

## ***Interactive comment on “Cyanobacterial species richness and *Nostoc* highly correlated to seasonal N enrichment in the northern Australian savannah” by Wendy Williams et al.***

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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 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 land-

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scape 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

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estimates of rates of N-fixation,  $\delta^{15}\text{N}$  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  $\delta^{15}\text{N}$  (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  $\sim 5.2 \text{ kg ha}^{-1}$  seasonally. This estimation must be treated with caution as in the field there are multiple environmental variables that could result in this figure

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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  $6 \text{ kg N ha}^{-1} \text{ year}^{-1}$  (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  $\delta^{15}\text{N}$ " 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.

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