



## **Dust to Crust: Rangeland regeneration through biocrust inoculation of Mitchell grass (*Astrebla lappacea* (Lindl.) Domin)**

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**Key words:** biological soil crust; bio-priming; germination; grasslands

### **Abstract**

Biocrusts are a living complex of microscopic organisms (cyanobacteria, bacteria, fungi, algae, lichens, liverworts, mosses) that cover the upper soil between vascular vegetation in arid and semi-arid rangelands. As providers of ecosystem services, they are a nature-based solution for landscape regeneration, but site- and species-specific knowledge is often missing. We tested the hypothesis that biocrusts differ in their effect on grass germination. Our focus was Mitchell grasslands with tussock grass *Astrebla lappacea* because degraded semi-arid western Queensland requires effective regeneration techniques. We used characteristic descriptions of biocrusts in testing the effects of biocrust as soil surface or as separate seed inoculum on germination success in glasshouse and laboratory experiments. Germinations increased slightly on a biocrust surface compared to a sand-only control. Although differences were not statistically significant, germination was greater than in sand in 71.4% of inoculum treatments and rate was significantly faster in one-third of treatments. With promising early findings, we conclude that biocrusts can improve germination of *A. lappacea*, with optimisation in next-steps research such as inoculum field experiments across landscapes. Biocrusts hold promise as a nature-based solution to address the global problem of degraded rangelands.

### **Introduction**

Mitchell grasslands are dominated in their natural state by *Astrebla* tussock grasses and cover about 57 million hectares of Australia's rangelands ("Mitchell Grasslands", 2021). *Astrebla lappacea* (Lindl.) Domin. (Curly Mitchell grass) is one of four abundant *Astrebla* species. It is a highly desirable perennial grass that provides ecosystem stability, having a 20 to 30-year life span and considerable drought tolerance (Lambert et al., 1990). Death of *Astrebla* tussocks is accentuated by heavy grazing during drought, which causes the problematic colonisation of annual grasses in the interstitial spaces when *Astrebla* cover is reduced (Orr & Phelps, 2013). Regeneration techniques are required to successfully and cost-effectively restore these landscapes. Focussing on nature-based solutions, we studied the role of biocrusts in the target grasslands. Biocrusts provide a multitude of ecosystem services including stabilising soils, carbon and nitrogen fixation and cycling, and water cycling (Weber et al., 2016). Biocrusts influence vascular plant establishment due to their position in the upper soil layer and surface where seeds are dispersed (Bowker et al., 2022; Weber et al., 2016). However, plant germination responses

to biocrusts are variable and are site- and species-specific (Havrilla et al., 2019; Peter et al., 2016). Research into the effects of biocrust surfaces and inoculums on the germination of other species has been conducted (Bowker et al., 2022; Jiménez-González et al., 2022; Muñoz-Rojas et al., 2018; Peter et al., 2016; Serpe et al., 2006) with mixed findings. To fill knowledge gaps relating to Mitchell grasslands, we explored the interactions of biocrusts and *A. lappacea* seed, and tested in a first step how different types of biocrusts affect germination success. This study aimed to test two hypotheses:

1. Biocrusts enhance germination of *A. lappacea*.
2. Differently developed biocrusts have different effects on germination of *A. lappacea*.

## Methods

Biocrusts were collected from 21 sites across western Queensland between April and May 2024. Mean annual rainfall varies across the region between 380 and 530 mm. The wettest months are November to March with 70% of annual rainfall (Climate Data Online, 2024). Sites spanned nine land types and consisted of intact, open woodlands and grasslands to degraded areas with low or no ground cover and soil erosion. Spatulas were used to collect the top 5 mm of soil surfaces with biocrust. *A. lappacea* seed was collected from wild populations near Julia Creek (141.7442°E, 20.6909°S) in May 2024, dried and stored at room temperature. Germination tests using a sub-sample of 20 naked caryopses were undertaken in a Thermoline Scientific STAR700 Climatron with LED lights set at 12 hourly diurnal cycles (32°C day, 25°C night). The number of seeds that had germinated (5 mm radicle visible) were counted over 30 days.

