Draft Final report

Boosting natural regeneration of the nitrogen capital in grazing lands

Project code:	B.PAS. 0502
Prepared by:	Dr Wendy Williams, Prof Susanne Schmidt and Team* The University of Queensland
Date published:	Unpublished Draft

PUBLISHED BY
Meat and Livestock Australia Limited
PO Box 1961
NORTH SYDNEY NSW 2059

Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government to support the research and development detailed in this publication.

Abstract

This project focussed on how rangeland management influences the capacity of biological soil crusts to replenish soil nitrogen lost in export of livestock production. Using multi-tiered cutting-edge science spanning from gene-centric analysis to satellite imagery, we explored tools to quantify the presence, composition, and function of biocrusts in long-term fire and grazing trials over multiple seasons. Imaging can distinguish biocrusts from degraded bare and vegetated soil. Biocrust-topsoil has 4-times more nitrogen and carbon than bare soil confirming their essential role in rangelands. N input from biocrusts of 5 kg per hectare represents a substantial input that accounts for approximate one sixth to half of the annual pasture N demand in extensive northern Australian ecosystems. Bacterial genes responsible for nitrogen fixation emerged as a sensitive indicator, which responds to landscape condition and season, confirming that management modulates nitrogen fixing capacity.

Stocking rates that maintain good land cover support nitrogen fixation via biocrusts, which are most productive when sheltered by grass. Informed by land condition and climatic conditions, where wet season spelling can facilitate nitrogen inputs during peak biocrust activity (Williams et al., 2018). Fire regimes using moderate temperatures will speed biocrust recovery while reducing litter buildup and boosting nitrogen fixation. Based on previous research well-developed biocrusts in healthy landscapes generate annually 5 kg nitrogen per hectare. This conservative estimate means that per square km, biocrusts provide 25-50 tonnes nitrogen for pasture with 1-2% dry matter nitrogen. The findings can augment good industry practice by harnessing biocrusts to annually replenish nitrogen in grazed rangelands. Rapid and cost-effective restoration of biocrusts could support the rapid recovery of rangelands after disaster, and there are insights on how this could be accomplished.

*Team includes a range of contributors to this project and report that are listed at the beginning of each chapter and tabled in Acknowledgements

Executive summary

Background

Biocrusts form the biodiverse living layer of microscopic organisms that colonise the upper centimetre of soils in rangelands and other drier regions as dense soil cover between vegetation. Dormant and dark coloured in the dry season, biocrusts are green and active in the wet season. Biocrusts reduce erosion and increase water infiltration in healthy landscapes but are less developed or absent in degraded areas. Following fire, drought, or flooding, biocrusts are the first responders to recolonise bare soil. This project examined the capacity of biocrusts for biological nitrogen fixation as they harbour specialist bacteria, including comparatively large cyanobacteria.

Addressing the industry's interest in soil health and expanding producers' understanding of biological nitrogen fixation beyond legumes, biocrusts were studied in long-term trials focussing on fire (Victoria River Research Station, NT) and grazing regimes (Wambiana Grazing Trial, QLD).For the first time in Australian rangeland soils, a multi-tiered technology-driven approach quantified and characterised biocrusts, their organisms and the bacterial genes responsible for nitrogen fixation and nitrogen cycling processes. The overarching aim was to evaluate how rangeland management and biophysical conditions affect the presence of biocrusts and their potential to fix nitrogen and thereby regenerate nitrogen that is removed in livestock production. Since few Australian producers knew about biocrusts, communication raising awareness was a key project aim.

Objectives

The key objective was to identify how pasture management affects the capacity of biocrusts to generate nitrogen as natural fertiliser so that producers can maximise the capacity to replenish nitrogen in soil for pasture growth. The project addressed these questions:

- Can image analysis (proximal, drone/UAV, satellite) quantify biocrusts to upscale from patch to landscape?
- Do biocrusts have preferred habitats within landscapes?
- Do biocrusts on different soil types differ in species composition and function?
- How do fire and grazing impact biocrust presence and capacity to fix nitrogen?
- Can DNA analysis of biocrust organisms provide information on nitrogen fixing capacity?
- Can analysis of bacterial genes quantify biocrust processes, especially nitrogen fixation?

- Which management recommendations emerge from the findings?

Methodology

An integrated methodology was used to upscale from molecular precision to landscape-level industry relevance. We:

- chose two long-term research sites with >25-year fire and grazing treatments and two soil types at each site to discern the impact of management on biocrusts presence and nitrogen fixing capacity. Fire regimes contrasting no-fire, with 2,4,6-year early and late dry season burning. Grazing regimes are ungrazed control, moderate and heavy stocking rates, and wet season spelling.
- quantified the presence/absence of biocrusts at plot and landscape levels in dry and wet seasons using imagery (smart phone, multi-spectral sensing with UAV and satellite).
- used landscape function analysis that categorised landscapes into four microsites (bare soil, between grasses, under grasses, under litter) to distinguish biocrust types and function.
- assessed biochemical indicators of biocrust development and function.
- analysed biocrust DNA to identify communities and nitrogen fixing bacteria.
- analysed bacterial DNA with powerful bio-informatics methods to quantify genes responsible for nitrogen fixation and all nitrogen conversions.
- quantified nitrogen fixation with laboratory assays ($^{15}N_2$ tracer, acetylene reduction).
- communicated project discoveries in producer-relevant forums from the onset of the project.

Results/key findings

This project provides unparalleled insight into the presence and function of biocrusts. The principal question of '*how much nitrogen can biocrusts generate for pastures*' has to consider the net effects of biocrust nitrogen input, uptake by pasture, and nitrogen loss from soil.

- In degraded landscapes that lack vegetation cover and are eroding, nitrogen input by biocrusts is lowest. Such landscapes have negative feedback: low nitrogen input, low uptake by pasture, and high risk of nitrogen loss.

- In well-managed landscapes with suitable vegetation cover, nitrogen input from biocrusts is highest. Such landscapes have positive feedback: high nitrogen input, high uptake by pasture, and low risk of nitrogen loss.

- Nitrogen input is maximised when biocrusts are undisturbed in the wet season during the peak nitrogen fixation period.

Summarised results and key findings

Multispectral imaging and remote sensing to identify biocrusts	Imaging distinguishes biocrusts from bare soil and from pasture in dry and wet seasons.
	Satellite imaging is cost-effective and readily available but is restricted to 3-meter resolution, while UAV imaging is more costly but achieves 6 cm resolution.
	Chlorophyll was tested as a potentially cost-effective marker for nitrogen fixation with green cyanobacteria as a major nitrogen fixing taxon in Australian biocrusts. But with many non-green N-fixing bacteria present, chlorophyll is not a proxy for fixation potential but facilitates the multi-spectral sensing of active green biocrusts in the wet season.
	This means current satellite-based pasture assessment can be expanded to include biocrusts and can be refined with UAV imaging where required.
Landscape function analysis to quantify biocrust presence	Bare soils have no or low biocrust presence due to unfavourable conditions with excess light, low moisture, high physical forces promoting erosion.
	Between and under grass hummocks, biocrusts are most prominent and active. These sheltered microsites have favourable conditions (attenuated light and physical forces, higher moisture). Under leaf litter, biocrust is less active with light being a limiting factor.
	This means desirable land cover that has adequate pasture supports and promotes biocrusts function.
Soil nitrogen and carbon as indicators for biocrusts	Amount of nitrogen and carbon in the top cm of soil at the four microsite types was not a universal indicator of biocrust presence. Rather, both elements occur in biocrust, soil organic matter and indicate past events that subtract or add nitrogen and carbon (e.g., erosion, manure deposition).

	Comparing two microsites 'bare soil' and 'soil with biocrust' revealed up to 4-times higher nitrogen and carbon in 'soil with biocrust', confirming biocrust functions for erosion prevention and nitrogen and carbon inputs.
	This means biocrust cover on soils preserves and likely increases soil nitrogen and carbon stocks.
DNA metabarcoding identifying bacteria	DNA analysis of biocrust communities confirmed a universal presence of nitrogen fixing bacteria.
	DNA analysis did not distinguish biocrusts under distinct fire management, but soil type, microsite and season were the drivers of differences in bacterial communities.
	Grazing regimes altered biocrust communities together with soil type, microsite, and season. This highlights that grazing intensity had a stronger influence on the composition of biocrusts and soil microbiomes, than the fire management studied here.
	This means bacterial communities in biocrusts change in response to environmental conditions, and management can promote biocrusts with desirable functions.
Gene-centric and ^{15}N tracer analyses to estimate nitrogen fixation	Newer methodologies characterising all genes within a community identified significant impacts of grazing and fire on the composition of bacterial genes including nitrogen fixing potential and nitrogen conversion processes (ammonification, nitrification, denitrification etc).
	The presence of nitrogen-fixing genes was highest in the wet season in biocrust growing at favourable microsites between and under pasture grasses.
	^{15}N tracer analysis showed nitrogen fixation rates ranked from highest to lowest with ungrazed > wet season spelled > moderate/high stocking rates without wet season spelling.
	This means biocrusts that are undisturbed in the wet season and grow in favourable microsites have the highest nitrogen fixation capacity and rates.

Benefits to industry

The project raised industry awareness of biocrusts so that producers can accommodate biocrusts in their land and grazing management decisions. The benefits are; (i) biocrusts are most prolific in rangelands that are carefully managed for pasture retention and (ii) biocrust can annually

regenerate soil nitrogen to ensure soil health and fertility and support pasture growth for long-term sustainable use of rangelands and livestock production. This requires:

(1) optimising stocking rates in line with existing and emerging recommendations,

(2) wet season spelling informed by paddock condition, i.e., more degraded paddocks will benefit from more frequent and/or longer wet season spelling.

(3) considering soil type because biocrusts are more vulnerable in sandier soils than clayey soils, with degraded land on sandy soils taking longer to recover biocrust cover and function.

(4) optimised fire regimes that reduce leaf litter build up while ensuring speedy recovery of biocrusts to be active in the wet season.

With producers aiming to maximise pasture production, managing biocrusts allows controlling erosion and maximising input of the 'renewable nutrient nitrogen' which is quantitatively the most important nutrient, accounting for 60-70% of soil-derived nutrients. A confident estimate is that well-developed biocrusts generate annually 5 kg nitrogen per hectare or 500 kg N per square km. Modelling predicts that N contributions by biocrusts improve pasture quality on a seasonal basis. When biocrusts access sufficient moisture during the wet season, they are both protected by pasture cover from harsh conditions, and from trampling during peak nitrogen fixation in the wet season. Higher nitrogen inputs may occur under optimal conditions, in line with estimates elsewhere. The conservative estimate of 500 kg N/km^2 would provide sufficient nitrogen for 25-50 tonnes of pasture with 2 to 1 % dry matter N.

Future research and recommendations

This project took comprehensive steps to examine biocrusts in the context of Australian rangelands and their management. Situating the project at two research stations with fire and grazing management enabled a dual focus on:

(i) develop methods to detect and analyse biocrusts and their nitrogen fixation potential, and

(ii) discerning biocrust responses to environmental and management variables.

(iii) develop modelling program (e.g. CLEM) to further elucidate industry benefits.

(iv) incorporate biocrusts into land management including natural capital and restoration goals.

We are confident that the overarching principles for biocrust responses to pasture management and environmental factors (seasons, soils) are universally applicable.

However, specific implications for managing biocrusts across a wider range of land conditions, locations (climate, soil), and management (e.g., regenerative grazing) demand attention and are discussed in the conclusions of this report.

Table of contents

Boosting natural regeneration of the nitrogen capital in grazing lands 1

Abstract 2

Executive summary 3

Background 3

Objectives 3

Methodology 4

Results/key findings 4

Summarised results and key findings 5

Benefits to industry 6

Future research and recommendations 7

1. Background 16

1.1 Dryland ecosystems and the role of biocrusts 16

1.2 Grazing management in dryland ecosystems 17

1.3 Land degradation and soil fertility 17

1.4 The importance of biocrusts 18

1.5 How soil renews and delivers nitrogen 19

1.5.1 Nitrogen is a renewable nutrient 19

1.6 Nitrogen processes within biocrusts 23

1.6.1 The soil nitrogen bank 24

1.6.2 Five kg nitrogen input per hectare (0.5 t per square km) is a lot in northern extensive grazing systems 25

1.6.3 How much nitrogen is typically available to northern native pasture systems? ..25

1.6.4 Nitrogen losses and inputs in grazed native pasture systems 26

1.7 Biocrust and fire effects on seeds germination 28

2. Objectives 29

2.1 Quantifying the nitrogen-generating capacity of biocrusts. 29

2.2 Identifying the environmental and management factors that drive the presence and activity of biocrusts in different land types. 30

2.3 Developing and modelling different scenarios for N-smart grazing management practices. 31

2.4 Generated knowledge prospective to improve nitrogen Best Management Practices. 31

2.5 Developed high quality information products for end users that have made 150 pastoralists aware of the opportunity that biocrusts present for N-smart BMP. 31

3. Methodology 32

3.1 Key research questions 32

3.2 Approach 32

3.3 Research sites 35

3.3.1 Victoria River Research Station (VRRS) Kidman Springs, NT 35

3.3.2 Wambiana Grazing Trial, North QLD. 37

4. Biocrust structure and taxonomic composition 38

4.0.1 Sample collection 38

4.0.2 VRRS Fire Trials 2019 38

4.0.3 Wambiana Grazing Trial 2020 38

4.0.4 Biocrust microscopy 39

4.0.5 Scanning Electron Microscope (SEM) imaging 39

4.0.6 Community composition profiled by DNA sequencing 41

4.1 Influence of fire and grazing on biocrusts at VRRS 41

4.2 Results: Taxonomic composition of biocrusts at VRRS 41

4.3 Metagenomic insights of biocrust function 43

4.4 Structural form of biocrusts at VRSS 46

4.5 Biocrust composition across microsites 49

4.5.1 Bacterial community profile: Early 4-year fire microsites VRRS 49

4.6 Results: Biocrusts at Wambiana Grazing Trial 54

4.7 Biocrust metagenomic insights, moderate stocking, wet season spelling 56

4.7.1 Photosynthesis 56

4.7.2 Taxonomic profiles of biocrusts across microsites 57

4.7.3 Biocrust microsites: moderate stocking, wet season spelling 58

5 Biocrust recovery after fire at VRRS 61

5.0.1 Fieldwork (2019 and 2020) 61

5.0.2 How do we measure biocrusts across 32 fire plots? 61

5.0.3 Field sampling 61

5.0.4 Fire environment 64

5.0.5 Biocrust Resurrection and Recovery 64

5.0.6 Photosynthetic activity 65

5.0.7 Carbon, Nitrogen and Chlorophyll 65

5.0.8 Biocrust morphology and cyanobacterial diversity 66

5.0.9 Statistical analysis 66

5.1 Results: Biocrust resurrection and recovery 67

5.1.1 Effect of microsite 70

5.2 Effects of fire on biocrust nitrogen and carbon at VRSS 70

5.2.1 Calcarosol 70

5.2.2 Vertosol 72

6 Wet season resting from grazing boosts biocrust hotspots 75

6.0.1 Field methods 75

6.0.2 Landscape function 76

6.0.3 Ground cover 76

6.0.4 Statistical analysis 78

6.1 Results: Wet season resting boosts biocrust hotspots 79

6.1.1 Biocrust hotspots in duplex soils 79

6.1.2 Biocrusts in red-yellow earths 80

6.1.3 Landscape function across interspaces 81
6.1.4 Stability 82
6.1.5 Infiltration 82
6.1.6 Nutrient cycling 82
7 Metagenomic insights into biocrust function 84
7.0.1 Shotgun sequencing and gene-centric analysis of biocrust 84
7.0.2 Overall experimental design 86
7.0.3 Biocrust function evaluation on fire and grazing trials 87
7.1 Results: Nitrogen processes within biocrusts 87
7.1.1 N cycle genes across microsites of the 'Early 4-year' fire treatment 87
7.1.2 Bacterial community profile: Fire treatment, soil and season effects at VRRS .90
7.1.3 Explanation of results in terms of land management implications. 91
7.1.4 Effects of microsites and grazing on N at Wambiana grazing trial 92
7.2 Quantifying biological nitrogen fixation of biocrusts 96
7.2.1 Acetylene reduction 96
7.2.2 Stable isotope $^{15}N_2$ 97
7.3 Interim Results: Quantifying N fixation at Wambiana grazing trial .. 97
7.4 Bioavailability of nitrogen from biocrusts 98
7.4.1 Ion exchange resin methodology 98
7.4.2 In situ fire and grazing wet trial on calcarosol at VRRS 98
7.5 Results: Bioavailability of nitrogen from biocrusts at VRRS 100
7.6 In situ wet season study at Wambiana grazing trial 101
7.7 Results: Wambiana grazing trial 102
7.7.1 Duplex clays 102
7.7.2 Red-yellow earths 104
8 Nitrogen and carbon dynamics: fire and grazing 105
8.1.1 VRRS Sampling Program 105
8.1.2 Statistical Methods 108

8.1.3 Preliminary results and field notes....108
8.2 Results: Nitrogen and carbon dynamics at VRRS (2020) 110
8.2.1 Calcarosol N and C stocks (kg/ha) 110
8.3.1 Vertosol N and C stocks (kg/ha) 112
8.4 Results: Nitrogen and Carbon dynamics at Wambiana grazing trial 2020 113
8.4.1 Red yellow earth N and C stocks kg/ha 113
8.4.2 Duplex clay N and C stocks (kg/ha) 115
9 Fire, biocrusts and seed germination at VRRS..... 117
9.1 Seed germination and fire 117
9.1.1 Greenhouse seedbank germination 117
9.1.2 Statistics....119
9.2 Results: Germination before and after fire with biocrusts 120
9.2.1 Biocrust cover and seed germination....120
9.2.2 Microsite germination 121
9.2.3 Biocrust cover in pre and post fire samples 122
9.2.4 Germination pre and post fire....123
10 Rapid assessment of ground cover with mobile phones at VRRS 125
10.1 Field measurements 125
10.1.1 Biocrusts and ground cover....125
10.1.2 Biocrust field collections and pigment analysis 125
10.1.3 Taking RGB photos in the field. 126
10.1.4 Quantifying ground cover using decision tree-based segmentation model (DTSM) 126
10.1.5 Preparation of photos and defining Region of Interest (ROI) 127
10.1.6 DTSM application and accuracy evaluation 127
10.1.7 Statistical analysis....128
10.2 Results: Modelled seasonal cover 130

10.2.2 Modelled grass and litter cover compared to field measurements.....130
10.2.3 Relationships between microsites 131
10.2.4 Modelled biocrust cover compared to field measurements 133
10.2.5 Biocrust pigment content across microsites 134
11 Detecting biocrusts using remote sensing before and after fire..... 136
11.1 Large scale fire and grazing demonstration at VRRS.....136
11.2 Data Collection, Preparation, and Processing 138
11.3 Training Data Collection 138
11.4 Preparation of Planet Scope Data 138
11.5 Data Processing 139
11.5.1 Sample Point Allocation 139
11.5.2 Spectral Value Extraction 139
11.5.3 Calculation of Vegetation and Crust Indices 139
11.6 Statistical analysis.....140
12 Detecting biocrusts using remote sensing before and after fire. 141
12.1.1 Spectral response of land cover classes before the fire event 141
13 Large scale fire and grazing demonstration at VRRS 144
14 Communications 149
14.1 Video production.....149
15 Discussion and Conclusions 151
16 Key Findings 152
17 Future Research and Recommendations 152
18 Benefits to industry..... 153
18.1 Acknowledgements 154
19 References 155
20 Appendices 159
Appendix 1: Biocrusts – the living skin of rangelands soils..... 159
Video production for land manager and producer education 159

Appendix 2: Biocrust extension and education in the Northern Territory and Queensland 161

Next Gen Rangeland Management Courses 161

Katherine Show 2023: Biocrust display 161

Agriculture NT Facebook Post 161

Appendix 3: Resting from grazing in the wet season boosts biocrust hotspots 167

Appendix 4: Effects of grazing and fire management on rangeland soil and biocrust microbiomes. 183

Appendix 5: Using digital photography to monitor changes in biocrusts and ground cover in a savanna rangeland. 191

1. Background

1.1 Dryland ecosystems and the role of biocrusts

Drylands cover approximately 40% of the global terrestrial surface (Hartley et al., 2007) in regions with high temperatures and low rainfall and are key contributors to earth system processes and primary production (Metternicht and Smith, 2020). Ranging from arid, to semi-arid and dry-subhumid, dryland vegetation is characterised by discrete patchiness due to discontinuous cover of vascular plants (herbs, grasses, shrubs, trees) and often with inherently poor soil fertility in many regions (Bowker et al., 2014; Carberry et al., 2011; Maestre et al., 2016; Williams et al., 2021). A distinguishing trait is that biocrusts cover the soil surface between and under vascular vegetation, and these are the focus of this report.

The functionality of dryland ecosystems is impacted by changing land cover, climate and fire and the interactions between biotic and abiotic components, and functionality impacted by soil erosion, loss of biodiversity and loss of primary productivity (Maestre et al., 2016). In Australia's northern dry-wet savannas and semi-arid shrublands, seasonality influences vegetation structure and regional biodiversity with summer rains (monsoon in savanna) driving primary productivity over an annual cycle of a few months of a wet season, followed by a long dry season period.

Biocrusts are integral and often overlooked component of dryland systems with biodiverse communities of microbial and small organisms, including bacteria (cyanobacteria are often the most prominent organisms in Australian biocrusts), fungi, microalgae, lichen, liverworts and mosses (Williams et al., 2014). Biocrusts form an expansive protective cover on the soil surface, serving important ecological functions including soil stabilisation, carbon and nitrogen fixation, and are integral to carbon and nitrogen cycles (Elbert et al., 2012; Williams et al., 2018, 2014). Biocrusts green up and are metabolically active when sufficient moisture is present (wet season) and form a dark or reddish crust on the soil surface when dormant in the dry season. Nitrogen fixation by cyanobacteria and other diazotrophs provides a direct source of bioavailable (plant-available) nitrogen (N) for plants that fluctuates seasonally (Barger et al., 2016; Williams et al., 2018). Biocrusts survive fire and contribute to the post-fire bioavailable nutrient spike that has been observed in global savannas, but this is less well understood in Australian drylands (Weber et al., 2016). Cyanobacteria are particularly important in Australian biocrusts as their extracellular matrix (ECM) encapsulates colonies of microorganisms in a hydrophobic hardened polysaccharide coating

that, in response to humidity, temperature and rainfall, turns to hydrophilic slime (Billi and Potts, 2002; Potts, 1999; Williams et al., 2014). This enables cyanobacteria to survive desiccation throughout the dry season and resurrect in the wet season following abundant rainfall (Büdel et al., 2018; Williams et al., 2014). The ECM stores nutrients and partially disintegrates at the start of the wet season (Büdel et al., 2018), thereby releasing plant-available N (Williams et al., 2018). In savanna impacted by grazing, fire, monsoonal rainfall and drought, the ability of biocrusts to recover from disturbance including in abundance and diversity is likely instrumental for maintaining ecological functions.

