



## XII INTERNATIONAL RANGELAND CONGRESS AUSTRALIA 2025

### **How grazing management influences biocrust community composition in the Northern Territory rangelands**

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**Key words:** biocrust, community composition, rangelands, grazing management

#### **Abstract**

Biological soil crusts are an important indicator of the long-term productivity of grazed landscapes. Healthy biocrust communities can improve soil fertility by increasing soil stability, enhancing moisture retention, as well as facilitating both carbon and nitrogen cycling and fixation. The Rain Ready Rangelands project, funded through the Australian Government Future Drought Fund analysed biocrust health on three producer demonstration sites in central Australian woodlands and shrublands and the Barkly Tablelands Mitchell grass regions of the Northern Territory. In this paper we focus on a producer demonstration site located approximately 250 km northwest of Alice Springs in tall open Acacia shrublands with deep red earths, sandy red earths, red clayey sands and deep sandy loams over mixed short grasses.

Samples were collected under varying grazing intensities (with distance from water and inside vs. outside cattle exclosures) and with different grazing management (current vs. recommended grazing management). To detect the functional contribution of these communities baseline samples were collected and analysed for biocrust species composition, total carbon and nitrogen and DNA analysis. 16S rRNA marker gene sequencing was used to profile bacterial community diversity along grazing gradients from watering points, and inside and outside cattle exclosures.

Bacterial community composition shifted significantly with distance from water at the central Australian sites. Diversity was highest at the most disturbed site 50 meters from the water point at one of these locations, while community composition had not diverged inside vs. outside newly established exclosures after the first year. Changes in total carbon and total nitrogen were observed only with distance from water, with enhanced C and N adjacent waterpoints compared to all other distances. The ecological implications of these changes are being further investigated. We plan to monitor these sites through time to see if alternative management regimes and cattle exclusion results in different trajectories in biocrust community composition and function.

#### **Introduction**

This research took place in the Alice Springs region of the Northern Territory. As a part of a broader project called Rain Ready Rangelands, we established a Paddock Challenge demonstration site on a large cattle station covering

over 2700 km<sup>2</sup>. We aimed to illustrate the benefits of positive grazing management strategies such as adjusting stocking rates to safe carrying capacity on land condition by measuring vegetation and biocrust communities across sites.

Biological soil crusts, also known as biocrusts are often referred to as the 'living skin' of the soil surface (Weber et al. 2022). Inhabiting the upper centimetres of soil between and under vegetation in dryland and savannah ecosystems, biocrusts play a central role in primary production. Biocrusts functional influence on soil fertility and plant growth can provide us with an indicator of how management can promote drought resilient soils (Eldridge and Delgado-Baquerizo 2017; Williams et al. 2021). Hence, we measured biocrust responses to grazing intensity and management through sampling biocrusts, and soil C and N.

To ensure long term productivity of grazed landscapes, sufficient replenishment of nitrogen is required. Nitrogen accounts for a large proportion of the soil-derived nutrients that pastures require. Among other functions, cyanobacteria, bacteria and other microorganisms that make up biocrusts have the capacity to fix nitrogen and cycle nutrients (Belnap 2003).

Environmental DNA sequencing of 16S rRNA gene, at terrestrial field sites identifies bacteria at the genus and species levels, providing detailed information about overall composition and abundance of microbial communities. There was no current knowledge in this region of biocrust communities and the effects of trampling by livestock. Thus, we set out to discover the diversity of biocrusts and their associated microbial communities. As management entails very large areas in a climate controlled by low rainfall, we designed this component to encompass the landscape scale effects at a micro-scale.

## Methods

Samples were collected in April 2023 along two transect lines north and south of 2 long established bores (permanent ground water supplies for livestock). Samples were collected from deep red earths, sandy red earths, red clayey sands and deep sandy loams. Biocrust samples were collected from three land systems: Bushy Park (Broad alluvial drainage floors and floodplains with deep red earths), Kanandra (coarse textured brown alluvial plains of deep sandy loams and sandy red earths) and Sandover (Flat or gently undulating desert floodplains and levees with red clayey sands and deep sandy loams) (Perry et. al. 1962 & Grant 1983R). Pastures were dominated by mixed short grasses and forbs including *Enneapogon polyphyllus* (Oatgrass), *Aristida contorta* (Kerosene grass) and *Eragrostis kennedyae* (Lovegrass).