Glasshouse pot trials were conducted to test effects biocrust have when on the soil surface. Naked *A. lappacea* caryopses were imbibed in water for 18 hours. Three replicates for each of 21 biocrust sites plus a sand control were randomly allocated to pots and the surface covered by biocrust. One soaked seed was sown ~5 mm. Bottom-up watering was used and water availability reflected wet-dry cycles. Mean counts of pasture grass germination were used for analyses. Biocrust inoculum trials were conducted to determine whether differently developed biocrust affect germination of *A. lappacea* differently when applied directly to seed. The top 2 mm of each biocrust sample was passed through a sieve (~0.5 mm). Sieved biocrust (1.5 g) was combined with 1mL of DI water to create a separate inoculant for 21 samples. Sand was used for a control. Six *A. lappacea* naked caryopses were immersed in each inoculant and the control then sealed in Petri dishes using parafilm. Petri dishes were placed in the climatron. Equal volumes of DI water were used throughout the experiment to moisten inoculants. The number of germinated seeds were counted over 29 days. Germinated seeds were those meeting International Seed Testing Association guidelines (International Seed Testing Association, 2013).

One-way ANOVA with a Kruskal-Wallis test for non-parametric data tested whether germination with biocrust, compared with a sand control, was significantly different. Spearman's rho was used to determine effect size of correlations between biocrust characteristics and germination. A generalised logistic model with time as a covariate was fit for proportional data to extrapolate germination rates with biocrust inoculum. Significance was determined by examining the 95% confidence intervals of odds ratios and whether one was between the lower and upper confidence interval. Jamovi (The Jamovi Project, 2024) was used for statistical analysis.

## Results

### ***Experiment 1: Effects of biocrust surface on seed germination***

Mean counts of all pasture grass germination, including seeds already in the seedbank and sown *A. lappacea*, were analysed. Emergence of seedlings was greater than the control in 47.6% of treatments and 33.3% of treatments had germination equal to the control. Correlations between biocrusts characteristics and germination were analysed (Figure 1). Significant correlations ( $p < 0.05$ ) were observed between germination and both water availability in land types biocrusts were sourced from, and crust colour.

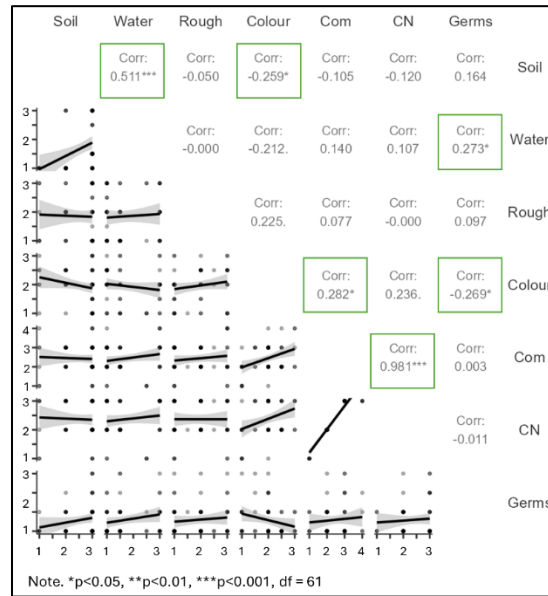


Figure 1. Correlation between biocrust characteristics and germination of pasture grasses in surface trials (Spearman's, asterisks indicate significance). Axes numbers represent classifications: Soil (1= Shallow, gravelly or sandy, 2= Texture contrast, 3= Heavy clay); Water (1= Low availability, 2= Moderate, 3= High); Roughness (1= Smooth, few pores, 2= Few pores, raised surface, 3= Rough due to liverworts, mosses or lichens); Colour (1= Light to intermediate, 2= Dark, 3= Green); Community (1= Light cyanobacteria, 2= Intermediate cyanobacteria, 3= Dark cyanobacteria with lichens, liverworts and/or mosses, 4= Dark dominated by lichens, liverworts and/or mosses); Carbon and Nitrogen (0= Bare ground, 1= Some organic matter/litter, little biocrust, 2= Light to intermediate cyanobacterial biocrust, 3= Dark cyanobacterial biocrust or dark biocrust with lichens, liverworts and/or mosses)