1.2 Grazing management in dryland ecosystems

In Australia, approximately 75-80% of the land mass is classified as rangelands and most of the area is used for livestock production. Extensive grazing is the most extensive use of land within dryland ecosystems and supports livelihoods of rural communities (Asner et al., 2004). 60% of the national cattle herd is located across northern Australia. With demand growing and the availability of suitable agricultural land limited, production has intensified (Maestre et al., 2016).

The introduction of hard-hooved livestock to Australian ecosystems impacts the structure and functionality of the ecosystem (native animals are smaller and have padded feet). Interactions between climate and grazing are a fundamental driver of ecosystem function within drylands and hold many implications for their management (Maestre et al., 2016). With increasing grazing pressure, species richness of flora and fauna declines, and overall land degradation increases (Eldridge and Delgado-Baquerizo, 2017). Heavy grazing impacts biocrusts and reduces their diversity, alters soil surface stability, and carbon and nitrogen fixation (Asner et al., 2004; Eldridge and Delgado-Baquerizo, 2017; Williams et al., 2008). Our project examined the effects of managing grazing lands with stocking rates and fire to explore how management can best harness biocrust for soil fertility regeneration and maintenance.

1.3 Land degradation and soil fertility

Declining soil fertility of agricultural land is a global problem (Reed et al., 2019), and dryland ecosystems under extensive cattle grazing are no exception. Soil fertility describes the soil's ability to provide anchorage, water, nutrients and beneficial organisms to vegetation, and is enabled by the soil's physical, chemical and biological integrity (Young et al., 2022). Poor land management,

together with climate extremes, can lead to a decline in soil fertility and erosion, and resulting land degradation means lower quantity and quality of pasture plants (Bastin et al., 2024). Degradation of grazing lands is a serious problem in Australia and globally with drylands degrading due to inappropriate human activities (land clearing, overgrazing, weed invasion, fire) as well as weather extremes (droughts, floods, wildfires, Asner et al., 2004; Hartley et al., 2007; Maestre et al., 2016).

Here we focus on grazing and fire in the context of soil fertility loss that stems from the decline in soil N stocks when nutrient is exported via cattle sales, exceeds the rate of N replenishment (Duniway et al., 2018). Nitrogen is our focus because it accounts for over 60% of the soil-derived nutrients that plants require for growth and as such, has a strong impact on the productivity and quality of pasture (Shah et al., 2020). Like carbon, N is replenished in ecosystems by biological processes, so-called 'fixation' that convert atmospheric dinitrogen into bioavailable N. To ensure long-term productivity, grazed landscapes must be sustainably managed, which demands nitrogen replenishment via biological N fixation.

1.4 The importance of biocrusts

Described as a "living skin" by Bowker et al., (2018), biocrusts are now broadly accepted as multifunctional and globally relevant communities (Bowker et al., 2014; Maestre et al., 2016). Biocrusts grow in the upper centimetres of soil between and under vegetation in drylands and savannahs and are regularly defined by their microscopic communities that include cyanobacteria, bacteria, algae, micro-fungi, lichens, liverworts, and mosses (Weber et al., 2022). They vary in composition and functionality. Among the numerous functions of biocrust in ecosystems, an essential one is fixation of atmospheric N, which enables biocrusts to colonise soils with inherently low N levels, including early succession substrates and degraded, N depleted soils. In addition to N input into soils, biocrusts provide physical protection to the soil surface (Belnap, 2003; Delgado-Baquerizo et al., 2013), have a strong influence on the hydrological cycle (Eldridge et al., 2010) and fix carbon to generate organic matter (Delgado-Baquerizo et al., 2013; Thomas and Dougill, 2007). The internal and external structure of biocrusts enable the community of organisms to play a major role in ecosystem function and soil stability, conferring increased resilience to global changes in temperature and rainfall (Delgado-Baquerizo et al., 2013; Eldridge and Greene, 1994). In the Australian rangelands biocrusts often cover all the soil surfaces between grass plants, thereby forming an integral part of the ecosystem.

Research gaps exist regarding the N fixation capabilities of bacterial species and their host organisms (e.g., lichen) within biocrusts despite the recognised importance of biocrusts for N input into ecosystems (Belnap, 2003; Román et al., 2021; Torres-Cruz et al., 2018). Specifically, the amount of N input and N mineralisation within biocrusts is lacking on a global scale (Barger et al., 2016). For this reason, it is essential to understand the fundamental processes involved in biocrusts and the interactions between biocrusts, soil, plants and managing the land for grazing.

1.5 How soil renews and delivers nitrogen

Nitrogen accounts for over half of the essential nutrients that plants acquire from soil. It is also a major ingredient of soil organic matter, a key component of fertile soil. Maintaining the soil's nitrogen capital is essential for sustainable production, and here we discuss the role of biocrusts in Australia's rangelands for replenishing nitrogen and protecting soil from degradation.

1.5.1 Nitrogen is a renewable nutrient

Both nitrogen and carbon are renewable nutrients that are not part of the initial makeup of the rocks that form soil. Rather, both are biologically generated. This is termed 'biological carbon and nitrogen fixation' which converts atmospheric gases into bioavailable forms of carbon and nitrogen. Nitrogen is fixed by specialist bacteria (including cyanobacteria), carbon by plants and the microscopic and microbial organisms that form biocrust.

Nitrogen-fixing bacteria grow on the soil surface in the biocrust, deeper in the soil as part of the soil's biological community, or in symbiosis with plants such as legumes. Once nitrogen has entered soil, it cycles through soil-plant-animal systems in many chemical forms, which include organic (e.g. protein) and inorganic (e.g. nitrate) nitrogen (organic in the chemical sense means that carbon is a part of a molecular structure).

Organic carbon and nitrogen are the main constituents of the soil's organic matter. Soil organic matter keeps soil physically, chemically, and biologically healthy, and enables sustainable agricultural production.

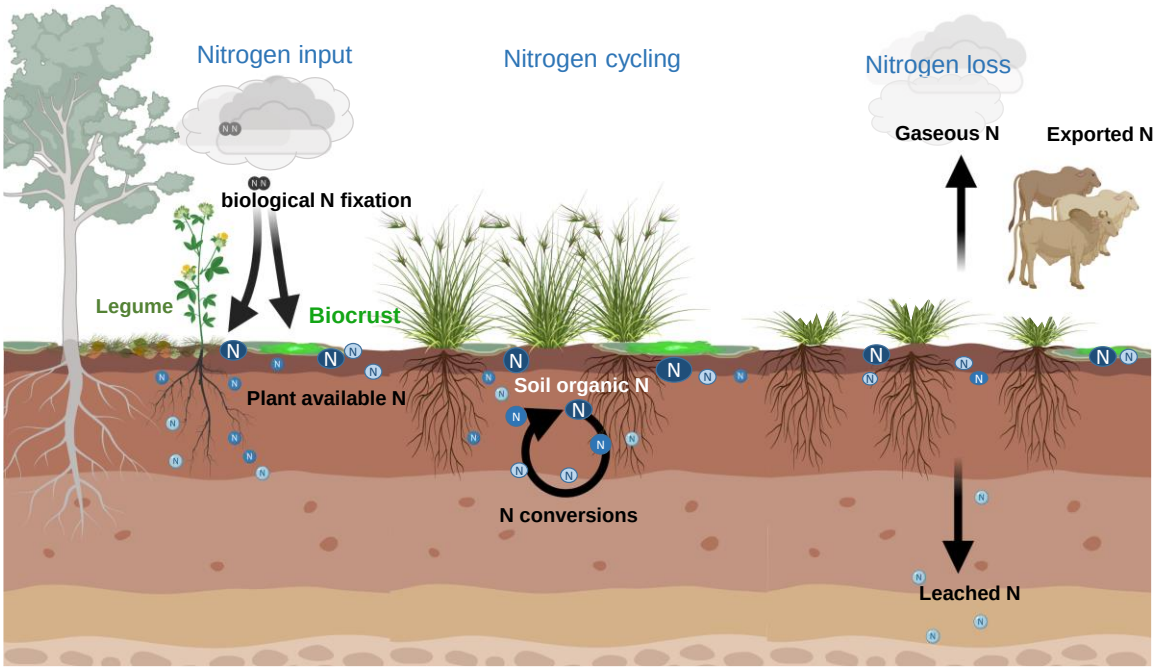
Soil organic nitrogen (SON) is the main nitrogen reservoir that supplies nitrogen to plants. Soil organisms feed on organic matter directly or consume small soil organisms such as bacteria or fungi. These processes release organic nitrogen (e.g. protein, amino acids) and inorganic nitrogen

(ammonium, nitrate). Bioavailable nitrogen is loosely bound to soil particles from where root-excreted chemicals can remove it or is dissolved in soil water. Dissolved nitrogen can also be washed into the deeper soil by rain and thereby lost from the site.

When more nitrogen and soil organic matter is lost than replenished, soil degradation occurs. The diagram below shows the three nitrogen paths: input from biological N fixation, cycling through biological matter and soils, and nitrogen losses as leaching, gas or exported stock (Fig. 1.5.1).

The various soil nitrogen pools are outlined in the table below. The most common form of soil analysis estimates the 'plant available nitrogen', the so-called exchangeable pool, using a strong salt solution that removes ammonium and nitrate from soil exchange sites which are quantified. This analysis is an estimate and generally does not include organic nitrogen, although amino acids and protein fragments can account for a large portion of the exchangeable soil nitrogen pool and can be bioavailable directly, or after conversion, by soil microbes.

Figure 1.5.1 Illustration of the nitrogen cycle demonstrating inputs and losses.



The relevance of nitrogen and its forms in soil are multi-fold:

1.5.2 **Nitrogen availability** is a major factor driving vegetation growth and productivity. It is an inherent trait of production systems that is linked to biophysical conditions (water

availability, temperature, soil type, availability of other nutrients). Nitrogen accounts for ≈70% of soil-derived nutrients for plants (legumes are an exception, receiving air derived nitrogen from symbiotic bacteria) and is therefore the most important nutrient for plants. With insufficient nitrogen, plants cannot photosynthesise to realise their growth and are stunted. Nitrogen cycling is part of nitrogen availability because nitrogen enters soil including via nitrogen fixation of bacterial, dead organic materials and manure. Nitrogen is metabolised by soil organisms and absorbed by plants and recycled to soil from vegetation and animals.

Nitrogen availability and nitrogen forms are connected because nitrogen supply and transformations result in various outcomes. For example, excess available nitrogen can be generated in situations when soil organisms are active (e.g., start of wet season rain) and vegetation is lacking (following dry season, drought, fire or overgrazed). When too much bioavailable nitrogen accumulates in soil, it can be lost. Most loss prone is nitrate as an end product of nitrogen conversions which readily leaches from soil (more information below). In contrast, in vegetated biodiverse landscapes, nitrogen is efficiently absorbed by vegetation and losses are low as roots of herbaceous and woody vegetation mop up nitrogen from different soil depths.

In degraded landscapes, negative feedback means that little carbon and nitrogen are recycled into the soil, soil organisms starve and decline, and nutrient availability declines. Biocrusts are the first to colonise degraded land so that eroded landscapes start rebuilding organic matter by adding nitrogen so that vegetation reestablishes.

1.5.3 **Nitrogen input** occurs via biology nitrogen fixation of specialist bacteria that convert inert atmospheric nitrogen gas to bioavailable nitrogen. All ecosystems contain nitrogen fixing bacteria but how much nitrogen they fix will depend on the biophysical conditions. High input occurs when legumes (e.g., Acacia wattle, clover, bean etc) have a strong presence in a system. In rangelands, biocrusts are a second source of nitrogen input with often a high potential for nitrogen fixation through photosynthesising bacteria. These bacteria are green (cyanobacteria) gain energy from light just like plants, to fuel nitrogen fixation. Cyanobacteria are visible as green, red, or dark film on soil surfaces, and are prominent in Australian biocrusts.

1.5.4 **Nitrogen loss** from soil via erosion, leaching or gaseous losses (including through fire) and by removing livestock, diminishes productivity and degrades landscapes when net loss exceeds nitrogen input. When examining losses from soil, the form of nitrogen determines how vulnerable to loss it is as some are held tightly while others are leached or volatilised. The broad forms of nitrogen are organic and inorganic (mineral) outlined below.

1.5.5 **Nitrogen forms** (chemical structure that result from biological conversions) which are relevant for how nitrogen is retained by soil and how available it is to vegetation:

Organic nitrogen - Complex organic nitrogen (e.g., fresh and decaying organic matter, soil organic matter) is that largest soil nitrogen pool and the most stable. It is not immediately plant accessible but can become available when mobilised by soil organisms. This pool is part of the soil organic matter pool and has on average a 10:1 carbon:nitrogen ratio (i.e., 10 units of carbon per 1 unit of nitrogen). Thus, nitrogen stabilises soil organic matter, ensure soil fertility and carbon sequestration. Organic nitrogen is a soil store with low risk of leaching but can be lost via erosion and fire. It is activated through enzymatic digestion (bacteria, fungi, plants), consumption and excretion (e.g. worms, insects). For example, certain fungi grow on/ in plant roots (mycorrhiza) digest organic matter and feed nitrogen to their plant host. From this organic nitrogen pool, smaller organic nitrogen molecules (e.g., amino acids the building blocks of protein) are generated by soil microbes which plants can easily absorb. Small organic nitrogen is a transient pool that feeds both plants and soil organisms.

Inorganic (mineral) nitrogen - Small organic nitrogen is further processed (mineralised) by soil organism to generate inorganic nitrogen, i.e., ammonium and nitrate. This mineral pool can be small in systems with well-matched nitrogen delivery and absorption rates by plants. It is large in degraded systems where nitrogen supply outstrips demand by vegetation. A key difference between ammonium and nitrate is that ammonium has a positive charge that binds it to the soil, while nitrate's negative charge makes it highly mobile in soil. Nitrate accumulates in soil when high availability of organic nitrogen and ammonium fuels the activity of specialist nitrifying bacteria. This situation occurs when vegetation is lacking or inactive and does not absorb organic and ammonium nitrogen, which risks nitrate leaching from soil or conversion to nitrogen gases by soil bacteria.

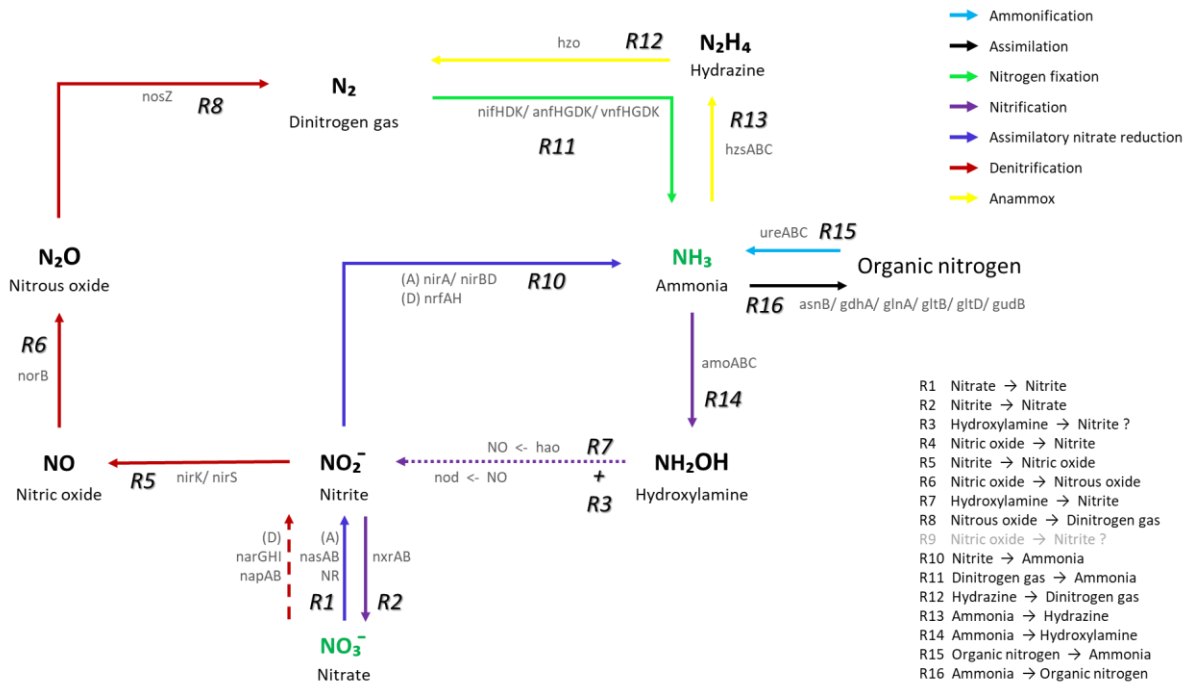
Plants readily absorb ammonium and nitrate and often grow best with both forms present. Some plants are better able to use nitrate (e.g., certain herbs store nitrate for later use) while others (including certain grasses) prefer amino acids and ammonium over nitrate. Plants can influence the nitrogen forms around their root: e.g. by inhibiting nitrifying bacteria, plants reduce nitrogen losses, an example is African gamba grass in northern Australian savanna (Rossiter-Rachor et al. 2009).

In summary, for this project, input, cycling and the losses of nitrogen are a central consideration as various scenarios can occur. Low input and low losses of nitrogen may have similar net outcome for the nitrogen budget as high input and high losses. The relative input of nitrogen into a landscape is directly related how nitrogen is retained and recycled. In line with other management decisions promoting productive landscapes, retaining sufficient vegetation is central to maximise nitrogen input by biocrusts, and nitrogen recycling and retention.

1.6 Nitrogen processes within biocrusts

Understanding the function of nutrient cycles in relation to the uptake of biologically available nitrogen and other nutrients by plants is paramount to several of our key research questions. Here, we enumerated the relative frequencies of 57 microbial genes involved in nitrogen cycling (Fig. 1.6.1) including those associated with inputs (nitrogen fixation) and losses (denitrification - nitrous oxide emission (N_2O)) to the atmosphere, as well as conversion into nitrate via nitrification (NO_3^-) which is a highly mobile form of N that is easily washed from soil. The presence of these genes in our samples is indicative of their capacity to perform these functions. See illustration below (Fig. 1.6.1).

Figure 1.6.1 Processes involved in the nitrogen cycle and bacteria participation; bacterial genes involved in these processes.



1.6.1 The soil nitrogen bank

If soil was the Australian Reserve Bank, the nitrogen capital would increase with nitrogen fixation (in some regions also input of ammonia from air pollution or lightning). Nitrogen withdrawal occurs with biomass removal such as pasture grazing, nitrogen leaching, topsoil erosion, gaseous emissions as part of the nitrogen cycle and fire, and of course with removal of cattle and other stock. The SON pool are the bank's shares and gold. Both would not be used for daily needs, while the cash held at the counter or piggy bank is available to plants (exchangeable and dissolved pools).

Biocrust can contribute to both, the Reserve Bank and the piggy bank at different times. In the wet season, a significant amount of nitrogen is fixed by cyanobacteria, bolstering shares and cash. In the dry season, the biocrust dries and partially disintegrates. At all times, soil organisms feed on biocrust and excrete plant-available nitrogen. How much nitrogen is being fixed by different types of biocrust across a year's cycle, and its fate in the nitrogen cycle, is investigated in this project.

Nitrogen is a deal breaker for agricultural production, which is why most cropping and intensive livestock systems use nitrogen fertiliser to boost soil nitrogen stocks. This is not possible in extensive grazing lands, which have to rely on biological nitrogen fixation for input. Better understanding of

nitrogen inputs and transformations in soil will inform grazing management to maximise the benefits from ecological processes.

Experimental data shows that the estimated annual N input from biocrusts of 5 kg per hectare represents a substantial input that accounts for approximate one third to half of the annual pasture N demand in extensive northern Australian ecosystems (see below). Note, N fixation also occurs through legumes in native pastures and from lightning strike.

1.6.2 Five kg nitrogen input per hectare (0.5 t per square km) is a lot in northern extensive grazing systems

Global estimates of N inputs from biocrusts vary from annually 5 kg N per hectare in north Queensland tropical savanna (Williams et al., 2018) to 41 kg N/ha in the Negev desert in Israel (Russow et al., 2005). N-fixation rates are heavily influenced by the biocrust community structure, number of active days (when soil surface is wet) and disturbance (grazing, fire, drought) (Williams et al., 2018). The N contribution by cyanobacteria and biocrust community matches legume pastures in central Queensland that, over a 4-year period, fixed on average 5 to 40 N kg N/ha/year, with up to 68 kg N in some years (Armstrong et al., 1999) .

1.6.3 How much nitrogen is typically available to northern native pasture systems?

The annual uptake of N by native pastures is typically 5 to 30 kg N/ha/year. This compares with average N uptake rates of 20 to 95 kg/ha/year in improved legume pastures in central Queensland (Armstrong et al. 1999). Nitrogen uptake in native pastures has been extensively quantified across northern Australia in pasture growth sites, known as SWIFTSYND sites(O'Reagain and Scanlan, 2013) (Day and Philp 1997). In the Victoria River District, annual N uptake rates by pastures averaged between 15 and 30 kg N (M. Cobiac, Technical Bulletin 324, NT, 2006).

Annual N uptake can be much lower on less fertile land types. In the Barkly region, Cowley et al. (unpublished) found on a spinifex/perennial grassland type that the maximum N uptake was only 8 kg N/ha/year. Similarly, the maximum N uptake in woodlands at Kidman Springs and Katherine was 5.5 to 18 kg/ha/year (Dyer et al. 2004 unpublished report).

Nitrogen uptake measured in the Northern Territory is consistent with levels at the Wambiana grazing trial (5-21 kg N/ha/year, Table 1.6.1 from O' Reagain et al. 2008 unpublished report;

O'Reagain and Scanlan, 2013) and across 46 sites in Queensland (Day et al. 1997) where N uptake was 13 to 24 kg N/ha/year (excluding brigalow and exotic pasture sites).

Table 1.6.1 Mean, maximum and minimum N uptake between 1999 and 2005 for SWIFTSYND exclosure sites on five land types at the Wambiana grazing trial. NB: Values for the Box and Ironbark are the means of two individual sites.

N Uptake	**kg/ha/yr**		
Land type	Mean	Minimum	Maximum
Box	12	6	21
Ironbark	8	5	11
Blackbutt	12	5	17
Brigalow	15	5	21
Coolabah	12	6	17

1.6.4 Nitrogen losses and inputs in grazed native pasture systems

Nitrogen is lost through several process in grazed systems including runoff, erosion, leaching, and volatilisation from dung and urine and through gaseous emissions from ingested pasture (mostly belching) and through removal of livestock and fire (Piñeiro et al., 2010).

Table 1.6.2 Nitrogen budget for northern NT grazed pasture systems with average stocking rates 11 AE/km^2 where AE = Adult Equivalents (compiled by R. Cowley, unpublished).