The exclosure was erected in a representative area of productive country, aiming to explore differences between the paddock/watered area treatment versus no grazing. The fenced cattle exclosure was located 1 km from a Bore. Biocrust monitoring sites were strategically located along transects at varying distances from a watering point (50 m, 500 m, 1-2 km, 6 km). Three types of biocrust samples were collected for DNA, C and N and biocrust microcosms.

Quadrats (n=3) were randomly placed on the ground at each distance from water and photographed. DNA samples were collected using a 50 mL Falcon Tube and a spatula. In each Falcon Tube 3 sub samples of the top 1 cm of soil were collected and combined (n=3 per quadrat). Equipment was cleaned with alcohol wipes between each sample. Total C and N samples were prepared by using a spatula to collect three subsamples 10 x 10 x 5 cm depth (n=3 per quadrat). Biocrust microcosms were collated by collecting 4 surface subsamples using a spatula and placed in a petri dish (n= 3 per quadrat). This methodology was repeated twice at each site, giving us three composite samples of each type from every site location (n=12 per site).

Environmental DNA was extracted using the Qiagen DNeasy® PowerSoil Kit. Amplicon sequencing of the 16S rRNA gene was amplified by polymerase chain reaction (PCR) using the primers 799F (5'-



AACMGGATTAGATACCKG-3') and 1193R (5'-ACGTCATCCCCACCTTCC-3') (Lane, 1991) for bacteria profiling, and sequenced using the MiSeq System (Illumina) platform. The taxonomy for the 16S rRNA gene reads from sequencing was assigned using blastn from QIIME2 against the SILVA (v138.1) (Quast et al. 2012) database.

Total carbon (C) and total nitrogen (N) in soil samples was performed using the Elementar Vario Macro cube analyzer in CN mode at UQ lab....

## Results

### *Distance from water effect on Central Australian biocrust microbial communities*

The composition of biocrust communities varied with distance from water (PERMANOVA  $R^2=65\%$ ,  $F=10.9$ ,  $P<0.001$ ), although alpha diversity remained consistent. The community composition at a distance of 50 meters from the water was significantly different ( $P < 0.001$ ) from all other distances (Figure 1).

Although not statistically significant, the 6 km biocrust communities did separate out from all other distances, whereas samples from between 500 m and 1-2 km from water showed significant overlap in composition (Fig. 1). In total, 60 operational taxonomic units (OTUs) were identified with a relative abundance greater than 1% in the biocrust bacterial community. ANOVA analysis ( $p < 0.05$ ) on the relative abundance revealed that 47 of these 60 OTUs exhibited significant changes based on their distance from the water.

The biocrust composition was represented by members of the Acidobacteriota, Actinobacteriota, Armatimonadota, Chloroflexota, Firmicutes, and Proteobacteria. From a general overview, several members of the Acidobacteriota and Chloroflexota, were reduced in their relative abundance at 50 m from water, in contrast to several members of Firmicutes that increased their relative abundance at the shortest distance from water.

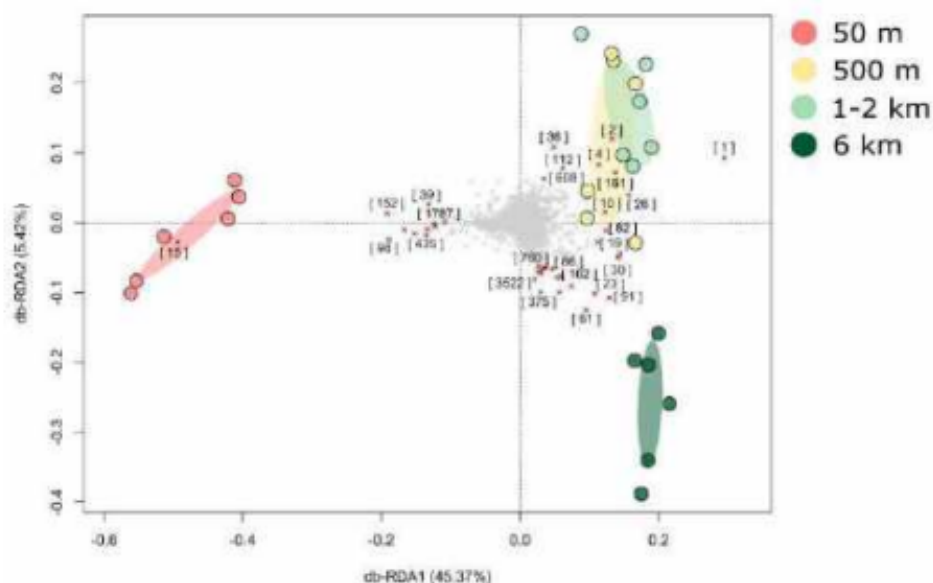


Figure 1. Separation of biocrust community composition between 50 m and 6 km from water clearly evident in Distance-based Redundancy Analysis (db-RDA) ordination plot. Constraint by distance as per PERMANOVA analysis. Discriminating OTUs are shown in brackets.