**Experiment 2: Effects of biocrust inoculum on seed germination**

Germination tests in paper towel revealed that 5% of *A. lappacea* seeds from the sub-sample germinated. Emergence was greater than or equal to the non-insulated control in 71.4% of biocrust inoculum from the 21 treatment sites. When data was extrapolated using a generalised logistic model, for treatments with increasing probability of germinating over time, one-third of sites were significantly faster compared to the control. Treatment sites grouped by land type revealed 77.8% of land types reached greater than 50% germination within the 30-day testing period. Community composition affected mean germination. Germination was greatest with light cyanobacterial inoculum (58.33 ± 35.36%). Intermediate cyanobacterial had 36.36 ± 19.46% and dark cyanobacterial crust with liverworts, mosses and lichens had 42.86 ± 16.27%. Dark biocrust dominated by liverworts, mosses and lichens had equal germination to the control (16.67%). Whilst cyanobacterial biocrust inoculum enhanced germination, mean germination for various biocrust communities was not significantly different to the control in these experimental conditions (p>0.05).

**Discussion**

In this study we aimed to develop an understanding of how biocrust and *A. lappacea* interact and determine whether biocrusts at various successional stages affect seed germination differently. Germination of the studied seeds was 5%. Naked *A. lappacea* caryopses usually have around 90% germination rate when dormancy is not a barrier (D. Phelps, personal communication, October 16, 2024). In our study, seed was harvested in May and experiments performed in August, so dormancy is a likely reason for the low germination rates in preliminary germination tests. Despite this, we found some support for our hypothesis that biocrusts affect germination. One way biocrust may enhance germination is through improved water relations. There is conjecture over effects of biocrust on water capture, movement and retention. Like our findings, others (Canton et al., 2020; Ming et al.,

2024) found that cyanobacterial biocrusts decreased run-off and retained water for longer than bare soils, especially in upper layers. Adessi et al., (2018) attributed increased water retention to extracellular polymeric substances (EPS); there is agreement that EPS contributes to better soil structure, aeration and water infiltration. In our study, soil texture influenced water availability as the sand control had higher evaporative rates (NP observations), resulting in poorer biocrust development, and reduced water retention. With less water available in the sand control, germination rates were lower than biocrust treatments.

We also found some evidence to support the hypothesis that differently developed biocrusts affect germination differently. Significant differences in germination in our experiments are likely due to differences in biocrust colour. Biocrusts are generally darker than the parent material they grow on and exhibit lower albedo, especially biocrusts with organisms like cyanobacteria that contain more chlorophyll and UV protecting pigments such as scytonemin (Kidron et al., 2022). Higher albedo associated with light cyanobacterial biocrusts likely accounted for increased germination in our experiments (Fig.1). We discovered that germination success was enhanced to varying degrees when seeds were primed with biocrust inoculum. This supports previous studies that have demonstrated that 'bio-priming' seeds with cyanobacteria enhanced germination in some species native to Western Australia (Chua et al., 2020; Muñoz-Rojas et al., 2018). In our study, the number of germinations may have been lower in some land types, but the speed at which germination occurred was higher. This would have the potential for seed to germinate and establish before water runs out. Biocrust enhanced germination rates for 71.4% of site inoculums and, for most treatments, inoculum-seed mixture remained moist, so water availability was probably not a barrier for germination in those with lower rates. Our observations are supported by studies where no significant difference in water retention was observed when induced biocrusts were ground up rather than being left intact (Colica et al., 2014). We found effects of biocrust inoculums were site-specific and this is consistent with findings of other studies (Havrilla et al., 2019; Peter et al., 2016).

Overall, biocrust enhanced germination of *A. lappacea*, although not significantly, and minor differences in germination were observed for differently developed biocrusts in the context of this study. Conclusions are based on limited data and replications as few biocrust samples were available so should be considered preliminary in this field of research. Additionally, identification of genera or species was outside the scope of this study and more testing for inoculums is recommended. Given the effects of biocrust are site- and species-specific, further research into the effects of biocrusts on germination of *A. lappacea* in field experiments is essential for development of effective nature-based solutions to rangeland regeneration.

### Acknowledgements

This research was supported by the University of Queensland in collaboration with Desert Channels Group. Thank you to Roxane Blackley and Desert Channels for sample collection and Chris Pezet for sharing his knowledge of Ardnaree and allowing access to the property for sample collection.

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