Nitrogen loss/input	Source of loss or input	Annual N inputs & losses (kg/ha)	Reference	Notes
Input	fixation by legumes	2	Norman & Wetselaar (1960); Langkaamp *et al.* (1979)	Varied between papers from 2- 12 kg/ha, so took lower point. 12 kg/ha is for acacia densities double total stem density at Kidman Springs, where mostly not acacias, although *Lysiphyllum* may fix N?
Input	fixation by biocrusts	5	Williams *et al.* (2018)	From Nth Qld, may vary with rainfall, soil type, disturbance
Input	rainfall	1.5	Cook (1994) fate of Nutrient during fires in tropical savanna Aust Journal Ecol; Wetselaar & Hutton (1963)	Cook assumes 15% of N lost to fire - 0.56 kg/ha/yr; 1.5kg/ha/yr Wetselaar & Hutton (1963)
Input	cattle supplements	0.45	Varies with SR and region	Urea @ 30g/day / hd 200 days per year @ 46% N for VRD. Less for Barkly
Loss	fire 1/4 years	-1.25	Cook (1994) fate of Nutrient during fires in tropical savanna	Relationship between fuel load and losses N transfer = -0.15+0.0046*fuel load (same units), assume fire 1/ 4 years; but Norman and Wetselaar (1960) - 4.5kg/ha/fire = -1.25kg/ha/yr if burnt every 4 yrs
Loss	leaching	-0.485	Based on Pineiro *et al.* (2010) leaching + volatisation - other volatisation estimates	
Loss	erosion and runoff	-0.85	Runoff N from Cowie (1993) in Radford *et al.* 2007 is 3.4kg/ha/year on grazed brigalow, where SR =3-5 x NT rates	3.4kg/ha/year in pasture catchment
Loss	cattle removal	-0.34	Assuming 3AE/kmsq removed at 2.6%N, but varies with SR, which varies with land type & region	Depends on stocking rate and turnoff. 1.6 kg N/ha/yr in Radford *et al.* (2007) at much higher SR = .35 kg/ha at 15AE/kmsq
Loss	cattle emissions N_2 and NH_3	-0.43	12% of N consumed Dean *et al.* (1975) Nutrient removal by cattle from a shortgrass prairie	
Loss	dung and urine volatilisation	-0.13	Dean et al. (1975) 25% of N consumed to urine and 45% to faeces and the soil. 14% of N in urine patches is volatised.	Compares well with Augustine *et al.* (2013) though Augustine *et al.* at lower SR
Loss	redistribution of N to near waters and camps	-0.73	Augustine *et al.* (2013), at lower SR than here. Need to rework figures for Kidman / NT	This could be substantial following Augustine *et al.* (2013). still in the paddock, but redistributed to where cattle spend time near waters and camps
Net N		**4.7**		

Cowie (1993) showed N losses due to runoff in a grazed pasture catchment in central Queensland were 3.4 kg/ha/year (reported in Radford et al., 2007). Losses due to livestock removal vary with

stocking rates and turnoff but were estimated to be 1.6 kg/ha/y for a Brigalow pasture (Radford et al. 2007). At the Toorak grazing trial on Mitchell grass in western Queensland, the highest stocking rate lost on average 3.2 kg/ha/y of nitrogen from the top 10 cm of soil (Pringle et al., 2014).

Nitrogen inputs to extensive grazing systems occur predominantly through rainfall and lightning, feed supplements, and fixation by legumes and biocrusts. An N budget based on N losses and inputs from the literature and northern NT levels of pasture growth and stocking rates is shown in Table 1.6.2.

Inputs from biocrusts at 5kg/ha would hence entirely offset N losses due to runoff and product removal at these rates.

It shows that the N balance is roughly equal to the inputs from biofilms at around 5kg/ha/yr net input of nitrogen. This suggests biocrusts have a significant impact on maintaining nitrogen in these systems.

1.7 Biocrust and fire effects on seeds germination

Plants of the northern Australian savanna have adapted to the harsh conditions, with wet summer rains that flood the land and seasonal winter droughts that deprive plants of water. To ensure that they germinate in the right conditions native plants often have seed dormancy which prevents germination until certain conditions are reached (Ralph, 2003). Some of the important seeds for plants occurring in the study regions and their known dormancy and germination characteristics are detailed in Section 9.1.1. The seed coat can cause dormancy in plants by preventing water or oxygen exchange with the embryo or simply preventing the seed embryo from growing larger. The coats would get broken down by fire, microorganisms, weathering, or passing through an animal over time or can be scarified as a treatment to overcome it. After ripening is required for some plants, especially native grasses, where the embryo is not fully formed as soon as seeds are dispersed but requires some time to become mature. Various chemical inhibitors can also be present and will need to be overcome before the seed can germinate. Multiple dormancies can be present in seed and will all need to be overcome for successful germination (Ralph, 2003; Adkins et al., 2002).

2. Objectives

The purpose of the project has been to advance knowledge that can be used to increase the nitrogen regeneration of northern grazing lands. The scope of the project explored the impacts of fire and grazing on biocrusts at two locations and across four soil types: (1) Victoria River District (Victoria River Research Station, Kidman Springs, NT) and, (2) Wambiana grazing trial (near Charters Towers, QLD). The five key objectives are summarised in Fig. 2.1. The objectives are driven by the knowledge gaps identified in understanding the contribution of biocrusts as:

- The natural capital of the rangelands
- Indicators of soil health and productivity
- The first responders after fire, drought, and floods
- A resilient microbiome that protects and enriches the soil

As the depth of our understanding of the biocrust microbiome increases, imparting this knowledge to land managers is central to our purpose. This can lead to informed management decisions that provide positive outcomes and maintains a stable and productive landscape.

2.1 Quantifying the nitrogen-generating capacity of biocrusts.

PARTIALY ACHIEVED

Two approaches were used; i) biocrust sampling and laboratory tests to quantify N production and direct measures in situ from soil samples including bioavailability of N forms; ii) DNA analyses of microbial community structure and diversity and gene-centric approach to confirm the presence of N fixing genes. Our conservative estimate is that well-developed biocrusts in healthy landscapes generate annually 5 kg nitrogen per hectare and potentially up to 20 kg. This conservative estimate means that per square km, biocrusts provide 25+ tonnes nitrogen for pasture with 1-2% dry matter nitrogen.

Figure 2.1 Summary of key objectives

2.2 Identifying the environmental and management factors that drive the presence and activity of biocrusts in different land types.

ACHIEVED

Key drivers of biocrust activity include a combination of landscape function principles based on nutrient cycling, soil stabilisation, and infiltration. There were differences in biocrust community

structures between grazing and fire treatments at a microsite level and functional genes involved in photosynthesis, C and N fixation.

2.3 Developing and modelling different scenarios for N-smart grazing management practices.

PARTIALLY ACHIEVED

Spatial and temporal scenarios based on a commercial scale fire trial at Victoria River Research Station using Planet Scope imagery defined the effects of fire and grazing on ground cover, carbon and nitrogen stocks generates the methodology for farm-based N-smart practices. Preliminary results are presented in Section 13.

2.4 Generated knowledge prospective to improve nitrogen Best Management Practices.

ACHIEVED

The impacts of grazing/land management practices on biocrust function:

(a) biocrust health using proximal and satellite imagery.

(b) Commenced and continuing use of a range of metrics including digital phone images, machine learning and satellite imagery.

(c) Continuing PhD research by Than Myint Swe due for completion in 2026.

2.5 Developed high quality information products for end users that have made 150 pastoralists aware of the opportunity that biocrusts present for N-smart BMP.

ACHIEVED

Communication with industry has been a focus of our project attending relevant conferences, workshops and participating in local farm-based information days. These were held in conjunction with regional organisers such as Departments of Agriculture and Southern Queensland Landscapes (QLD), Livestock Industries (NT) and MLA (Beefup, NT).

3. Methodology

3.1 Key research questions

1. HOW MUCH NITROGEN IS GENERATED BY BIOCRUSTS IN DIFFERENTLY MANAGED GRAZING LANDS?
2. ARE THERE HOTSPOTS OF NITROGEN PRODUCTION, AND WHAT CHARACTERISES THOSE HOTSPOTS?
3. HOW DO THESE CHARACTERISTICS VARY IN THE TWO REGIONS AND ASSOCIATED LAND TYPES THAT ARE THE PROJECT FOCUS?
4. CAN WE IDENTIFY THE 'GOLDILOCKS PRINCIPLE': THE RIGHT AMOUNT AND TIMING OF DISTURBANCE (GRAZING, WET SEASON SPELLING, FIRE) FOR BIOCRUSTS TO THRIVE AND MAXIMISE NITROGEN REGENERATION?
5. HOW CAN WE INTEGRATE THE KNOWLEDGE OBTAINED INTO EXISTING BEST PRACTICE PASTURE MANAGEMENT?

3.2 Approach

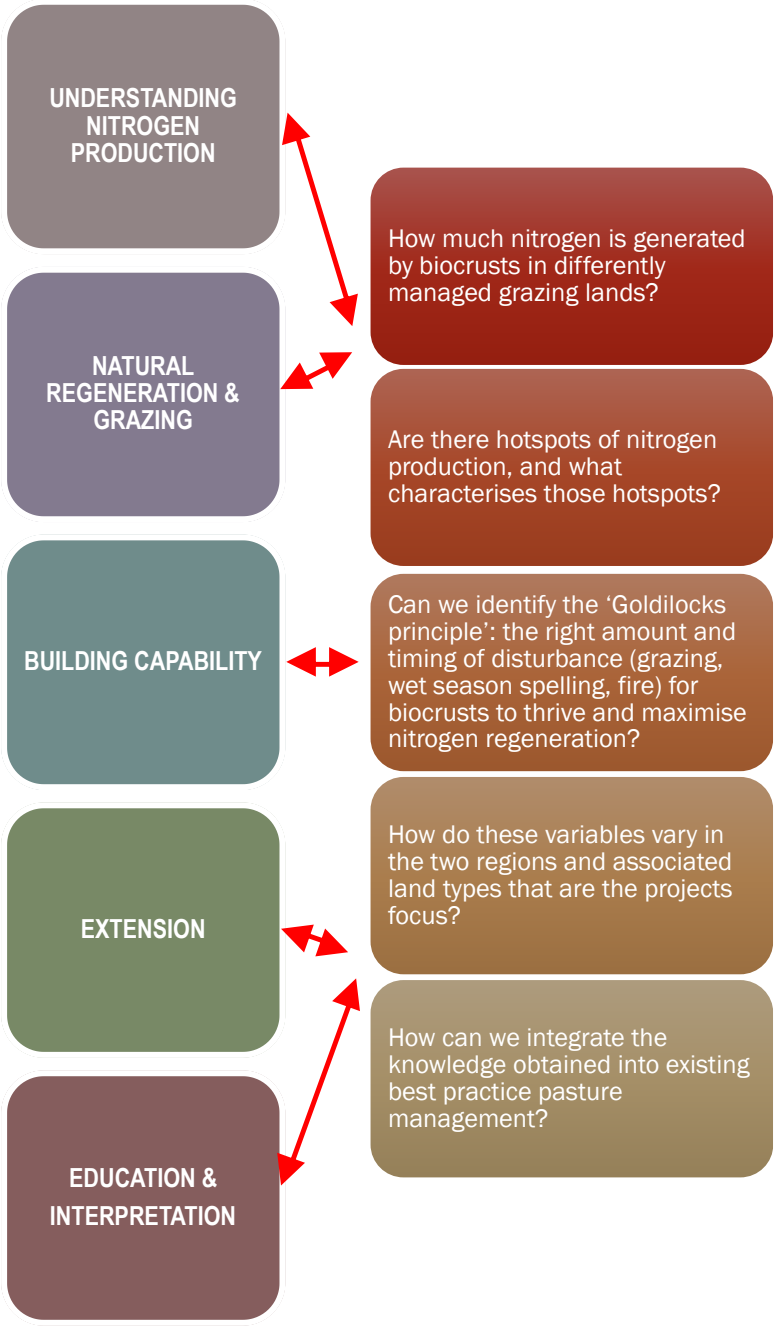
To define the research questions in terms of desired outcomes we focused on where they fit into the overall aims described in the five key questions (Fig. 3.2.1).

Landscape Function Analysis (LFA) has been developed to establish soil surface indicators for measuring and analysing the nature and severity of problems in a dysfunctional or degraded ecosystem (see Section 3.6, this report). The conceptual framework is based on the spatial organisation of the clumps of grasses and shrubs that capture, accumulate, and retain resources (called patches). The interspaces (or inter-patches) are the open areas between the grass patches and can be natural 'hotspots' for biocrusts, due to less competition for light, moisture, and litter.

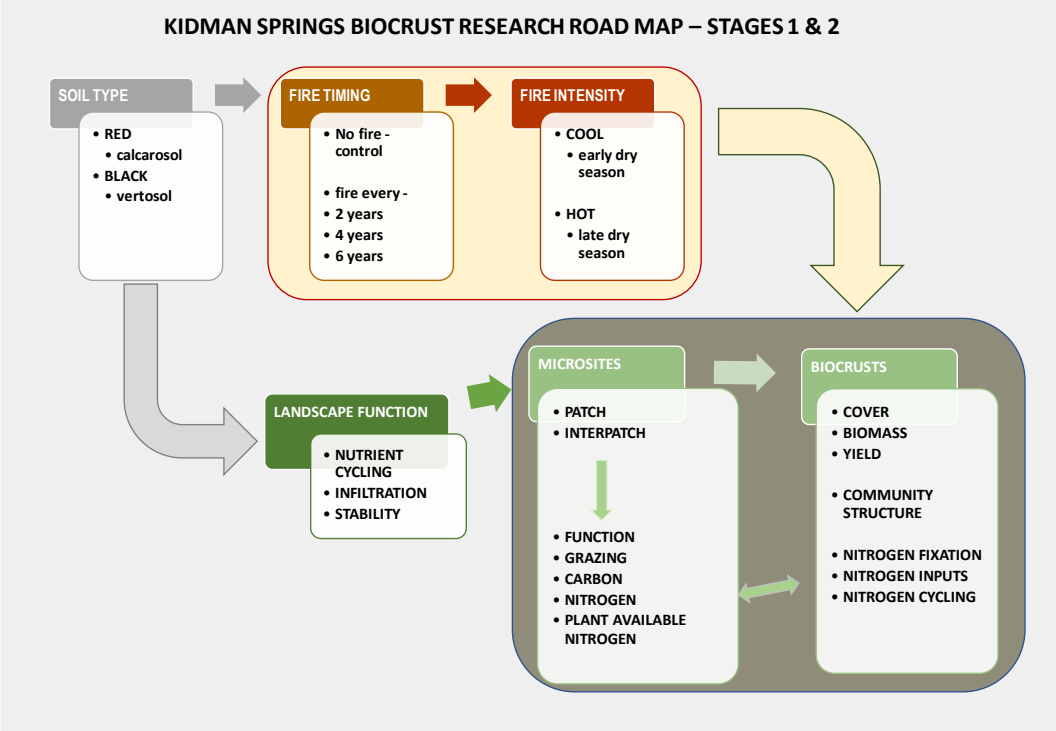
In these studies, we focused on the role of these biocrust hotspots in determining the three LFA indices: stability, infiltration, and nutrient cycling. These three indices are assessed by 11 soil surface indicators that are individually scored and provides the percentage level of each index. The indices are a relative measure and are independent of each other.

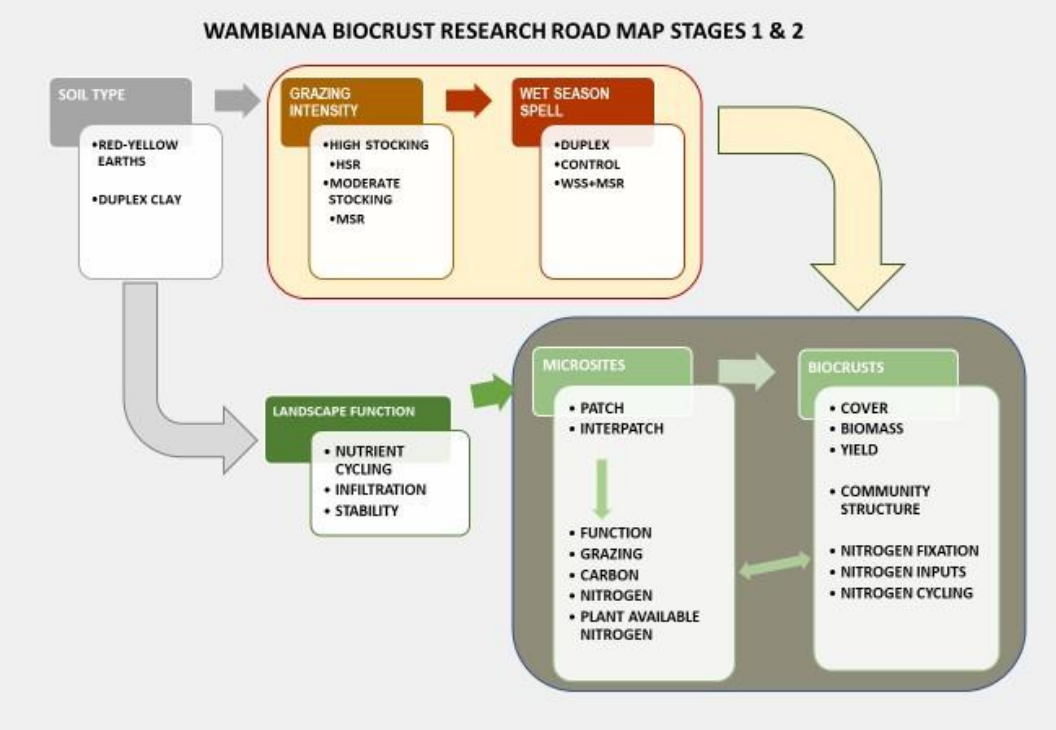
To achieve the answers to these key questions we established a research roadmap with a framework based around the principles of landscape function (described at the start of this section) and used this to develop our approach at both research sites (Figs. 3.2.2).

Figure 3.2.1 The overarching aims were to understand how nitrogen production from biocrusts could boost productivity. Importantly, this research must be communicated on several levels including across the landscape and to industry.



Figures 3.2.2 (a) Victoria River Research Station, Kidman Springs (NT) and **(b)** Wambiana Grazing Trial (QLD) roadmap to understanding landscape function.





3.3 Research sites

3.3.1 Victoria River Research Station (VRRS) Kidman Springs, NT

Known as Kidman Springs covers 31,400 ha in the Victoria River District south-west of Katherine (NT). Established in 1966 the research station run by the NT Department of Industry, Tourism and Trade is the NT's principle pastoral research station and is managed as a breeding operation. The Victoria River District contains three main soil types including cracking clays, calcareous red earths, and sandy red earths. As a semi-arid tropical rangeland, the district has a distinct monsoonal wet season, followed by a dry season.

The research was conducted at the Victoria River Research Station (VRRS), situated within the Kanrangpuru Country in the Victoria River district of northern Australia. The prevailing climate is characterized by a summer wet season spanning from November to April, followed by a drier season with minimal to no rainfall from May to October. The annual average temperature ranges from 20.1 to 34.9°C, with an average annual rainfall of 753.9 mm.

In 1993, two experimental sites were established, situated 4 km apart within separately grazed paddocks of native pastures within a Eucalyptus woodland ecosystem (Cowley et al., 2014). The long-term fire research project was established to investigate the impacts of various fire treatments. These treatments encompassed (1) different fire intervals at 2, 4, and 6-year cycles and (2) varying fire intensities, categorized as early dry season (cooler fires) or late dry season (hotter fires), in addition to unburnt control plots (Cowley et al., 2014). In total, there were sixteen experimental 4 x 4 grid plots established for each soil type, with each plot spanning 160 m x 160 m and separated by firebreaks (Figs. 3.3.1, 3.3.2).

The fire treatments included early (E) and late (L) dry season burns, with intervals between fires set at 2, 4, and 6 years (E2, E4, E6, L2, L4, and L6), as well as unburnt control plots. Early dry season (cool) fires were executed in the experimental plots during June, whereas late dry season (hot) fires were implemented in October. Furthermore, it is noteworthy that the Fire Graze study area, spanning an area of five square kilometres, represents the largest research zone for controlled burning within the VRRS, with the first burning event taking place in October 2022.

Figure 3.3.1 Conkerberry Paddock fire plots where each plot is burnt to the range of fire regimes detailed above (UAV imagery June 2023).

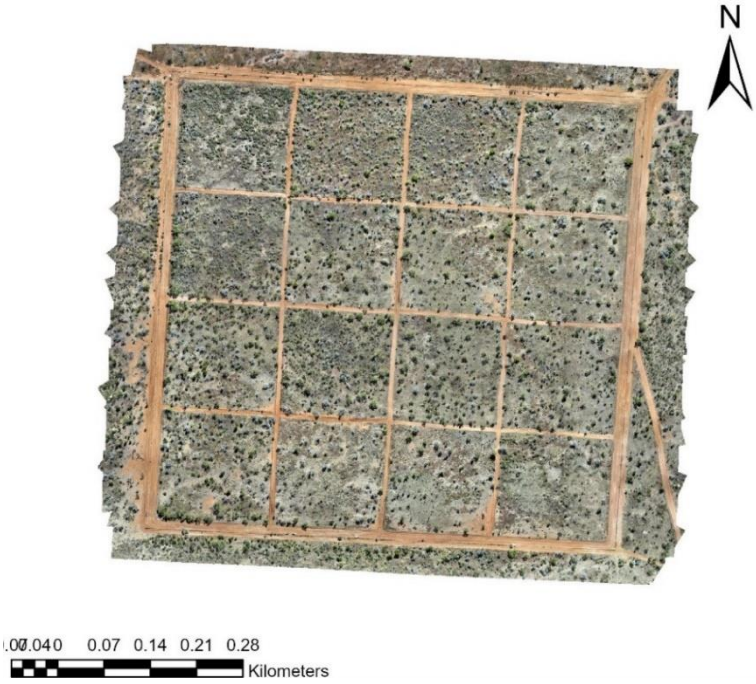
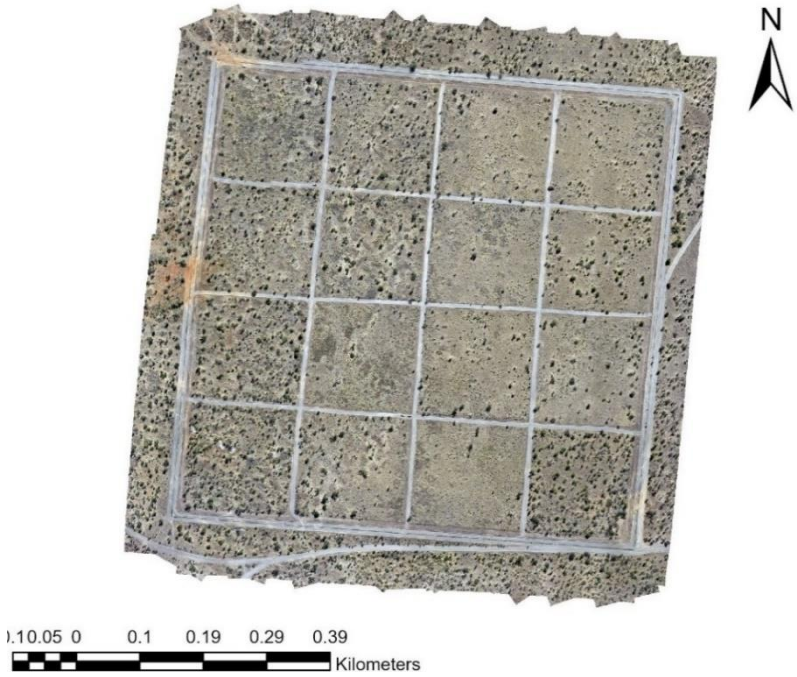


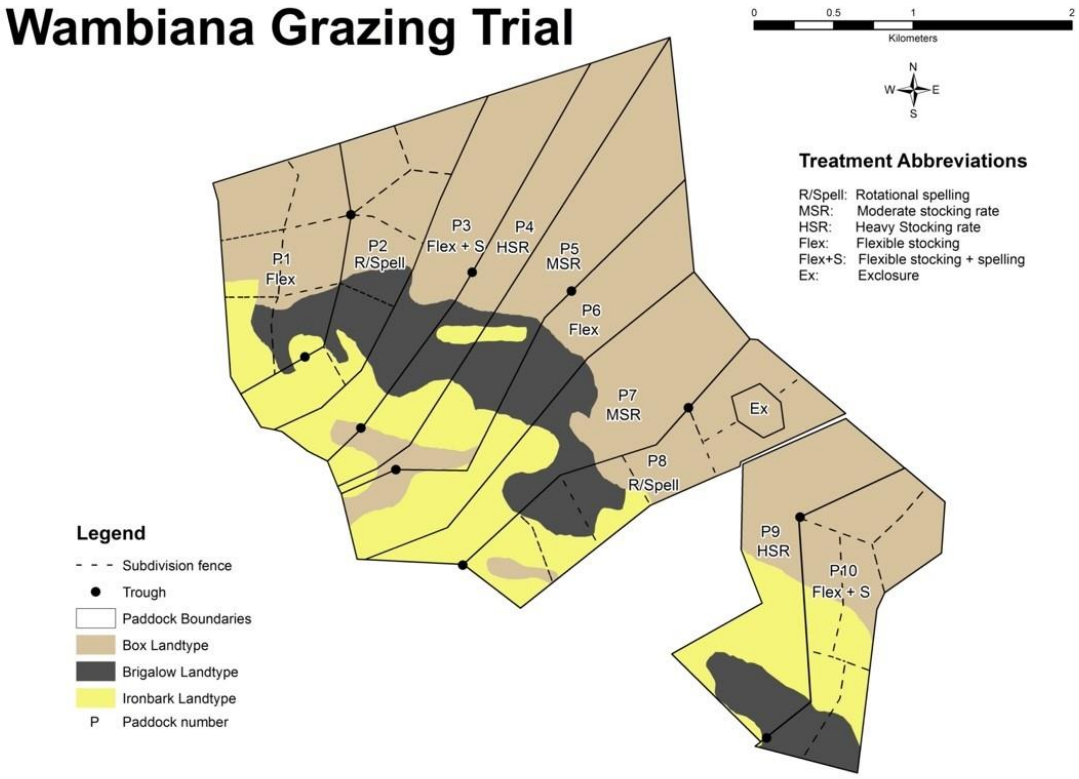
Figure 3.3.2 Rosewood Paddock fire plots where each plot is burnt to the range of fire regimes detailed above (UAV imagery June 2023).