### *Distance from water effect on total C, total N, and C:N ratio*

Total nitrogen and carbon (but not C:N ratio) varied with distance from water and at 50 m they were both more than double the levels found at all other distances (Table 1, Figure 2).

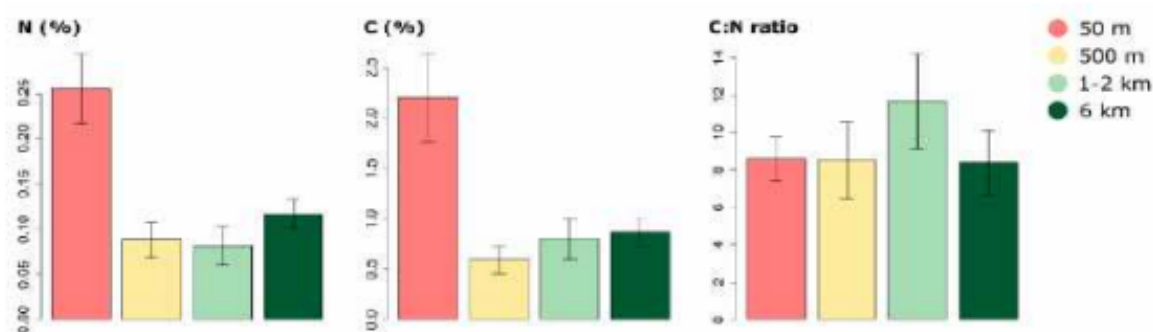


Figure 2: Bar graph of total carbon (%), total nitrogen (%), and C:N ratio by distance from water (50 m, 500 m, 1-2 km and 6 km). Error bars represent standard errors

Table 1. Total carbon (%), total nitrogen (%), and C:N ratio by distance from water (50 m, 500 m, 1–2 km and 6 km). Means and Standard Deviation, different letters represent significant differences as per post hoc analysis ( $P < 0.05$ )

Distance	Total Nitrogen (%)		Total Carbon (%)		C:N ratio	
	Mean	SD	Mean	SD	Mean	SD
<i>Mount Denison</i>						
50 m	0.26	0.09	2.21	1.06	8.57	2.86
500 m	0.09	0.05	0.60	0.33	8.51	4.98
1-2 km	0.08	0.05	0.80	0.51	11.66	6.20
6 km	0.12	0.04	0.87	0.34	8.39	4.22

### Effect of excluding grazing on biocrusts

The exclusion effect (inside versus outside) on alpha diversity and composition was evaluated across all sites. There was no effect of the first year of exclusion on alpha diversity (Shannon's index) and composition (results not shown).

### Discussion

The biocrust microbial community in extensive grazed rangeland paddocks was relatively stable in its composition and diversity with the exception of heavily trampled high use areas immediately adjacent waters.

The study on biocrust community composition found an increase in the relative abundances of several Firmicutes populations at the closest distance from the water points (50 m). While Firmicutes are known as plant growth-promoting bacteria (Amaresan et al. 2020), they have also been found to effectively remove ammonium and total nitrogen from wastewater (Yue et al. 2024), which is possibly related to the increased N content at the 50 m point from water.

The higher levels of total C and N at the water points are likely due to animal manure and urine inputs, as cattle often congregate and camp close to the water points. The animals graze out several kilometres daily, harvesting resources as they graze pastures and browse from the surrounding paddock. The stocking density and time spent were highest closest to water, leading to a translocation of resources to campsites and water points, which accumulates these resources from long distances into a smaller site closer to the water (Augustine et al. 2013). Given that only the heaviest grazing and trampling impacts immediately within the 50 m vicinity of stock water points had differences in C and N, it is unsurprising that exclosures surrounded by moderate to low grazing had little impact on soil C and N.

## Acknowledgements

This project was funded through the Australian Governments Future Drought Fund Resilient Soils and Landscapes.

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