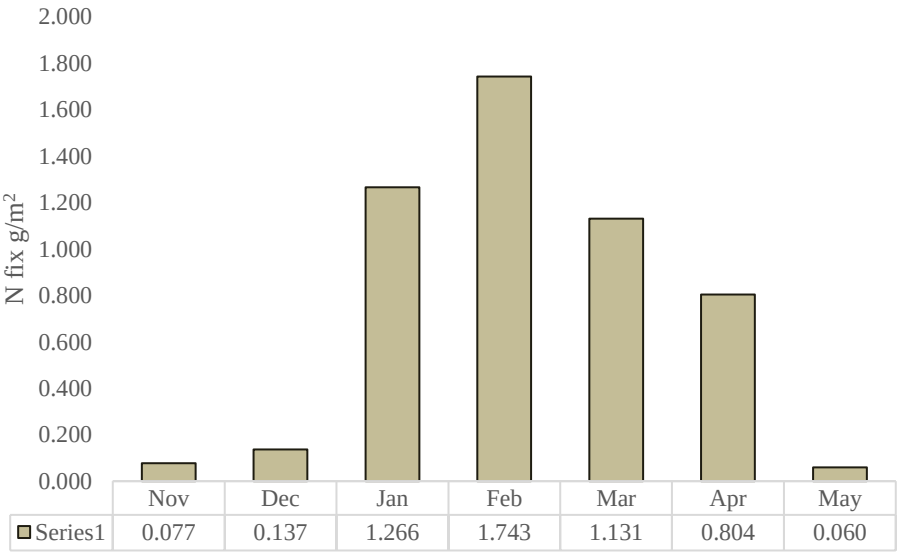

2.000
1.800
1.600
1.400
1.200
1.000
0.800
0.600
0.400
0.200
0.000
N fix g/m2
Nov
Dec
Jan
Feb
Mar
Apr
May
Series1
0.077
0.137
1.266
1.743
1.131
0.804
0.060

Figure 9