3.3.2 Wambiana Grazing Trial, North QLD.

The Wambiana Grazing Trial was established on Wambiana Station near Charters Towers in 1997 to test and develop evidence-based management strategies to manage for rainfall variability. It provides a typical grazing landscape encompassing 1000 hectares of land within the Burdekin catchment. In collaboration with the Department of Agriculture and Fisheries, and Meat and Livestock Australia, researchers have developed experimental paddocks to provide examples of contrasting management strategies (Fig. 3.3.1). Key grazing strategies implemented at Wambiana grazing trial include industry recommended moderate set-stocking (MSR), heavy set-stocking (HSR), rotational wet-seasonal spelling (R/Spell) and two variable stocking strategies (Flex and Flex + S) on three main land types (Box, Brigalow and Ironbark) (O'Reagain and Scanlan, 2013). In this study we used MSR, HSR and R/Spell together with exclosures (no cattle) and selected the two main soil types in the region: red yellow earths and duplex clays.

Figure 3.3.3 Site layout of the Wambiana Grazing Trial, including 10x 100-hectare paddocks**.**



4. Biocrust structure and taxonomic composition

Biocrusts are defined by their physical structure, functional characteristics, habitat, and taxonomic composition. Biocrusts or biological soil crusts and biofilms result from an intimate association between soil particles and differing proportions of photoautotrophic (e.g. cyanobacteria, algae, lichens, bryophytes) and heterotrophic (e.g. bacteria, fungi, archaea) organisms, which live within, or immediately on top of, the uppermost millimetres of soil. Soil particles are aggregated through the presence and activity of these often extremotolerant biota that desiccate regularly, and the resultant living crust covers the surface of the ground as a coherent layer (Weber et al., 2022). Cyanobacteria are one of the most visible components of the biocrust community where they cover the surface with a polysaccharide rich slime that forms a skin over the soil and harbours the other smaller crust bacteria that are invisible to the eye.

4.0.1 Sample collection

4.0.2 VRRS Fire Trials 2019

In June and October 2019, burning of the shrub burn plots coincided (2, 4, 6 years, early and late dry seasons). At these times, we collected six replicate biocrust samples (1 cm depth) across both soil types (Vertosol and Calcarosol), both pre-fire and post-fire, from the patches (areas under grass plant canopies) and inter-patches (open areas between grass tussocks). A second set of samples was collected and stored at minus 20C for metagenomics studies of biocrust diversity and function. Thus, the post-fire samples for June were collected in October too but there had been no rain in between those dates. These samples were stored dry at The University of Queensland's laboratories and used for the initial biocrust composition tests (microscopic), the biocrust recovery trials, and a metagenomic analysis. In 2020 a second batch of samples were taken at the end of the wet season and used to compare inside an exclosure (burnt early every two years with no grazing) and, outside exclosure, burnt every two years with grazing.

4.0.3 Wambiana Grazing Trial 2020

Sampling for biocrust presence, diversity and composition was carried out across the grazing trial paddocks in mid-2020. We selected three main grazing treatments (Heavy, moderate and moderate

with wet season spelling) and controls (fenced, no stock, previously used as SwiftSynd sites). We sampled two different paddocks for each treatment. Two existing transects were used as a guide and sampling was carried out within 6 x 1 m quadrats along a 30 metre transect, three samples each from patches and inter-patches.

4.0.4 Biocrust microscopy

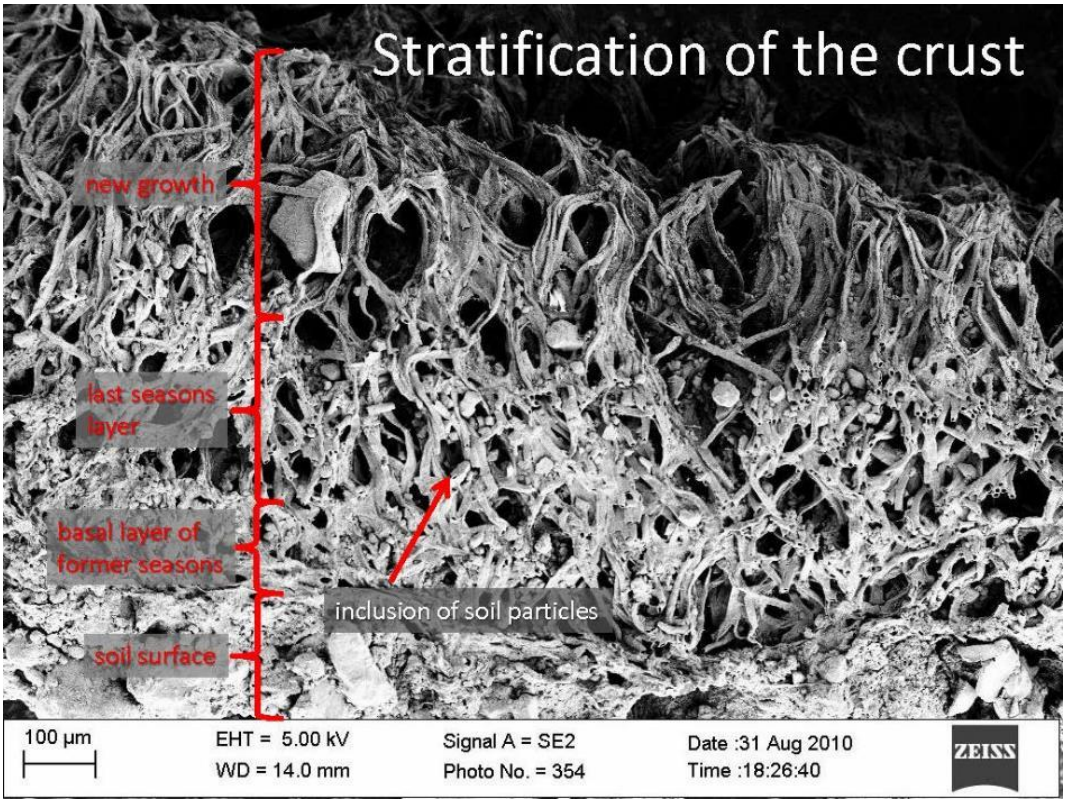
Cyanobacteria often grows as tufts on the soil surface with a basal layer of filaments anchored to the soil surface to provide stability. In this image the cyanobacteria have grown around the soil particles to create a strong network that will let water through but cover and protect the soil surface. In central and northern Australia where summer rain dominates at the beginning of each wet season the crust structure breaks down and fertilises the ground creating a new layer of compost that the new biocrust grows from over the next wet season. This is called stratification or layers of biocrust (Fig. 4.0.1).

The structure of the biocrust was examined using both high powered scanning electron microscopy and microscopic imagery of the key species of cyanobacteria that are visible at 100-400x magnification. Morphological qualities of cyanobacteria are used to confirm species identification. About one third of Australian cyanobacteria have not previously been identified and recorded in global taxonomic libraries so this is an important cross-check.

4.0.5 Scanning Electron Microscope (SEM) imaging

Representative biocrusts for imaging were selected from samples collected November 2020 one-year post fire for all burn treatments both before and after fire. The images were processed at the University of Queensland's Centre for Microscopy and Microanalysis. Double-sided carbon stickers were attached to round aluminium specimen stubs. Silver conducting paint was added to the stickers for enhanced stability of biocrust samples. Sections were made to appropriate sizes to fit on to stubs and placed using tweezers. After samples were prepared on stubs, they were coated with platinum, using the Safematic CCU-010 Compact Coating Unit. Ensuring the appropriate settings were in use, the chamber was pressurised before samples were coated for 10 seconds. Following platinum coating, samples were positioned on the viewing stage of the Hitachi TM4000Plus Tabletop Scanning Electron Microscope (SEM). The same process was carried out for both VRRS, Kidman Springs NT and the Wambiana Grazing Trial QLD. This was an observational study.

Figure 4.0.1 Illustration of new season cyanobacteria (20x magnification) and SEM image of hydrated cyanobacteria filaments (150x magnification, scale bar 100 µm), (Büdel et al., 2018).



4.0.6 Community composition profiled by DNA sequencing

Biocrusts from each of the four soil types across both sites were categorised into four microsites considered relevant to potential variations in the biocrust composition.

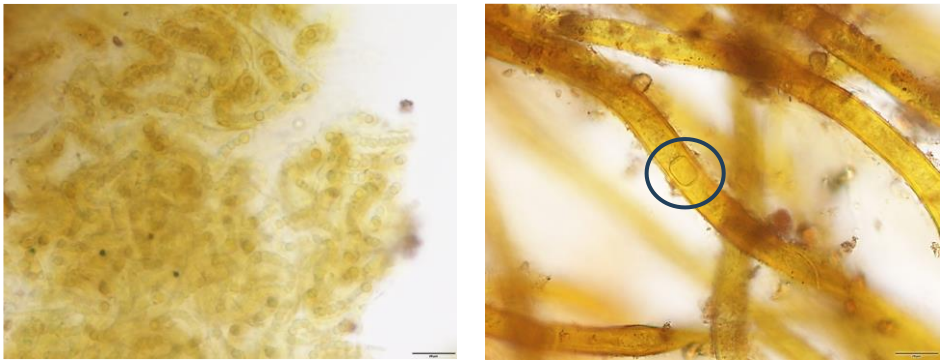
4.1 Influence of fire and grazing on biocrusts at VRRS

We returned to the site in wet season and collected more samples. Of these two samples were analysed shotgun (1 Gbps each) to work up methods. The adapter sequences were trimmed using Trimmomatic, assembled reads using metaSPADEs and then attempted to bin contigs into metagenome assembled genomes (MAGs) using metabat2. This approach failed to yield any MAGs due to the low quantity of data relative to the complexity of the samples. Hence, we explored what could be achieved using a gene centric approach. I used CCMetagen to generate a taxonomic profile of each sample. This indicates that there are more fungi, cyanobacteria and Rhizobia in the exclosure than in the grazed.

4.2 Results: Taxonomic composition of biocrusts at VRRS

In the initial microscopic inspection of the visible components of the biocrusts, cyanobacteria were prevalent (size and visibility) and were dominated by N-fixing species (Fig. 4.2.1). This appeared like a black skin (black from sunscreen pigmentation) covering the soil surfaces (Fig. 4.2.2).

Figure 4.2.1 Cyanobacteria recovery four years early burns, before fire (sample collection June 2019). (a) High level N-fixing capacity cyanobacteria Nostoc sp. (b) Stabilising N-fixer cyanobacteria Scytonema sp. with heterosysts (circled), the specialist N-fixing cells. Magnification 400x



(a) (b)

At Kidman Springs the biocrusts cover almost all the soil surfaces (Fig. 4.2.2) and are especially visible after fire and following the first rains. Although newly formed biocrusts are green in colour (due to chlorophyll), after about a day they will turn black or reddish in colour as they manufacture a pigment (scytonemin) to protect their photosynthetic apparatus from the harmful ultraviolet rays emanating from the sun. Once the plants grow and cover the ground, the biocrust cannot be seen unless exposed due to a natural bare patch (called interspaces or inter-patches) or damage from excessive trampling by cattle. In the paddock one can pull the grass aside and see the biocrust growing on the soil surfaces. It is brittle when it is dry and soft and flexible when it is wet. At this time the emerging grass plants can easily push through the biocrust to germinate (Fig. 4.2.2).

Figure 4.2.2 Biocrust regrowing (dark patches on soil) mid wet season after burning. New grasses recovering with access to plant available nutrients supplied from biocrusts.

4.3 Metagenomic insights of biocrust function

A focus throughout this research has been on a gene-centric approach to understanding the nature and function of the biocrust. We focused on the different soils, seasons and microsites. Biocrusts were shown to have the capacity to carry out the complete nitrogen cycle (Section 7.1) underpinning their central role in nutrient cycling.

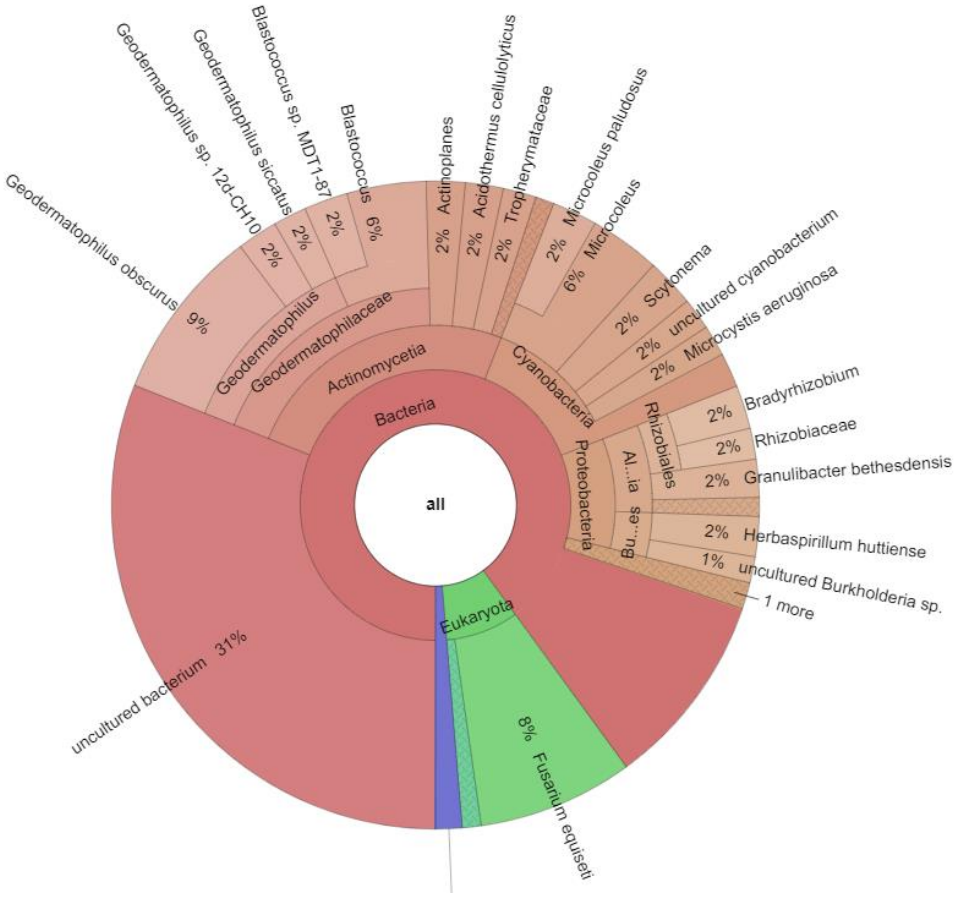
Our first investigation into the functional role of biocrusts was based on a DNA based microstudy of the impact of fire and grazing at VRSS. We focused on a 2-Yearly Early burnt site on the calcarosol soils and examined the composition of the whole biocrust inside (no cattle) and outside a small exclosure (Fig. 4.3.1). These results demonstrated the broad diversity of the biocrusts organisms present (Fig. 4.3.2 a,b; Fig. 4.3.3 a,b).

Figure 4.3.1 (a) Aerial view of cattle exclosure in 2-year early burn site where DNA samples were taken to compare the effect of fire on biocrust function with and without cattle. Biocrust is blackish cover on the ground withing the exclosure and lighter colour outside. **(b)** After the previous fire in this paddock.

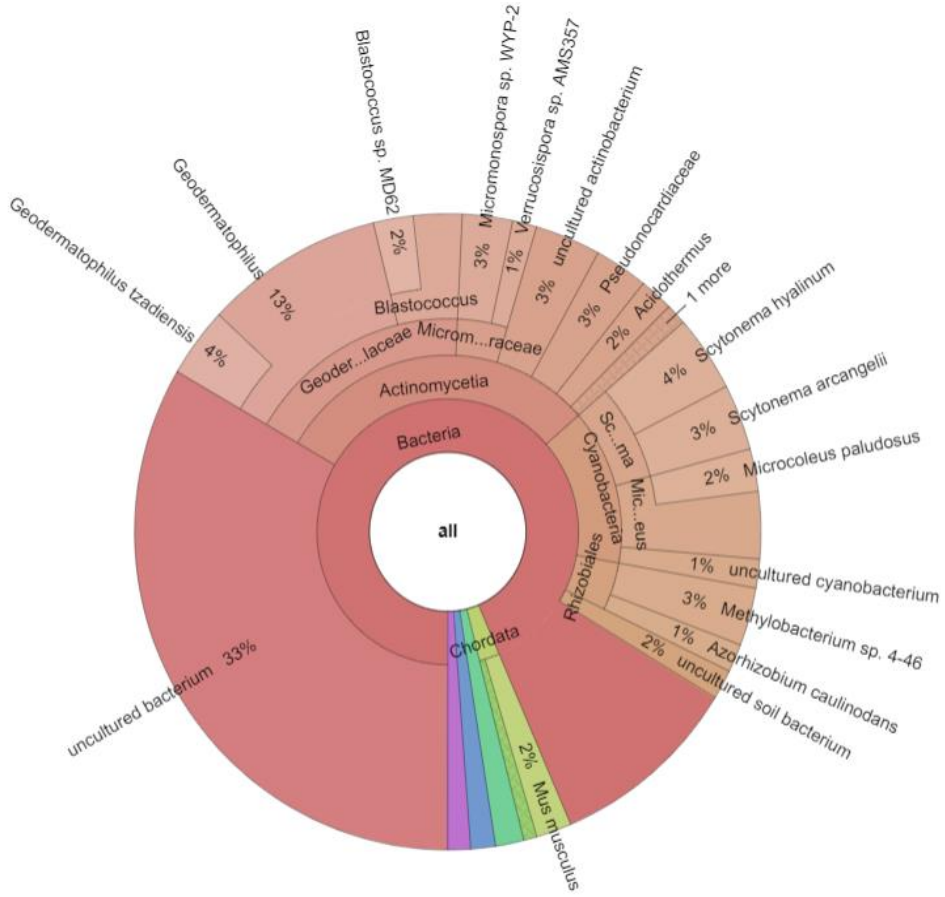
(a)

(b)

Figure 4.3.2 (a) Illustration of bacterial composition and function of biocrusts ***inside*** *the exclosure 2-year early fire* with no cattle disturbance and **(b)** outside the exclosure with cattle present.



(a)



(b)

Figure 4.3.3 Illustration of bacterial composition and function of biocrusts *inside and outside 2-year early fire*. Note that about one third of bacteria are unknown. Chordata was mouse and snake DNA recorded.

OUTSIDE EXCLOSURE

CYANOBACTERIA
- 89% N-fixers
- 15% Bacteria
- 14% Overall

RIZOBIALES: N-FIXERS
- 5% Bacteria
- 4% Overall

ACTINOMYCETIA
- 32% Bacteria
- 30% Overall

UNCULTURED BACTERIUM
- 36% Bacteria
- 33% Overall

CHORDATA

INSIDE EXCLOSURE

CYANOBACTERIA
- 80% N-fixers
- 15% Bacteria
- 13% Overall

PROTEOBACTERIA
- 11% Bacteria
- 10% Overall

ACTINOMYCETIA
- 28% Bacteria
- 25% Overall

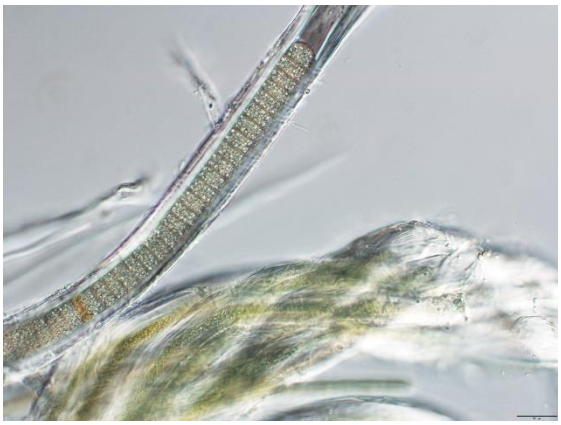
UNCULTURED BACTERIUM
- 34% Bacteria
- 31% Overall

EUKARYOTA

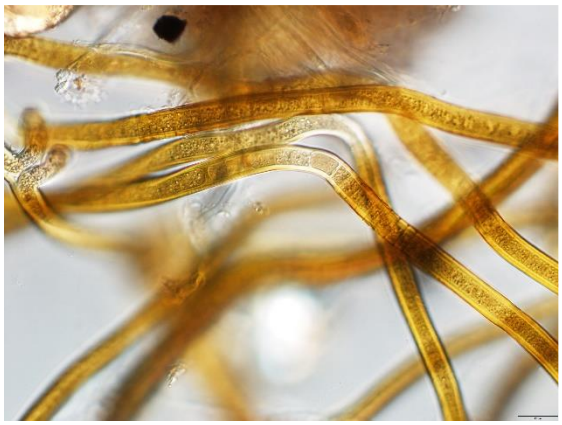
4.4 Structural form of biocrusts at VRSS

The visible biocrust organisms are mainly filaments of cyanobacteria that can be observed gluing the soil particles together in a network formation (Fig.4.4.1; Fig. 4.4.2). These images were taken after samples were watered to demonstrate the apparent fire effects even after a recovery phase. Overall, the late-fire treatments appear to have less visible filaments (Fig. 4.4.9 on). Later it was discovered there were seasonal effects especially in the vertosol soils. It is clearly visible in the Scanning Electron Microscope photos (SEM) that biocrust suffers damage and loss of structure post-fire.

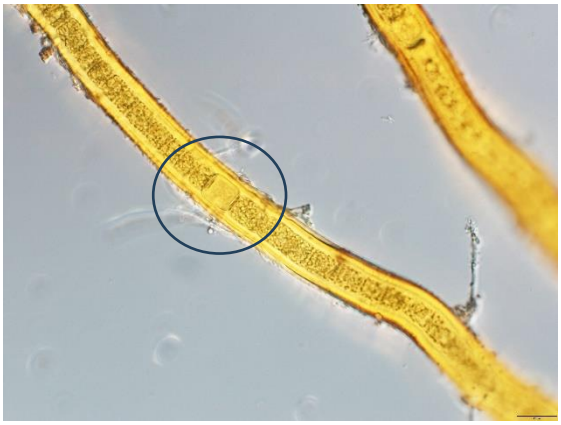
Figure 4.4.1 Micrographs of new cyanobacteria recovering following fire from early-four-year burn: (a) Porphyrosiphon sp. (above) with Microcoleus sp. (below), both early colonisers, (b-d) Scytonema sp. with specialist N-fixing cells (circled) that exclude oxygen, (d) illustrates the mass of filaments that aid stabilising the soil surfaces.



(a)



(b)

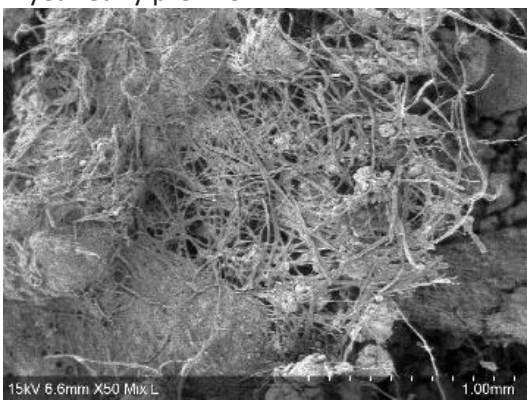


(c)

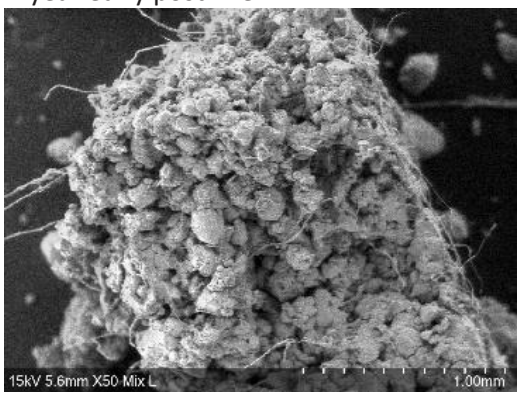
(d)

Figure 4.4.2. SEM images (50x magnification) of the upper millimetres of biocrust 15 months post-fire for early fire treatments in vertosol soils (Nov, 2020).

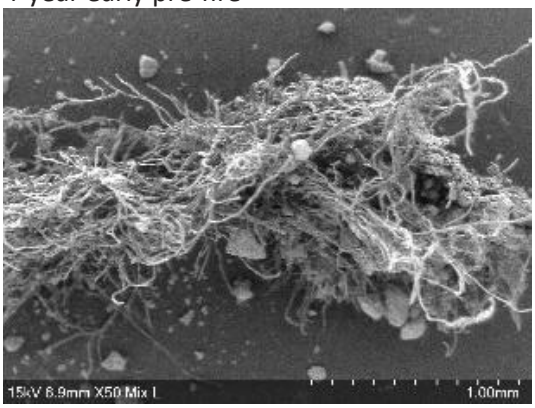
2-year early pre-fire



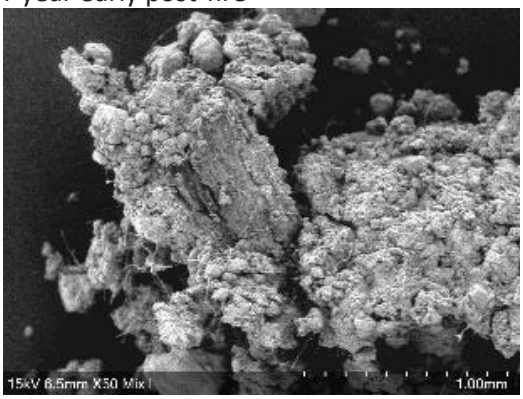
2-year early post-fire



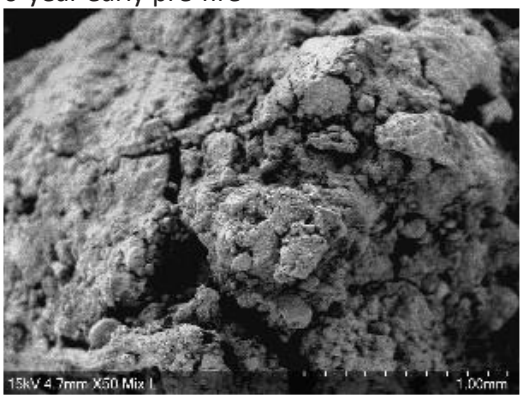
4-year early pre-fire



4-year early post-fire



6-year early pre-fire



6-year early post-fire

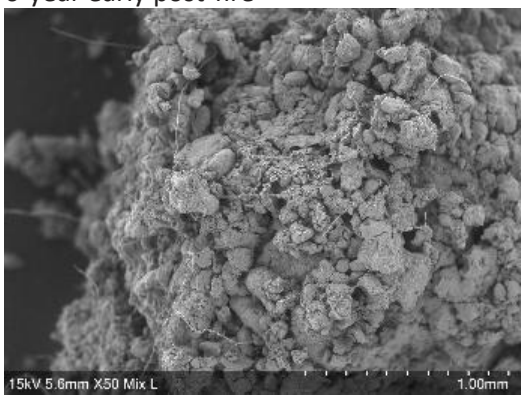
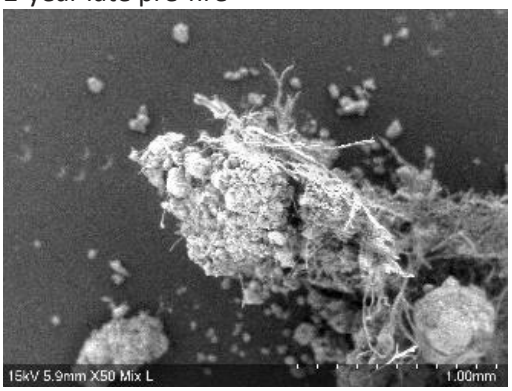
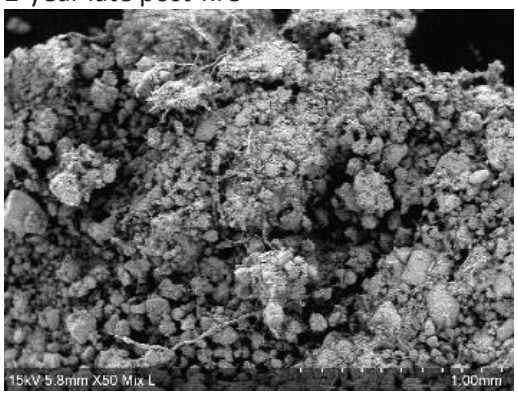


Figure 4.4.3 SEM images (50 x magnification) of the upper millimetres of biocrust for late fire treatments in Vertosol soils.

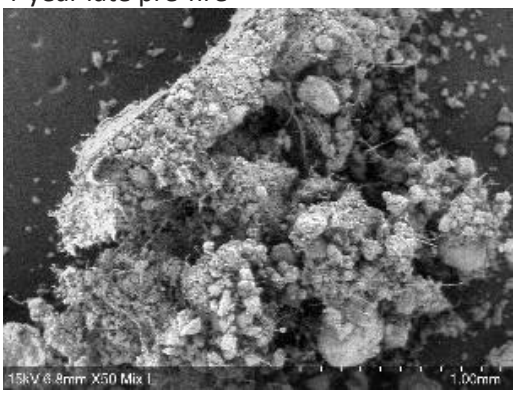
2-year late pre-fire



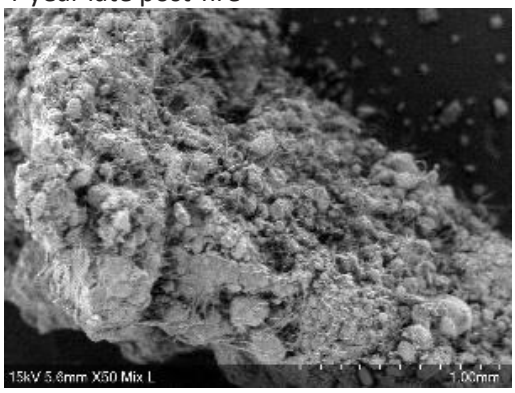
2-year late post-fire



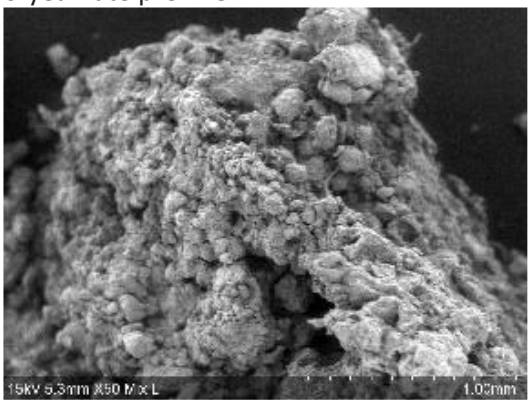
4-year late pre-fire



4-year late post-fire



6-year late pre-fire

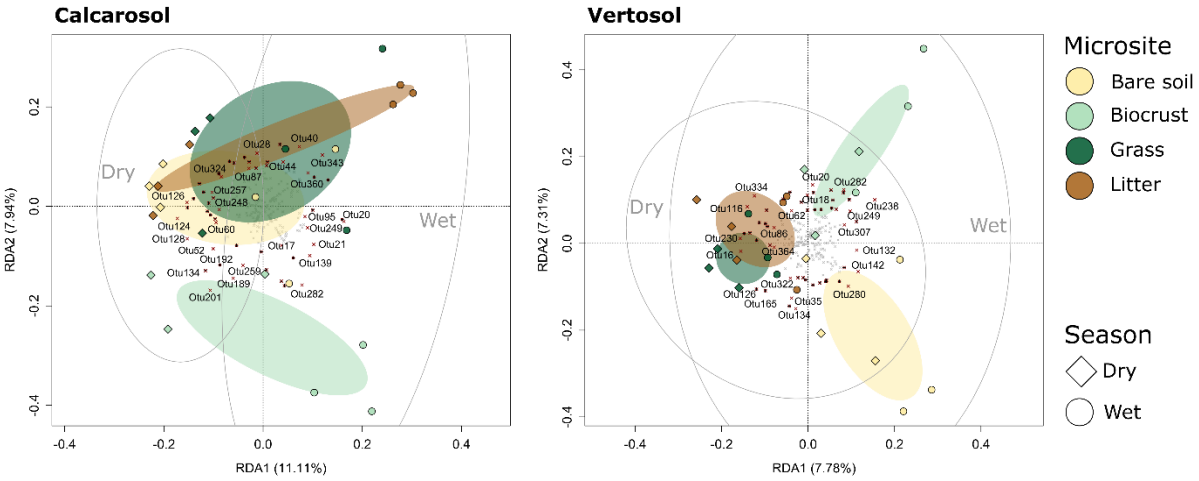


6-year late post-fire

4.5 Biocrust composition across microsites

The RDA (Fig. 4.5.1) shows how closely related the OTU's are for the different microsites. Quite different communities occupy under grass plants and out in the open spaces between grass plants. In the vertosol there is a marked separation between bare ground and biocrusts indicating there would be a loss of ecosystem function in the bare ground. Interestingly the biocrust was less likely to contain the same taxa as the other microsites demonstrating the exposure and heat adaptations required by these communities.

Fig. 4.5.1 Distance-based Redundancy Analysis (db-RDA) ordination plots highlighting differences in the composition of bacterial communities in the four-year early burn treatment between microsite (coloured ellipses), and seasons (empty ellipses). The most discriminating OTUs are shown in the plot. In both db-RDAs, the response was a Hellinger transformed OTU table, and the constraints (predictors) reflected the significant predictors identified using PERMANOVA. Hence, for both soils, the constraints were the main effects of microsite and season.



4.5.1 Bacterial community profile: Early 4-year fire microsites VRRS

Bacterial community profiles can be classified as groups of closely related individuals or OTUS (operational taxonomic units. Here we carried out a focused study for the current recommended fire regime for four-year burns. Bacterial communities were diverse with dominant representatives from 10 phyla (Fig. 4.5.2). There was a significant difference in the observed number of operational taxonomic units (OTUs) (Sobs) between soils, but not seasons, or microsites (Table 4.5.1). On the other hand, the composition of bacterial communities changed with soil, season, and microsite (Table 4.5.2). This represented the proportions or relative abundance of bacterial communities, where the OTUs (closely related individuals) changed with soil, season, and microsite (Table 4.5.3).

We identified 27 OTUs for both soils, 20 OTUs for vertosol, and 13 OTUs for calcarosol that significantly changed ($p >= 0.05$) because of microsites. These results describe the changes in community structure and composition across soil types, microsites (e.g. open areas and shaded areas, wet areas and drier areas). Therefore, we would anticipate this would drive the functional process of the biocrust across the landscape. The charts in Figures 4.3.4, 4.3.5 are an example of the different functional groups of bacteria that occupy the biocrust ecosystem.

The operational taxonomic units (OTU) listed in Figure 4.5.2 (e.g., indicated by arrow, Acidobacteriota, Pyrinomonadaceae increases abundances in the wet season on both soils; Pyrinomonas species have been isolated previously from savanna soil (Wust et al., 2016) and present one of the more common taxa in the studied biocrusts. Another group with high and varied abundance are Geodermatophilaceae in the Actinomycetia which grow on rock and soil surfaces and are unusually resistant to oxidative stresses that characterise hot and dry environments, in our microbe menagerie, these taxa are most more common on calcarosol (Normand et al., 2014). The most common organism groups into Rubrobacteria, a taxon with members that have a tolerance to high temperatures and ionising radiation. By comparison, cyanobacteria abundance with known N fixing capacity is lower and responds to season. However, cyanobacteria by far, make up the physical biomass of the biocrust occupying the surface of the soil forming a protective skin and habitat for other microscopic bacteria.

We found that similar bacterial taxa dominate the biocrusts but there are stronger changes in cyanobacterial presence in the wet season in the biocrust microsites (highlighted by the red box Fig. 4.5.2).

The heatmap represents the relative abundance of bacterial communities found in the Early 4 years burnt plot and how they change with soil type, in between seasons and the different microsites. The heatmap shows the mean values of three sample repetitions, and the taxonomy is summarised at family level. The taxonomy shown in the heatmap is the more representative bacterial communities (OTUs) by over 0.5% of the total bacterial community. From a total of 420 bacterial communities (OTUs), 120 were observed over 0.5%, summarised to 70 families (Fig. 4.5.1).

Figure 4.5.2 Heatmap summarising the frequencies of bacterial OTUs present at ≥0.5% mean relative abundance grouped at family level within microsites. Relative abundances are Hellinger transformed for the four year early burnt treatment.

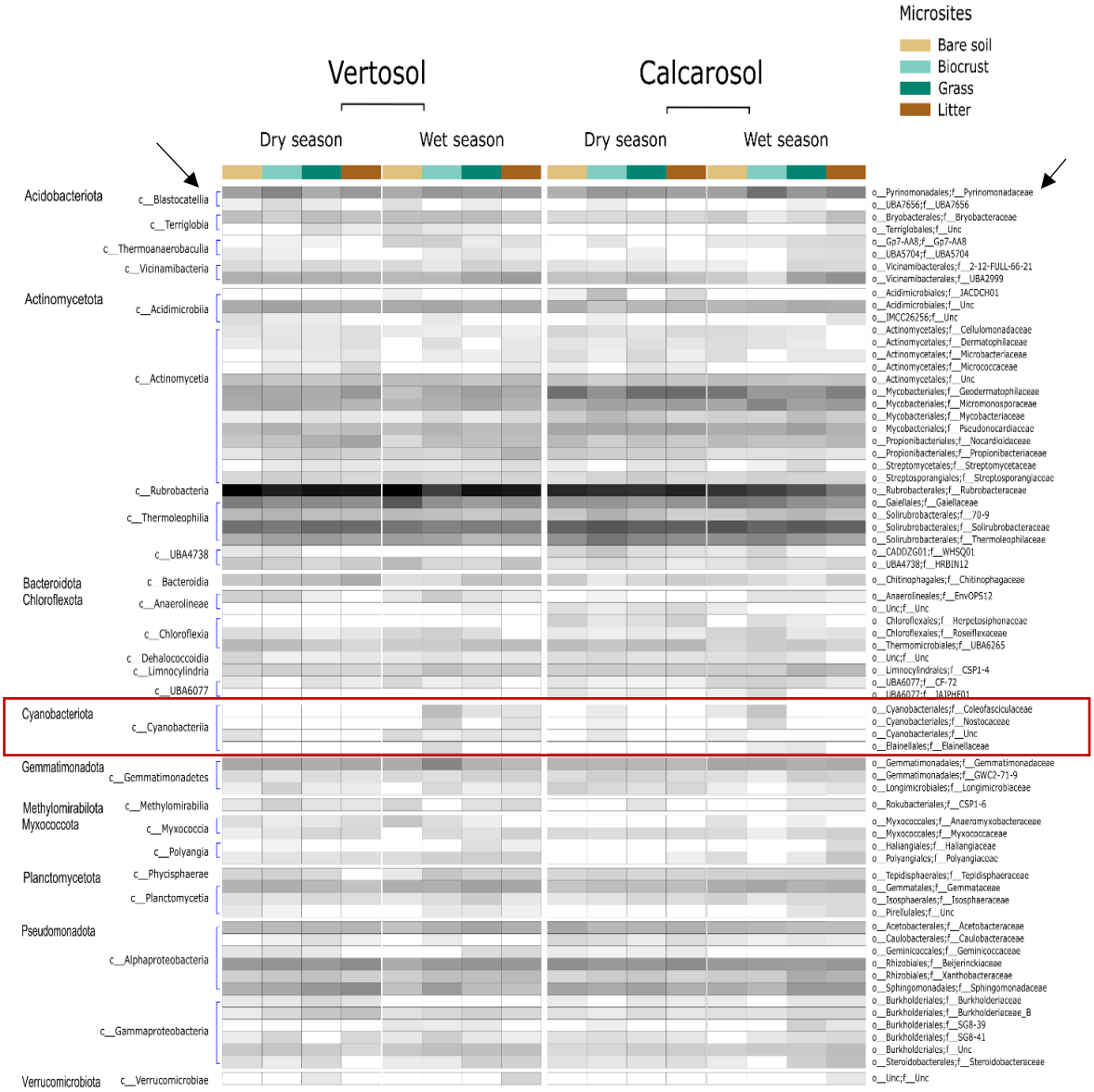


Table 4.5.1 Analysis of variance summary for soil, season and microsites for each alpha diversity metric in the fire experiment. Metrics include the observed numbers of OTUs (Sobs) and Shannon's Diversity Index (Shannon).

Predictor	Sobs		Shannon	
	F value	*P* value	*F* value	*P* value
Both soils				
Soil	7.732	0.009 **	14.358	0.001 ***
Season	0.108	0.744	0.398	0.532
Microsite	1.224	0.317	1.285	0.296
Soil:Season	0.346	0.561	0.025	0.874
Soil:Microsite	0.808	0.499	0.706	0.556
Season:Microsite	0.116	0.950	0.266	0.849
Soil:Season:Microsite	0.089	0.966	0.191	0.901
Within calcarosol				
Season	0.036	0.852	0.035	0.855
Microsite	0.844	0.489	1.380	0.285
Season:Microsite	0.141	0.934	0.275	0.843
Within vertosol				
Season	0.394	0.539	0.403	0.535
Microsite	1.166	0.354	0.567	0.645
Season:Microsite	0.068	0.976	0.213	0.886

ANOVA | Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

The composition of the bacterial communities' changes significantly with soil, season, and microsite, including showing interactions between soil and microsites (Table 4.5.2). This means that species changes occur that matches changes in function for N fixation with soil and season.

It is worth noting that the cyanobacteria genus *Nostoc* is a highly productive N-fixing cyanobacteria that was mostly evident in its presence during the wet season and more so in the vertosol soils. Furthermore, we believe that this is the first record for *Elainella* in Australia, a new tropical cyanobacterium that is also capable of N-fixation (Jahodářová et al., 2018). Additionally, Coleofasciculaceae is a recently described desert dwelling genus that includes important crust forming motile species (cyanobacteria that can move up and down the soil profile) (Moreira C Fernandes et al., 2021). These cyanobacteria are central to the development of a stable biocrust dominated by N-fixing species and housing other N-fixing bacteria.

Table 4.5.2 Results from PERMANOVA models summarising the main and interactive effects of soil (vertosol and calcarosol), microsite and season (dry and wet season) on the composition of bacterial communities, as well as the effect of microsite and season within each soil separately for the four yearly early burnt treatment.

Predictor	**OTU relative abundances**		
	F value	R^2 value	*P* value
Both sites			
Soil	5.429	0.094	0.001 ***
Season	2.646	0.046	0.001 ***
Microsite	1.724	0.089	0.001 ***
Soil:Season	1.858	0.032	0.006 **
Soil:Microsite	1.231	0.064	0.067 .
Season:Microsite	1.234	0.064	0.051 .
Soil:Season:Microsite	1.108	0.057	0.197
Within vertosol			
Season	1.63	0.064	0.008 **
Microsite	1.57	0.183	0.001 ***
Season:Microsite	1.10	0.129	0.218
Within calcarosol			
Season	2.87	0.107	0.001 ***
Microsite	1.39	0.156	0.021 *
Season:Microsite	1.24	0.139	0.084 .

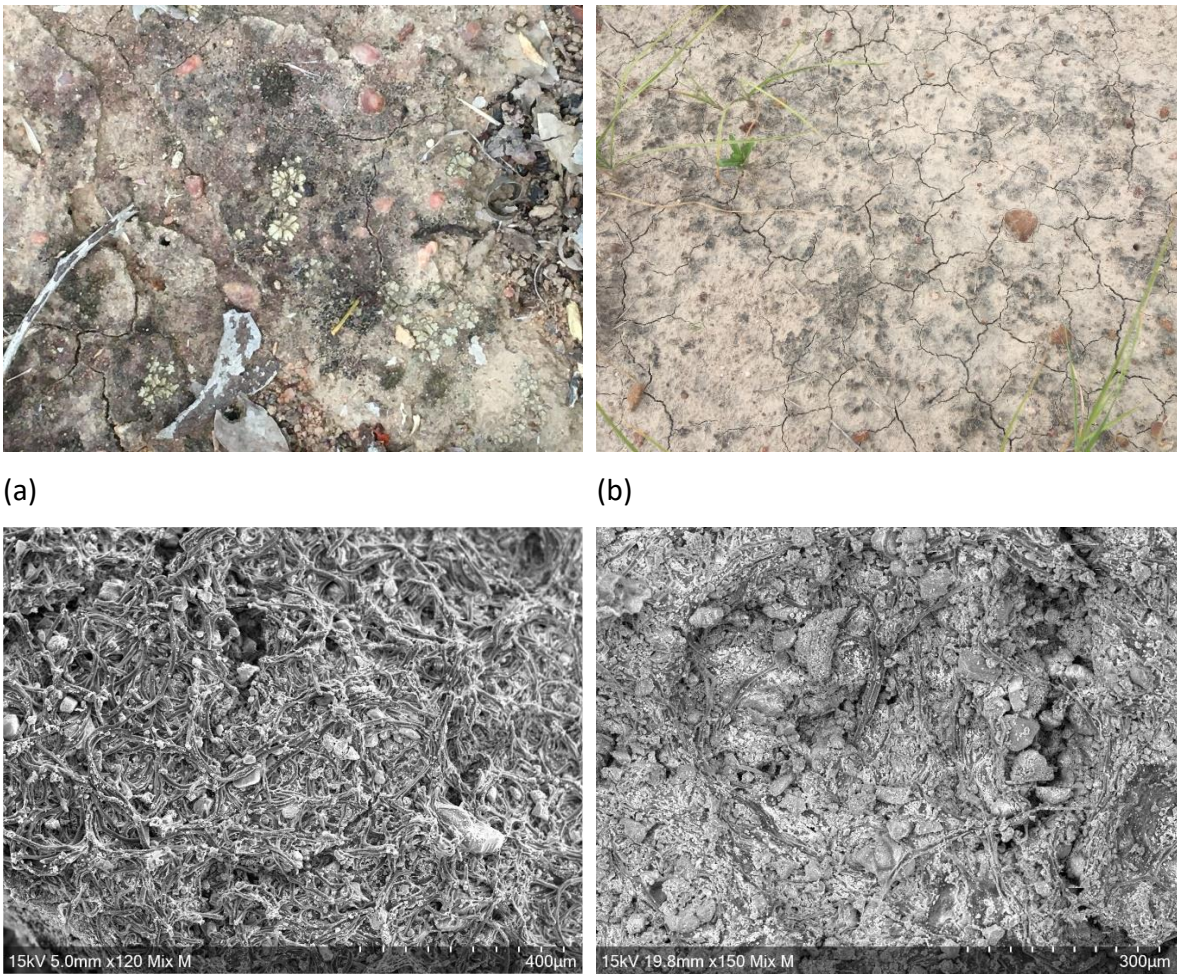
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

In the wet season cyanobacterial colonies on the calcarosol appeared to favour the open spaces rather than under the grass. In this case there would be less competition for light and from annuals. In the vertosol there is often more moisture over longer periods and surface water aids spread of cyanobacteria.

4.6 Results: Biocrusts at Wambiana Grazing Trial

The biocrusts found at Wambiana were dominated by cyanobacteria however the duplex clays included liverworts, mosses and microlichens (Fig. 4.6.1). Some microlichens have a symbiotic relationship with N-fixing cyanobacteria, therefore an important feature in the nutrient cycling capacity of these biocrusts. The red-yellow soils were sandy and dominated by cyanobacterium Symploca that had a distinct reddish colour (Fig. 4.6.2) and compared to the duplex biocrusts were generally very thin but important for soil stabilisation.

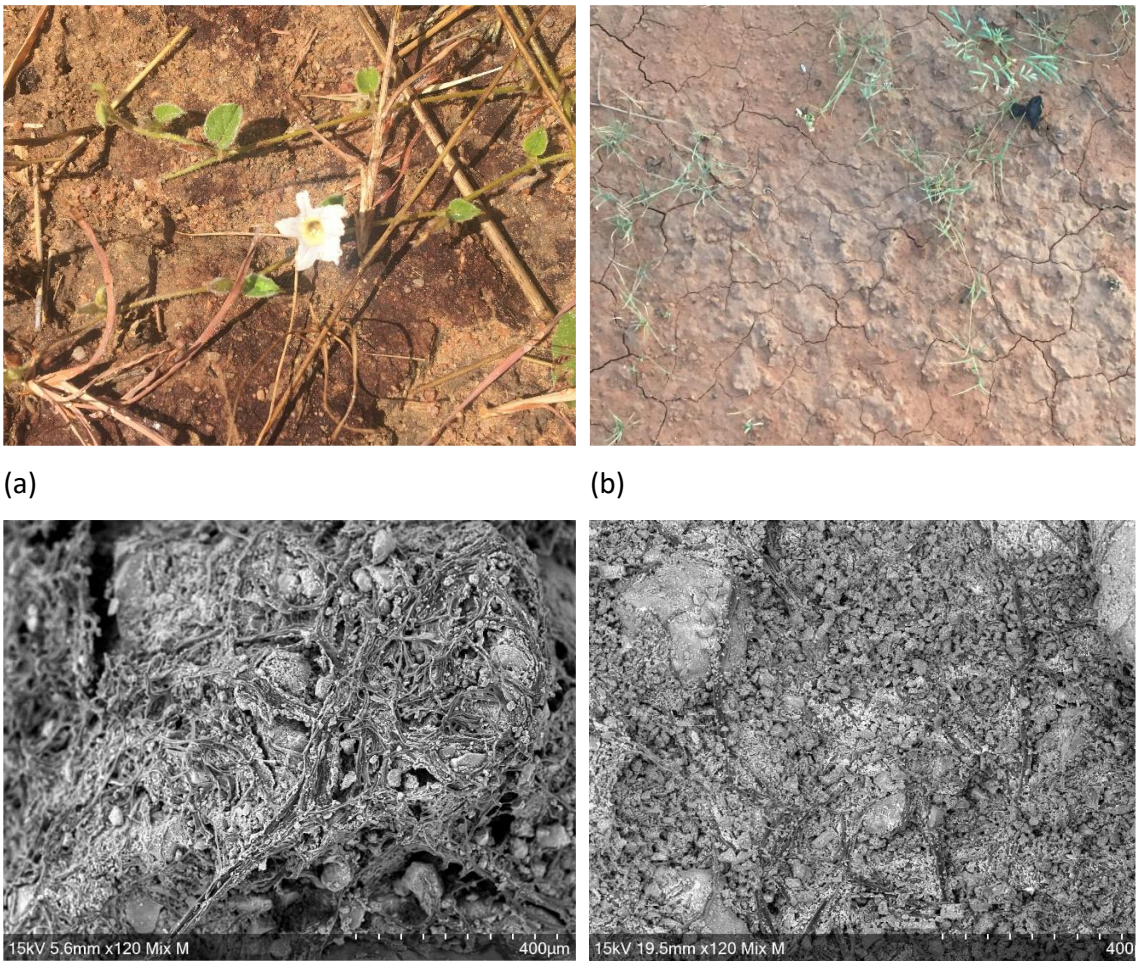
Figure 4.6.1 Duplex soils, photo and scanning electron microscope (SEM) images: (a) Biocrust in good condition (XCL) with cyanobacteria, lichens, liverworts, and mosses, (b) darkened patches represent poor quality cyanobacterial biocrust, (c) R/Spell cyanobacterial filaments with fine soil particles SEM 120 x mag. (d) HSR biocrust in poor condition, cyanobacterial filaments with soil 150 x mag (Williams et al. 2022).



(a) (b)

(c) (d)

Figure 4.6.2 Red-yellow earths, photo and scanning electron microscope (SEM) images: (a) Red coloured biocrust in good condition (XCL) dominated by cyanobacteria, (b) degraded cyanobacterial biocrust seen with faint discolouration on surface, (c) R/Spell cyanobacterial filaments with soil particles SEM 120 x mag. (d) HSR biocrust in poor condition, cyanobacterial filaments with soil 120 x mag (Williams et al. 2022).



(a) (b)

(c) (d)

4.7 Biocrust metagenomic insights, moderate stocking, wet season spelling

4.7.1 Photosynthesis

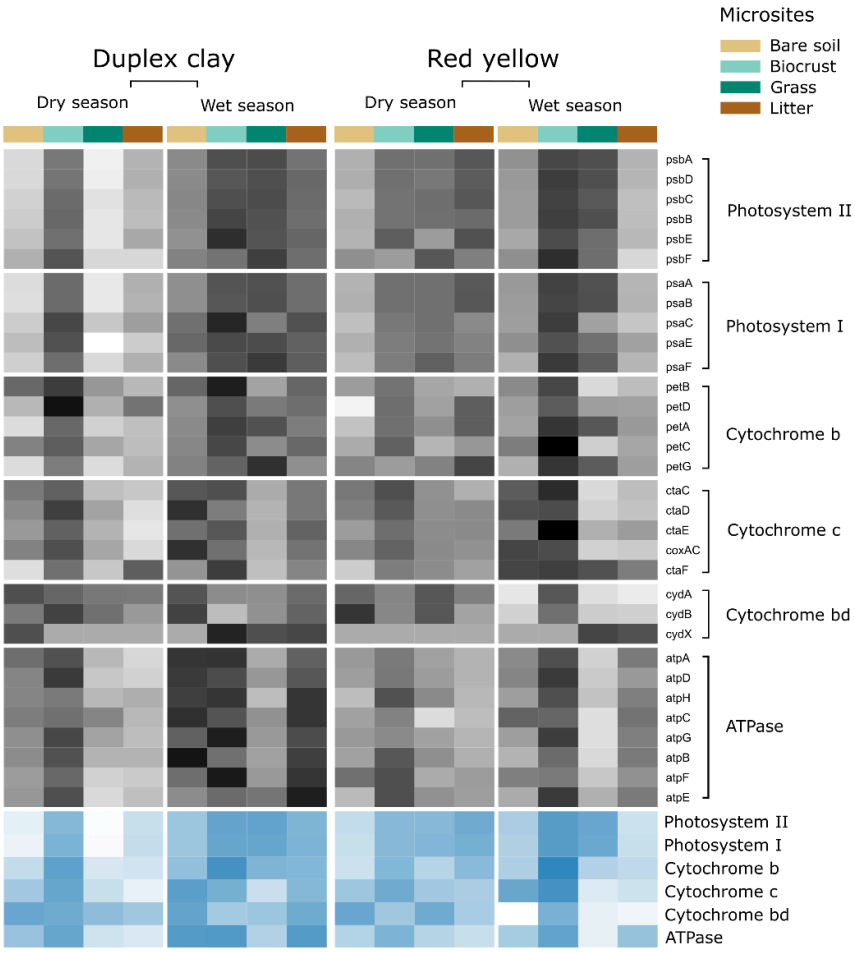
Season and microsite in both soils influenced the photosynthesis-related genes in the moderate stocking rate with wet season spelling (Table 4.7.1). The heatmap in the summary white-blue shaded showed that biocrust presents a higher frequency of photosynthesis-related genes and rises with the wet season in the red-yellow soil. While in the duplex-clay, it is higher in the biocrust in the dry season, with an increase for all the microsites in the wet season (Fig. 4.7.1).

Table 4.7.1 Results from PERMANOVA models summarising the main and interactive effects of soil (vertosol and calcarosol), microsite and season (dry and wet season) on photosynthesis related genes, as well as the effect of microsite and season within each soil separately.

Predictor	**Photosynthesis genes frequency**		
	F value	R^2 value	*P* value
Both sites			
Soil	3.948	0.036	0.039 *
Season	11.360	0.103	0.002 **
Microsite	9.578	0.260	0.001 ***
Soil:Season	10.459	0.095	0.002 **
Soil:Microsite	1.269	0.034	0.255
Season:Microsite	3.612	0.098	0.013 *
Soil:Season:Microsite	3.142	0.085	0.006 **
Within Red-Yellow			
Season	0.65	0.015	0.553
Microsite	5.27	0.361	0.003 **
Season:Microsite	3.77	0.259	0.005 **
Within Duplex-Clay			
Season	21.70	0.343	0.001 ***
Microsite	5.59	0.265	0.006 **
Season:Microsite	2.96	0.140	0.040 *

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Figure 4.7.1 Moderate stocking rate with wet season spelling Relative frequency heatmap of photosynthesis related gene frequency by microsites. Genes are in rows grouped by metabolic pathway and samples are in columns ordered by soil, season, and microsite. White-black shading represents the frequency per million bacterial reads while the white-blue shaded shows the sum of the square mean gene frequency across metabolic pathways. This heatmap has been row-scaled to show the relative abundance of each respective gene across microsites.



4.7.2 Taxonomic profiles of biocrusts across microsites

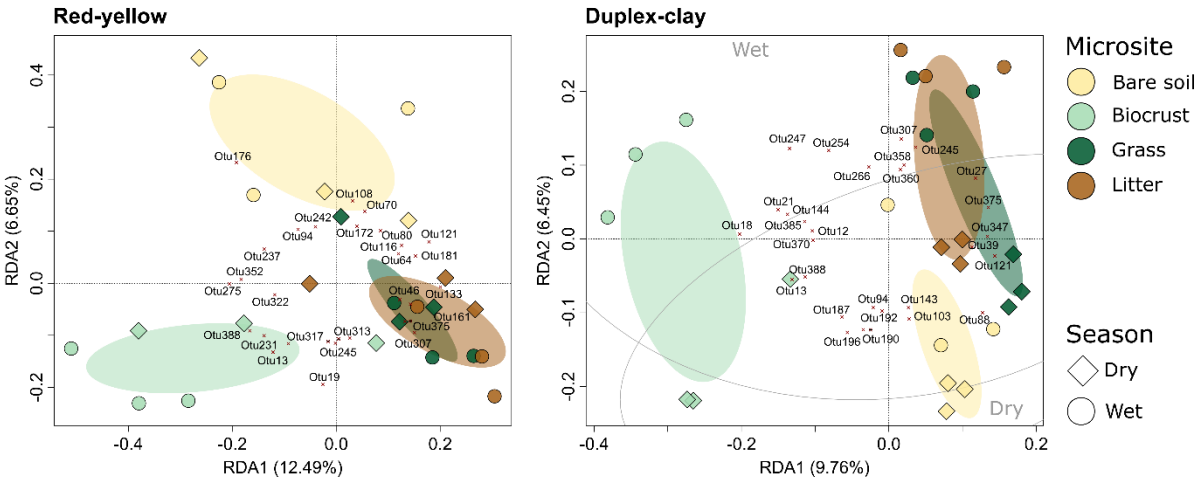
The relative abundance of bacterial communities found in the moderate stocking rate with wet season (rotational) spelling demonstrates how they change with soil type, between seasons and across the different microsites. The heatmap shows the mean values of three sample repetitions, and the taxonomy is summarised at family level. Cyanobacteria appear more highly represented in the red-yellow soils and more active in the wet season. Leptolyngbya at these sites are most likely dominated by unknown Microcoleus (sub-surface dwellers) and Symploca species that has the

distinctive red coloured crust. These cyanobacteria are known N-fixers with Nostoc often highly influential in the wet season (Williams et al., 2017).

The taxonomy shown in the RDA represents bacterial communities (OTUs) > 0.5% of the total bacterial community. From a total of 382 bacterial communities (OTUs), 122 were observed > 0.5% and condensed to 59 families (Fig. 4.7.2).

In both soils at Wambiana Grazing Trial the biocrust community is quite separated from the bare soil with grass and litter both overlapping but separated from the other microsites. This provides a clearer picture of how the bacterial communities are adapted to microsite niches. In this case, only a small number of OTU's are found in bare soil illustrating the lack of functional processes occurring in those microsites.

Fig. 4.7.2 Distance-based Redundancy Analysis (db-RDA) ordination plots highlighting differences in the composition of bacterial communities between microsite (coloured ellipses), and seasons (empty ellipses). The most discriminating OTUs are shown in the plot. In both db-RDAs, the response was a Hellinger transformed OTU table, and the constraints (predictors) reflected the significant predictors identified using PERMANOVA. Hence, for the red-yellow soil, the constraint was microsite, while for the duplex-clay soil, the constraints were the main effects of microsite and season.



4.7.3 Biocrust microsites: moderate stocking, wet season spelling

There were 382 Operational Taxonomic Units (OTUs) or bacterial communities across the four microsites at the moderate stocking rate with wet season spelling in Wambiana. There were significant differences in the observed number of OTUs (Sobs) between soils, but not season or microsites (Table 4.7.3). The proportions or relative abundance of bacterial communities' (OTUs)

changed with soil, season, and microsite (Table 4.7.3). We identified 40 OTUs for both soils, 25 OTUs for red-yellow, and 28 OTUs for duplex-clay that changed significantly (*p > 0.05*) within microsites.

Table 4.7.3 Analysis of variance summary for each alpha diversity metric in the fire experiment. Metrics include the observed numbers of OTUs (Sobs) and Shannon's Diversity Index (Shannon).

Predictor	**Sobs**		**Shannon**	
	F value	*P* value	*F* value	*P* value
Both soils				
Soil	5.240	0.029 *	6.222	0.018 *
Season	0.962	0.334	0.522	0.475
Microsite	0.141	0.935	0.333	0.801
Soil:Season	0.474	0.496	0.025	0.875
Soil:Microsite	2.618	0.068	2.461	0.081
Season:Microsite	0.651	0.588	0.441	0.725
Soil:Season:Microsite	0.296	0.828	0.019	0.996
Within Red-Yellow				
Season	0.064	0.804	0.149	0.704
Microsite	1.241	0.328	2.100	0.140
Season:Microsite	0.221	0.880	0.139	0.935
Within Duplex-Clay				
Season	1.048	0.321	0.433	0.520
Microsite	1.449	0.266	0.684	0.575
Season:Microsite	0.601	0.624	0.328	0.805

ANOVA | Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 4.7.4

Results from PERMANOVA models summarising the main and interactive effects of soil (red – yellow and duplex clay), microsite and season (dry and wet season) on the composition of bacterial communities, as well as the effect of microsite and season within each soil separately.

Predictor	**OTU relative abundances**		
	F value	R^2 value	*P* value
Both sites			
Soil	3.440	0.063	0.001 ***
Season	1.598	0.029	0.042 *
Microsite	2.196	0.121	0.001 ***
Soil:Season	1.062	0.020	0.321
Soil:Microsite	1.252	0.069	0.066 .
Season:Microsite	0.995	0.055	0.460
Soil:Season:Microsite	0.996	0.055	0.421
Within Red-Yellow			
Season	1.21	0.047	0.150
Microsite	1.90	0.221	0.002 **
Season:Microsite	0.97	0.113	0.477
Within Duplex-Clay			
Season	1.48	0.059	0.017 *
Microsite	1.50	0.179	0.001 ***
Season:Microsite	1.02	0.122	0.397

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

5 Biocrust recovery after fire at VRRS

5.0.1 Fieldwork (2019 and 2020)

Our biocrust and soil nutrient profiling has incorporated the pre-fire and post-fire measurements both across the soil surface and at shallow depths (5 cm) where we would most likely expect to find the concentrations of plant-available nitrogen. We are now assessing the species composition of the biocrust microbiome, how many nitrogen fixing bacteria are present and how much nitrogen is fixed over a wet season. During 2021 we plan to provide updates on preliminary results about biocrust regeneration following fire, and its role in nutrient cycling.

5.0.2 How do we measure biocrusts across 32 fire plots?

Biocrust organisms are quite small but at Kidman (a typical northern dry savanna), they are found covering the soil surface between the grass plants. Yet, they can be hard to see on the black soil and not always visible in the dry season. To measure biocrust presence and cover, we use several methods that included visual estimations of the biocrust presence and ground cover within four different microsites: litter, grass (basal area and canopy), biocrust and bare ground with no visible biocrust. Soil surface sampling was followed by laboratory extraction of chlorophyll and taking hyperspectral images of the soil surface. The latter provided a spectral measurement of the presence of biocrust chlorophyll. Nevertheless, biocrust cover, diversity and depth varied quite a lot across the landscape.

5.0.3 Field sampling

In 2019, the 12-yearly cycle occurred when all the trial plots were burnt in June (early cool fire) and October (late hot fire). Before the early burns (pre-fire) we sampled biocrusts across the two soil types and all the treatments (Fig. 5.0.1, Table 5.0.1, 5.02). Following the late burns in October (post-fire) we re-sampled all the plots except for the controls which were not burnt and there was no rain during this time. Thus, the pre-fire samples had not been burnt for 2, 4 and 6 years respectively and the post-fire samples had either been burnt four months prior or one week prior to sampling. During this time frame cattle had access to the plots, however following the late season fires, the paddocks are closed off for the entirety of the ensuing wet season.

Figure 5.0.1 Kidman Springs field trip team (July 2020). Collecting biocrusts and soil samples (top); The team (l-r) Harry Cosgrove, Steve Williams, Laiza Sherar (front), Wendy Williams and Madeline Dooley; (bottom right) biocrust cover measurements in quadrats.

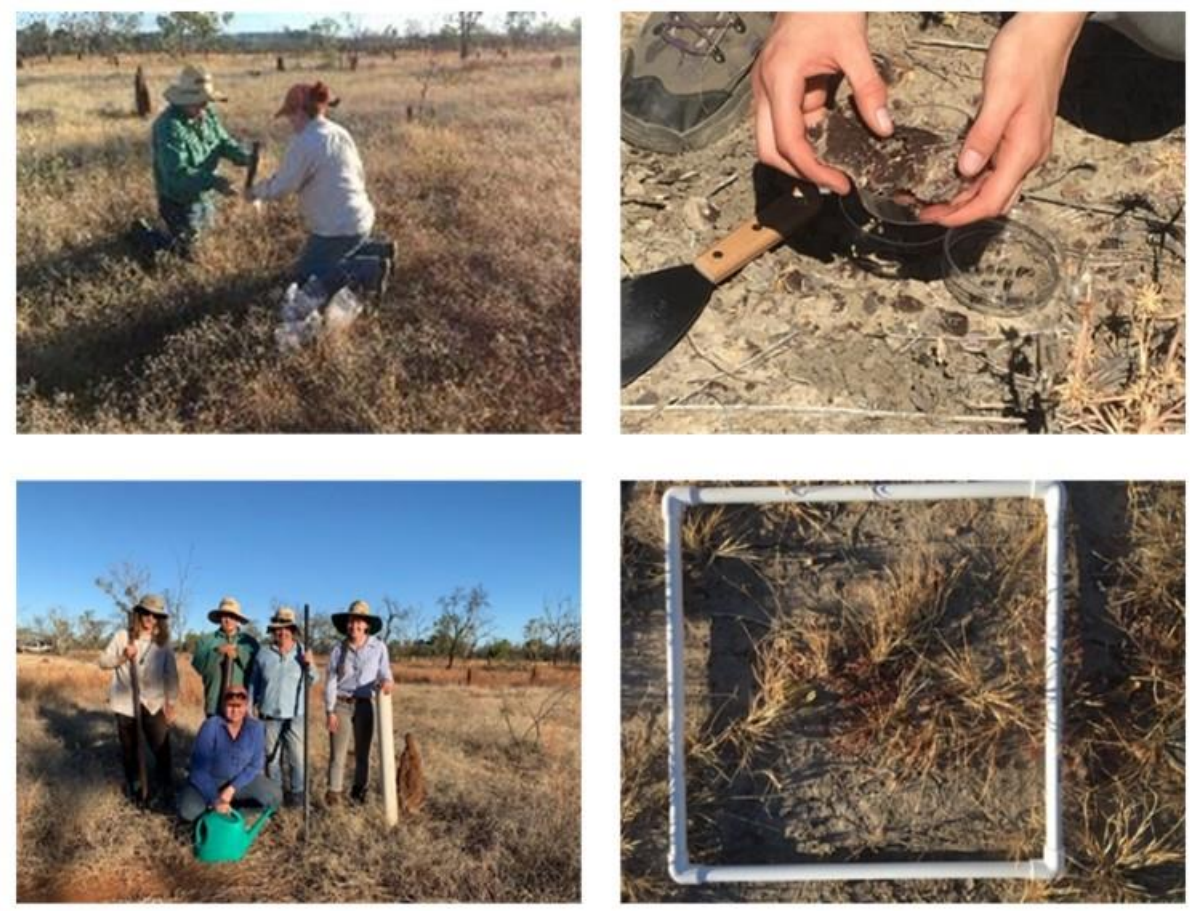


Figure 5.0.2 (a) Controlled burn at Plot 19 – Early 6-year (2019), (b) location of Kidman Springs in north-western Australia, (c) and (d) fire plot design where upper number represents the plot number and the lower letter represents fire season (early or late) and the number represents the fire interval (2, 4, 6 years or 0 – no fire); (e) post-fire biocrust sampling.

(a)

(b)

1 L6	2 E4	3 E4	4 E6
5 E6	6 L4	7 L4	8 0
9 0	10 L2	11 L6	12 E2
13 0	14 E2	15 L2	16 0

(c) Vertosol (black soil) plots

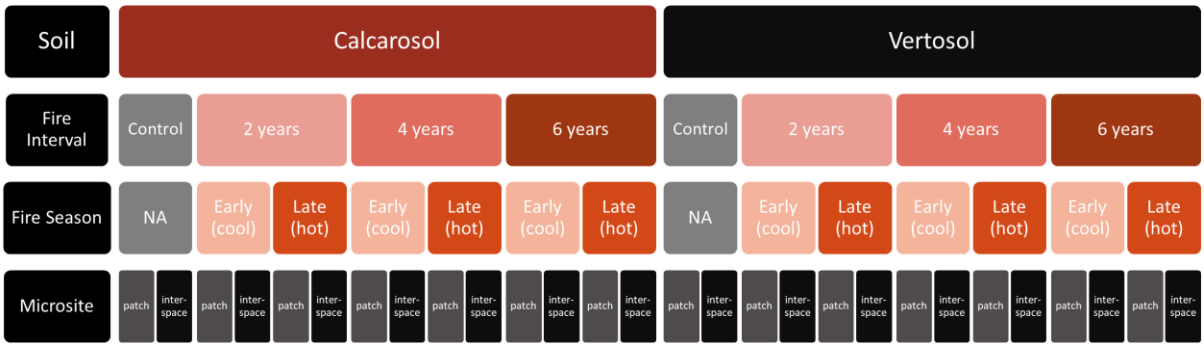
17 L6	18 0	19 E6	20 L4
21 0	22 E4	23 E2	24 L2
25 E6	26 0	27 E4	28 E2
29 0	30 L4	31 L2	32 L6

(d) Calcarosol (red soil) plots

(e) biocrust sampling post-fire

The sampling strategy was based on landscape organisation at two spatial scales: perennial plant patches where resources tend to accumulate and inter-patches (bare open areas between the patches). Biocrusts were prominent both within the patches (underneath grass and shrub canopies) and across the inter-patches. We established one 30 m transect on each of the two plot treatment replicates (positioned adjacent to permanent Transect 2). We then identified and marked three patches and three inter-patches contiguous to the transect. These areas also extended widthways and defined the sample collection sites. Five replicate samples of the biocrust or bare soil surface were cored to a depth of approximately 0.5 cm and placed in six-well plates. These were field dried (<5% moisture), sealed and transported back to the laboratory.

Table 5.0.2 Description of field samples collected from the two soil types, different fire intervals (years between) and fire seasons, early dry season (EDSF) and late dry season (LDSF), and microsites: patches=groups of grass plants, interspaces=open areas of bare soil often covered by biocrusts situated between grass patches. *Control (unburnt) samples were only collected during the pre-fire period.



Soil	Fire Interval	Fire Season	sample time	Landscape site	N= total no. samples
Calcarosol	2, 4, 6 years n=3	EDSF, LDSF x 2 x 2 plot reps n=4	Pre-fire Post-fire n=2	Patch Interspace n=2 x 5 reps =10	N= 6 x 2 x 2 x 2 x 5 reps = 240 + 20C =260 samples
Calcarosol	Control n=2 (plot reps)	No-fire n=1	Pre-fire* n=1	Patch Interspace n=2	(Control n=20)
Vertosol	2, 4, 6 years x 2 plot reps, n=6	EDSF, LDSF n=2	Pre-fire Post-fire n=2	Patch Interspace n=2 x 5 reps =10	N= 6 x 2 x 2 x 2 x 5 reps = 260 + 20C =260 samples
Vertosol	Control n=2 (plot reps)	No-fire n=1	Pre-fire* n=1	Patch Interspace n=2	(Control n=20)

5.0.4 Fire environment

To understand the impact of the fire on biocrusts we installed Thermochron temperature iButton loggers, operating temperature range 0 to +85°C (http://thermodata.com.au/), and Hastings Tinytag data loggers (https://www.hdl.com.au/data-loggers/) to record ambient temperature (in controls) together with soil surface in the fire plots (range 0 to +125°C), and iButtons were buried at 1 and 2 cm depth. These remained in place while the plots were burnt and retrieved several hours after. Due to the remoteness of the site these records were only taken for the late season fires (Oct 2019).

5.0.5 Biocrust Resurrection and Recovery

When cyanobacteria dry out for several months, they are in a desiccated state, and they do not photosynthesise or grow. Following rehydration, normally following rain, cyanobacterial resurrection takes place. A non-destructive method of measuring the resurrection status is measuring chlorophyll fluorescence and determining photosynthetic activity. In this study we rehydrated the samples to

saturation, placed them on trays in a growth chamber set at 28°C and 75% relative humidity on a 12-hourly light/dark cycle.

5.0.6 Photosynthetic activity

The regrowth of cyanobacteria that constituted the bulk of the biocrust was regularly monitored over 30 days (13 time periods) using a Pocket PAM (Pulse-Amplitude-Modulation, fluorometer Gademann Instruments and Walz, Germany) with an encased LED positioned within a few millimetres of the soil surface. The photosynthetic activity of cyanobacteria was determined by chlorophyll *a* (chla) fluorescence saturation pulse method (Raggio et al. 2014). A high intensity, short duration flash of light is given that effectively transiently closes all PSII (Photosystem II) reaction centres. The fluorescence yield and resulting maximal fluorescence, Fm´ value is compared with the steady-state yield of fluorescence in the light, (Ft) gives information about the performance of PSII (Maxwell and Johnson, 2000). The effective quantum use efficiency (describe by Schreiber et al., 1994) or PSII yield is:

$$\text{Yield} = (Fm' - Ft)/Fm'$$

Yield values range between 0 and 1 and prior studies have shown healthy cyanobacteria have reported yields 0.3-0.5 and seldom >0.6 while algae (0.65) and higher plants (0.8) are markedly higher (Sims et al., 2012). Yet the lower values can be an artefact of insufficient light intensity reaching PSII (Sims et al., 2012). In this study a positive yield value indicated photosynthetic activity while hydrated and yield increases were indicative of recovery (burnt sites) and resurrection. At the end of 30 days the samples were measured both on the surface and the sub-surface as there were visible cyanobacteria underneath the wells. Following this step, the samples were dried at 40°C for 48 hours and used for biomass and chemistry.

5.0.7 Carbon, Nitrogen and Chlorophyll

For each analysis three replicates of each treatment were processed, these were sub-sampled from sieved material following the micro-core pigment extraction (biomass). Total C and total N were determined with high temperature digestion using a vario MACRO Elemental Analyser (Elementar). For pigment content, the chlorophyll a extractions were carried out on the cyanobacterial soil crusts (Barnes et al., 1992) and calculated using Wellburn's (1994) equations.

5.0.8 Biocrust morphology and cyanobacterial diversity

The morphological features of the biocrusts are described from field samples with the aid of an electron microscope. Morphological features and measurements were carried out from wet mounts prepared from each sample set for both soil types, across all fire treatments and controls. The samples for the six treatments and control were rehydrated for 24 hours and examined using bright-field, phase contrast and differential interference contrast illumination systems with an Olympus BX51 compound microscope to a maximum magnification of ×400. Photomicrographs were obtained using an Olympus DP12 digital microscope camera. Identification was performed to a species level (where possible) in the laboratory using the closest available keys (Anagnostidis and Komarek, 2005; Komárek, 2013; Komarek and Anagnostidis, 1999). This work is in progress and detailed results will be published in 2025-2026.

5.0.9 Statistical analysis

All statistical analyses for nitrogen, carbon, chlorophyll a, and plant-available nitrogen were conducted using R Studio (version 4.0.2). We employed linear mixed models using the 'lmer' function to assess the effects of fire treatment and fire season in each plot, as well as the interaction between these variables. Type III ANOVA with Kenward-Roger's method was utilized to determine interactions across multiple variations. Estimated marginal mean values of nitrogen, carbon, plant-available nitrogen, and chlorophyll a were calculated using the 'emmeans' function from the 'emmeans' package in R (Lenth et al., 2024). Post-hoc tests were employed to generate contrasts, enabling pairwise comparisons between different fire treatments within each patch and interspace. All graphics were produced using the R package 'ggplot2' (Wickham, 2016). However, heat maps of carbon and nitrogen for Calcarosol and Vertosol in Victoria River Research station were created using GraphPad Prism version 10.2.2, GraphPad Software, Boston, Massachusetts USA, www.graphpad.com.

5.1 Results: Biocrust resurrection and recovery

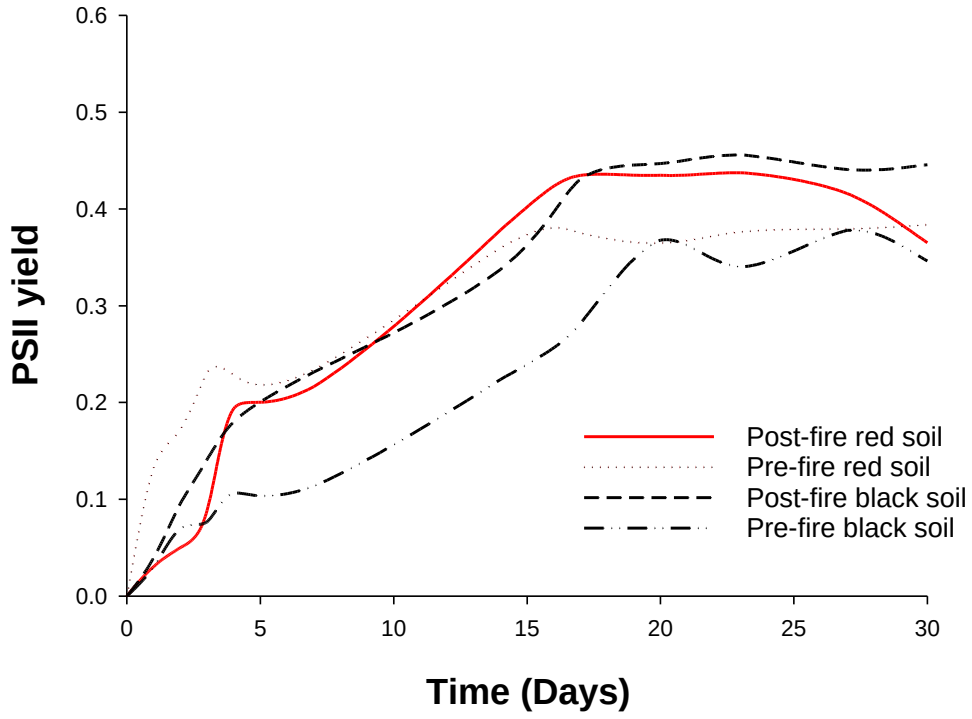
Biocrust recovery was measured on all surface soils for 30 days. Biocrust resurrection demonstrated by positive PSII yields for both soil types and all treatments occurred within the first four days. The pre-fire calcarosols were fastest to respond with an average PSII yield over the first four days of 0.244 (±0.15) and the pre-fire vertosols (0.115 ±0.15) were the slowest (Fig. 5.1.1). The final PSII yield at 30 days for the control plots was 0.399 (±0.04) for calcarosol soil surfaces and 0.363 (±.06) for the subsurface, and the vertosol soil surface was 0.429 (±0.04) and 0.399 (±0.03) for the subsurface.

For the red soil, there were no significant differences in final yields at the surface ($P > 0.14$), but for the subsurface, we found a significant burning by fire interval interaction ($F_{2,22} = 3.91$, $P = 0.021$). This showed no significant difference in PSII yield between pre-and post-fire for the 2-year burn, but significantly higher yield post-fire for both the 4- and 6-year burns.

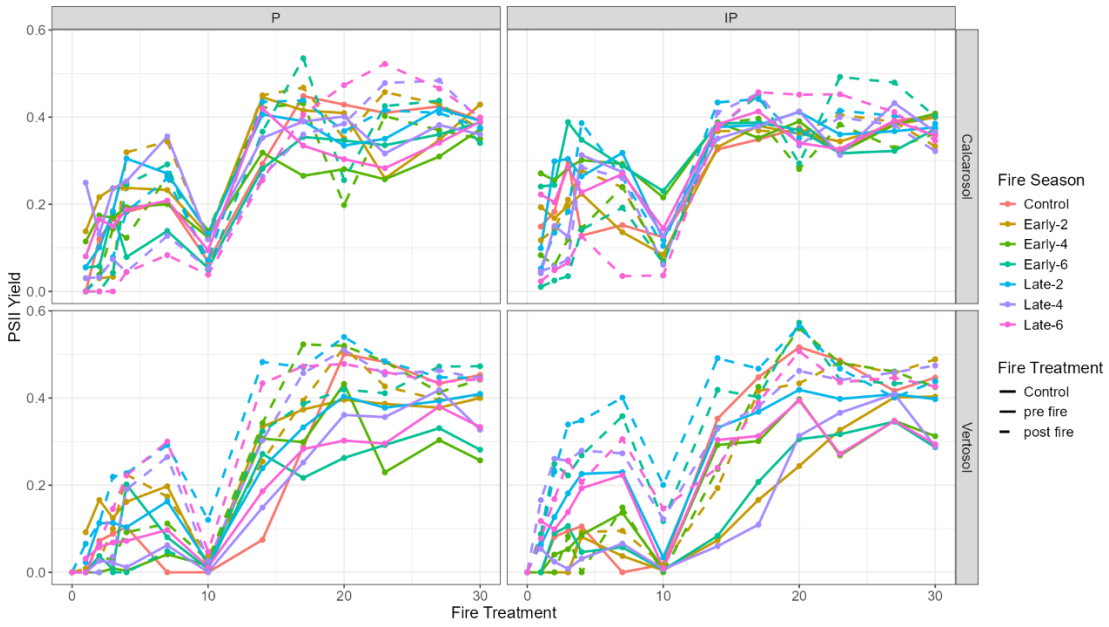
Unlike the calcarosol soil, we found a significant burning by fire interval interaction for surface cyanobacteria on the vertosol ($F_{2,22} = 15.0$, $P < 0.001$). This interaction showed that there was no significant difference in yield between pre-and post-fire for the 2-year burn, but significantly higher yield post-fire for both the 4- and 6-year burns.

Finally for the subsurface cyanobacteria on the vertosol we detected a significant burning by fire interval by season effect (F2,22 = 5.43, P = 0.003). Our analyses showed that under early burning, there was significantly greater yield post-fire under the 2-year fire interval, but not 4- or 6-year intervals. For the late burning, greater yield post-fire occurred only at 4 years, with no effects at either 2 or 6 years.

Figure 5.1.1 (a) Average photosynthetic yield for both pre-fire and post-fire biocrusts from calcarosol and vertosol soils tracked over 30 days. PSII yield values for cyanobacteria range between 0 and 0.6 and to 1.0 for plants (b) spread of recovery for all treatments across 30 days.

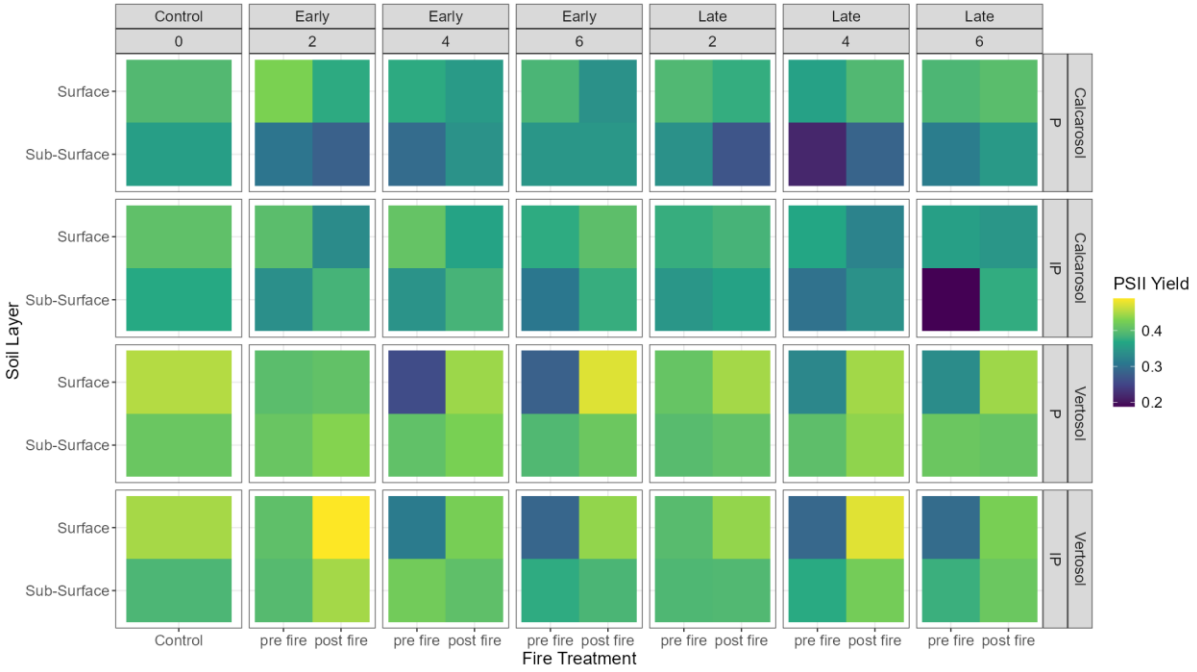


(a)



(b)

Figure 5.1.2 Heatmap for mean surface and subsurface photosynthetic yields (PSII Yield) at 30 days. This included patch (P) and interpatch (IP) and all treatments (controls, Early-2, Early-4, Early-6, Late-2, Late-4 and Late-6 yearly fire intervals). This covers pre-fire and post-fire biocrusts from calcarosol and vertosol soils at 30 days. PSII yield values for cyanobacteria range between 0 and 0.6 and up to 1.0 for plants.



The end yield (active photosynthesis) was in generally higher in the subsurface calcarosol and higher in the surface of the vertosol soil (depth 1-2 cm). Overall, the vertosol soil was functionally more productive than the calcarosol. (Fig. 5.1.3)

In the calcarosol there were no significant differences in the surface photosynthetic yields (Fig. 5.1.3) between treatments. In the subsurface before fire there were no significant differences between early dry season burning and late dry season burning (hotter fires). However, post-burning there were significantly higher photosynthetic yield in four and six yearly burns ($p < 0.05$) compared to other treatments, except for the two-yearly burns. After fire, there were no effects of interval or season of burn on post fire photosynthetic yields.

In the vertosol, pre-fire, there were no significant differences in photosynthetic yields between early and late burns for 2 yearly burn intervals, but they were significantly higher with late burns at 4 and 6 yearly burn intervals. Post-fire there were no effects of interval or season of burn on photosynthetic yields. Pre-fire, there was significantly greater PSII yield in the early burn, but only

at 4 yearly burn intervals. Post-burn, there was significantly greater PSII yield in the early burn, but only at two yearly burn intervals (Fig. 5.4.2).

5.1.1 Effect of microsite

Although we could not statistically analyse differences between soil types, the temporal trend in PSII yield up to 30 days was similar for pre-and post-fire and for patches and interspaces in the red soil (Fig. 5.1.1). Over 30 days, for both soil types we found no difference in PSII yield in the biocrusts for either calcarosol or vertosol soils ($P > 0.22$, (Fig, 5.1.1). However, for subsoils, we found greater yield in the inter-patches on calcarosol soils for early season burns ($F_{1,226} = 5.6$, $P = 0.019$). For vertosol subsurface soils, we found greater yields in the patches, but only for late season burns ($F_{1,226} = 4.62$, $P = 0.033$).

5.2 Effects of fire on biocrust nitrogen and carbon at VRSS

5.2.1 Calcarosol

In the calcarosol for the top cm of soil, it was interesting that the Control sites (unburnt over time) had the lower amounts of total nitrogen (TN) (Fig. 5.2.1). Similarly for plant available nitrogen (PAN), there was generally lower PAN in the unburnt Controls (Fig. 5.2.2). It is likely that TN would be leaching to greater depths due to an increase in trees, shrubs together with greater litter fall and incorporation at the unburnt sites.

Before fire, in both the biocrust under grass patches (P) and in exposed inter-patches (IP) TN was at the highest level in the early and late 2-year plots. TN trended down in early 4- and 6-years, closest to the unburnt control values that were generally less than burnt treatments. This was reversed post-fire with TN trending up in the early fires with more TN in early 6-yearly burnt in the patches, but trending down in the early burnt inter-patches. Overall, the late fires were lower in TN. Comparably, PAN also trended down with less frequent fire, especially in the late fires, although results were quite variable across the plots (Fig. 5.2.2)

In summary, for calcarosol soils, late fires (both before and after fire) trended down in TN and PAN compared to early burnt sites where post-fire the 4- and 6-yearly burnt sites had the greater increases in TN compared to other treatments (Fig. 5.2.2). Post-fire PAN was significantly higher in the 6-year early burnt biocrust under grass patches compared to all other treatments (Fig 5.2.2). This

is likely due to the higher concentrations of biocrust (Fig. 5.2.) as it was left for a longer time between fire disturbances.

Figure 5.2.1 Calcarosol: Total Nitrogen (TN) Mean (±SE) for unburnt controls, 2,4,6 early and late fires, pre- and post-fire. Samples were taken from patches, biocrusts under grass plants, and inter-patches, biocrusts on the exposed soil surfaces.

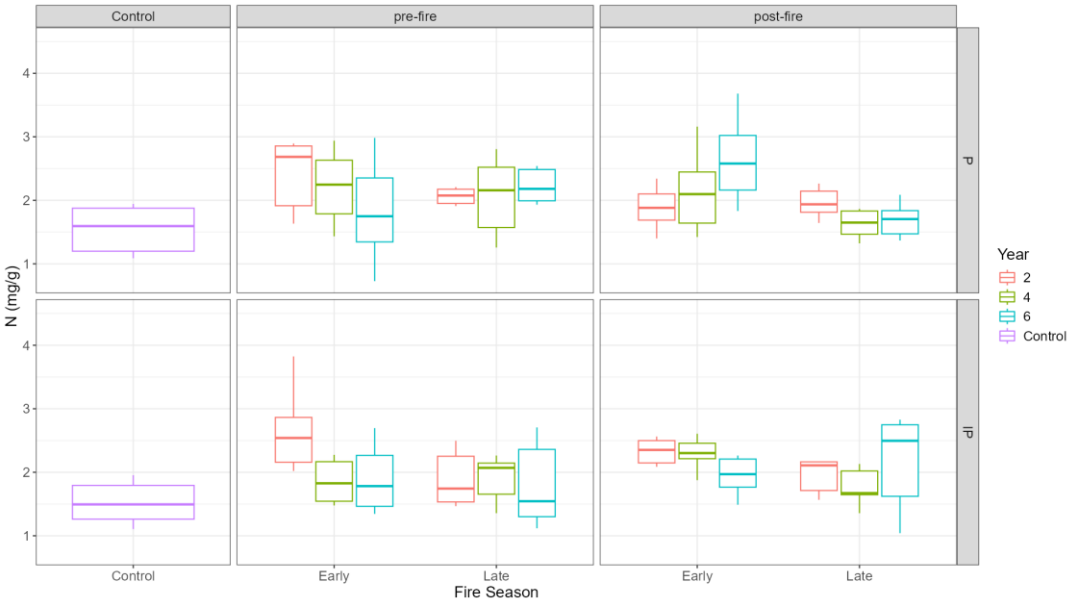
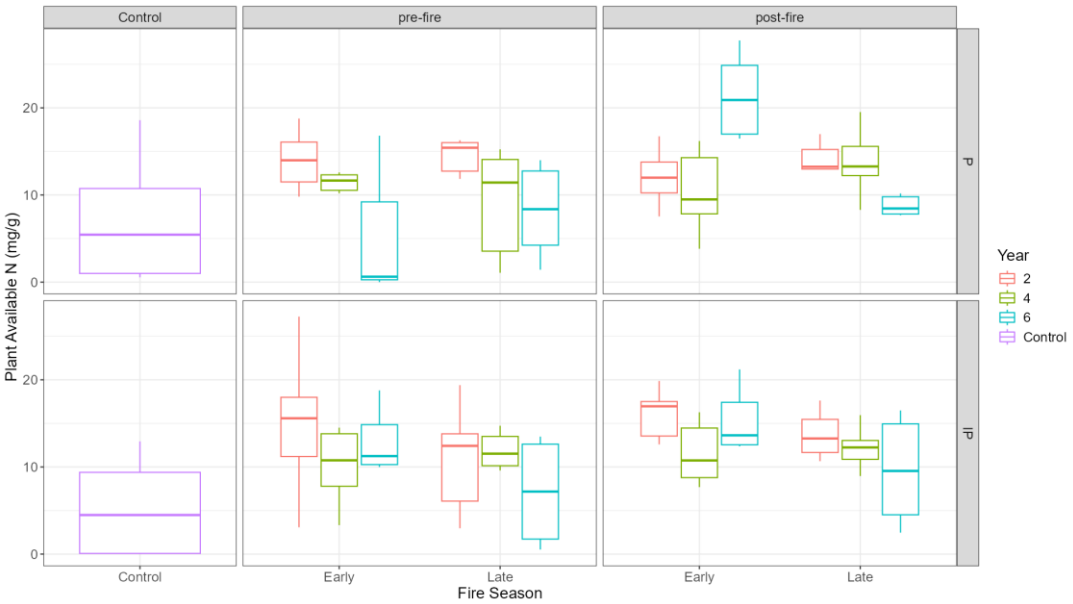
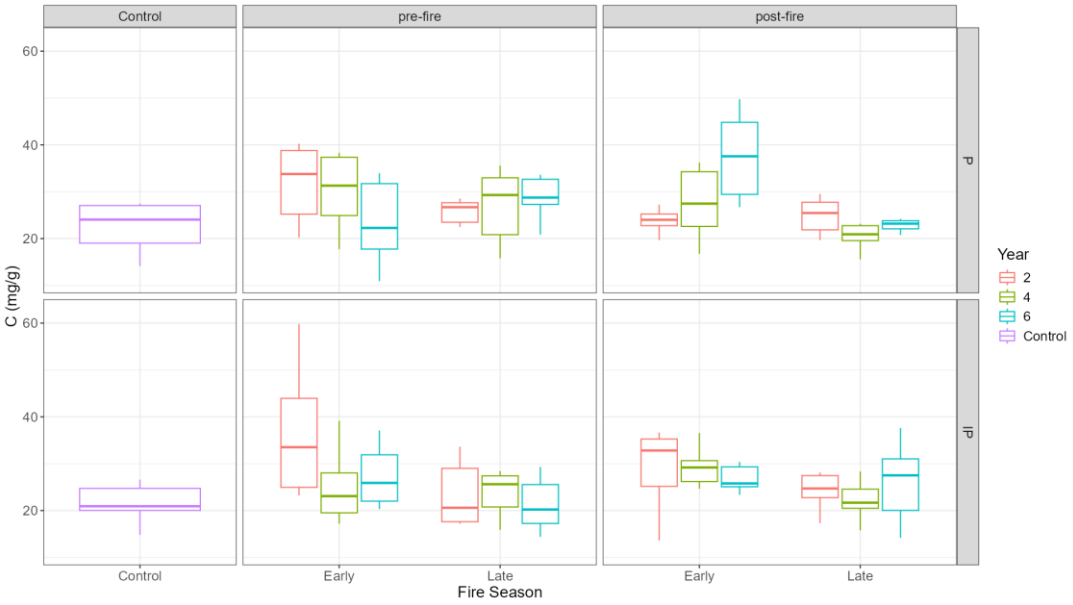


Figure 5.2.2 Calcarosol: Plant Available Nitrogen (PAN) Mean (±SE) for unburnt controls, 2, 4, 6 early and late fires, pre- and post-fire. Samples were taken from patches, biocrusts under grass plants, and inter-patches, biocrusts on the exposed soil surfaces.



In the calcarosol total carbon (TC) trended lower before burning (P) and higher after early fires (P). Overall TC was trending higher in the early fires post-fire (Fig. 5.2.3) and showed more potential to incorporate TC in early fires in the grass patches.

Figure 5.2.3 Calcarosol: Total carbon production from biocrusts in from underneath grass (P) and open spaces between grass plants (IP) before and after fire plus an unburnt control.



5.2.2 Vertosol

In the vertosol soils, mean TN did not appear to differ across plots and treatments although there was a wide spread of results especially in the pre-fire plots (Fig. 5.2.4). Mean values of TN in burnt treatments were similar to the unburnt Controls (Fig. 5.2.4). The early and late 4-year fires in the biocrust under grass patches seemed likely to have the greater potential to trend in an upwards direction compared to inter-patches.

There were higher levels of TC in the early 2-year burns after fire in the biocrusts under the grasses (Fig. 5.2.5). For all other treatments the general trend post-fire was lower TC as years between fire increased especially in the early post-fire under grass and late post-fire in the open bare ground. Overall, TC was highly variable across all treatments and microsites. TC processes are likely to be more stable at depth compared to the soil surface.

Figure 5.2.4 Vertosol soils: Total Nitrogen (TN) Mean (±SE) for unburnt controls, 2,4,6 early and late fires, pre- and post-fire. Samples were taken from patches, biocrusts under grass plants, and inter-patches, biocrusts on the exposed soil surfaces.

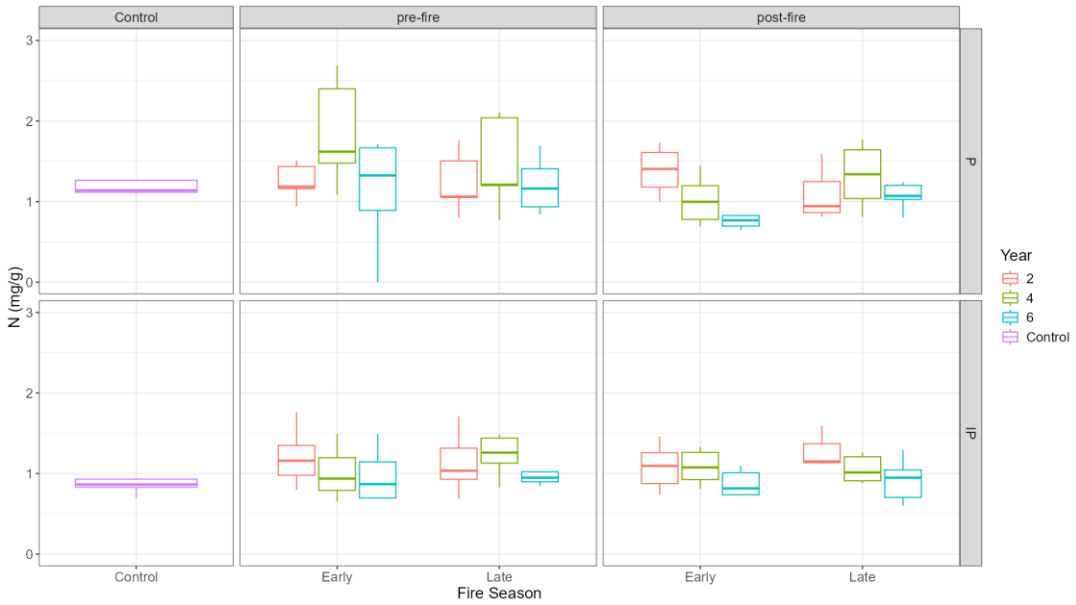


Figure 5.2.5 Vertosol: Total Carbon production from biocrusts in from underneath grass (P) and open spaces between grass plants (IP) before and after fire plus an unburnt control.

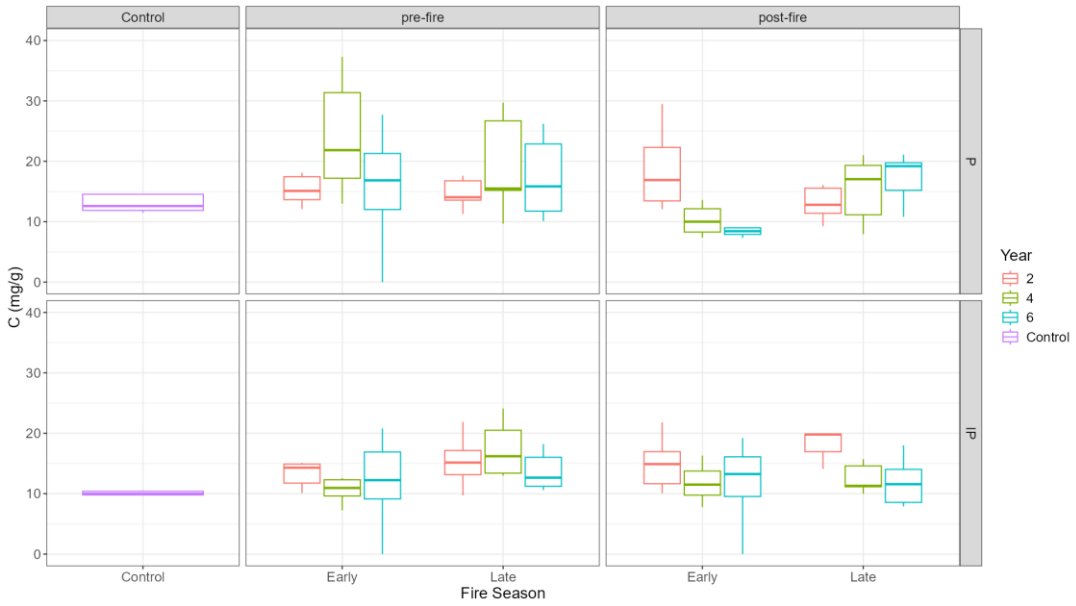
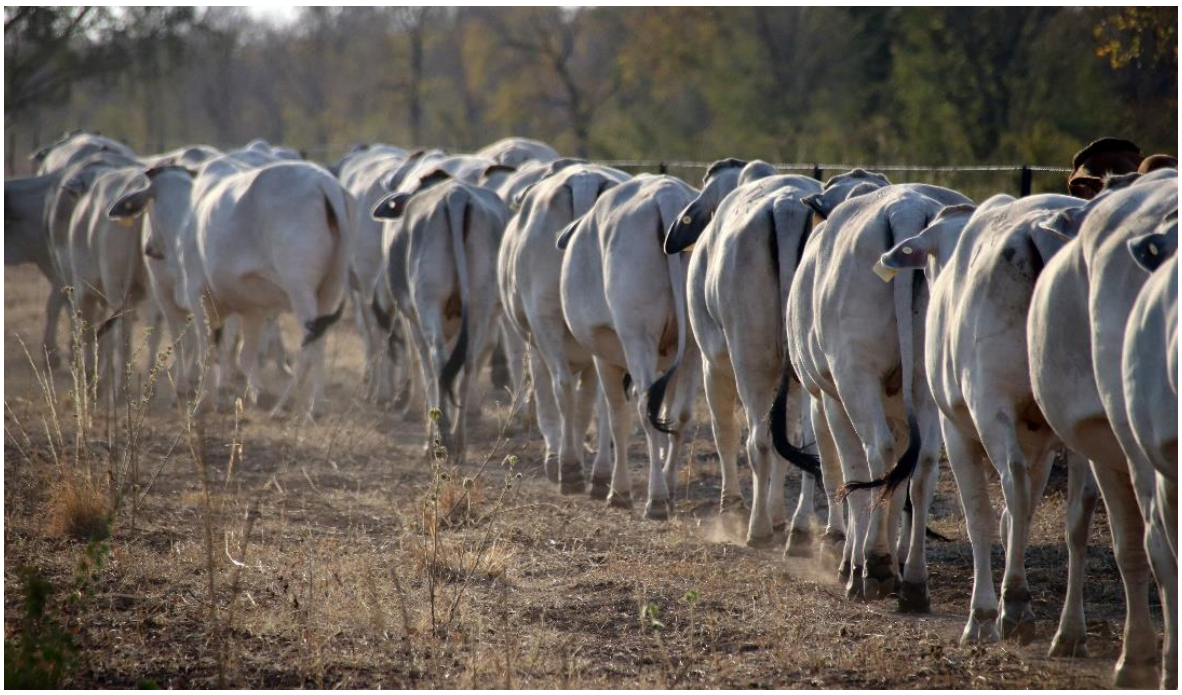


Figure 5.2.6 Resting from grazing post-fire for the first wet seasons provides a period of time with no disturbance where biocrusts can regrow. (Photo J. Eastaughffe, VRRS).



6 Wet season resting from grazing boosts biocrust hotspots

Excerpts from (Williams et al., 2021))

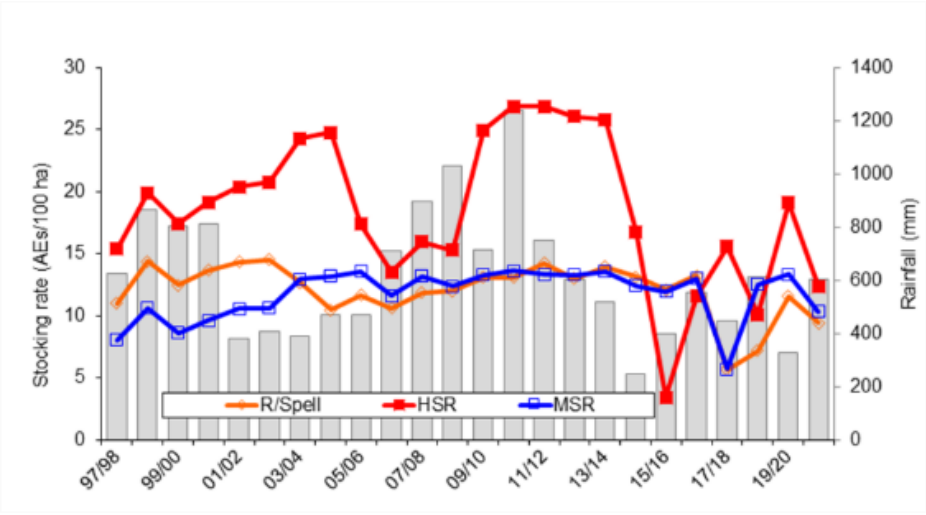
This study was published as scientific journal article, with full article provided as a PDF in Appendix 3.

6.0.1 Field methods

Each long-term grazing trial paddock has several permanent one-hectare monitoring sites consisting of five 100 m transects 20 m apart. Sites are stratified by soil type. Sampling was conducted in November 2020, following a season of well below average rainfall (384 mm) and a succession of five drought years, with 2014/15 the fourth driest year on record. Stocking rates against rainfall are shown in Fig. 3.6.1. We used the two replicate paddocks for each of the three treatments.

On each of the two soil types we selected one monitoring site. Here we selected two transects (50 m apart), then laid out a 30 m tape in the same direction as the 100 m transect. Alongside these 30 m lines, a 1 m^2 quadrat was placed at 6 m intervals (Fig. 3.6.2). There were two soil types of duplex soils (DC), and red-yellow earths (RY), two paddocks selected for each treatment (HSR, MSR, R/Spell, and XCL), two transects per paddock and, six quadrats per transect. Exclosure (XCL) treatments were fenced areas within these paddocks with no access for stock. In total, 24 quadrats per treatment per soil type were assessed.

Figure 6.0.1 Stocking rates expressed as adult equivalents (AE) per 100 ha for rotational/spell (R/Spell), heavy stocking (HSR) and moderate stocking (MSR) against rainfall records at the Wambiana grazing trial (Williams et al. 2022).



6.0.2 Landscape function

Landscape Function Analysis (LFA) has been developed to establish soil surface indicators for measuring and analysing the nature and severity of problems in a dysfunctional or degraded ecosystem. The conceptual framework is based on the spatial organisation of the clumps of grasses and shrubs that capture, accumulate, and retain resources (called patches). The interspaces (or inter-patches) are the open areas between the grass patches and can be natural 'hotspots' for biocrusts, due to less competition for light, moisture, and litter.

In this study, we focused on the role of these biocrust hotspots in determining the three LFA indices: stability, infiltration, and nutrient cycling. These three indices are assessed by 11 soil surface indicators (Fig. 3.6.2) that are individually scored and provides the percentage level of each index. The indices are a relative measure and are independent of each other. In this study we assumed the exclosures with no cattle grazing would be a benchmark for the best condition. The higher the index the better the condition. The LFA complete soil surface assessment (SSA) data sheet and detailed methodology is in the LFA manual. Our aim was to compare the different management strategies for each of the three indices that were representative of ecosystem function with a focus on the interspaces.

For each quadrat, the LFA attributes were recorded and ranked. Later they were separated into their dominant category: patches or interspaces. Only the interspaces were used in the data analysis and separately analysed as either biocrust dominant (cover >10% based on LFA category assessments) or bare soil dominant (where biocrust cover <10%). For each treatment and soil type there were at least five quadrat replicates used in the analysis. Quadrats that matched the criteria were analysed on separate Soil Surface Assessment (SSA) XL worksheets in the LFA program.

6.0.3 Ground cover

Ground cover was measured in each 1 x 1 m quadrat (Fig. 3.6.2) in two ways, firstly the overall grass cover was recorded. C. ovata patches were identified separately. This was followed by estimating the ground-level cover for each component as a total percentage of what was found within each quadrat. These categories comprised: biocrust, bare soil, basal area of grass plants, and litter cover and equalled 100%.

Figure 6.0.2 Box woodland transect on duplex soils (DC) with (a) heavy stocking (HSR) and (b) exclosure (XCL) no stock; Ironbark woodland on red-yellow earths (RY) with (c) HSR and (d) XCL (Williams et al., 2022).

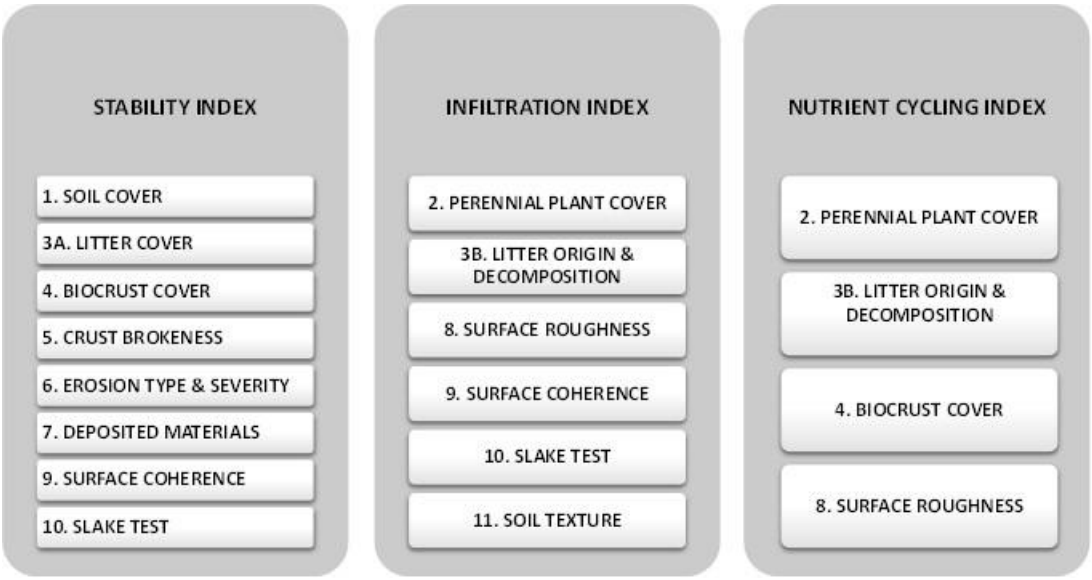
(a)

(b)

(c)

(d)

Figure 6.0.3 LFA indices and the different attributes measured that contribute to each one (Williams et al., 2022).



6.0.4 Statistical analysis

We examined the differences in biocrusts, bare soil, basal grass area and litter cover across all treatments using ANOVAs (Minitab V20, [23]) and applied Tukey's method to identify significant differences between treatments. To establish the three LFA indices for all quadrats, we processed the attributes in the LFA XL software, available online accompanying the manual [12] and detailed in Section 2.2.1. Once the indices had been calculated, we examined the differences in a General Linear Model with fixed factors to look at the effect on the three stocking levels for each variable. We then used Tukey's pairwise comparison tests to determine where significant differences occurred between treatments.

The above excerpts are from Williams et al., (2021).

6.1 Results: Wet season resting boosts biocrust hotspots

Results have been published in Williams et al., 2022 (see full paper in Appendix 3).

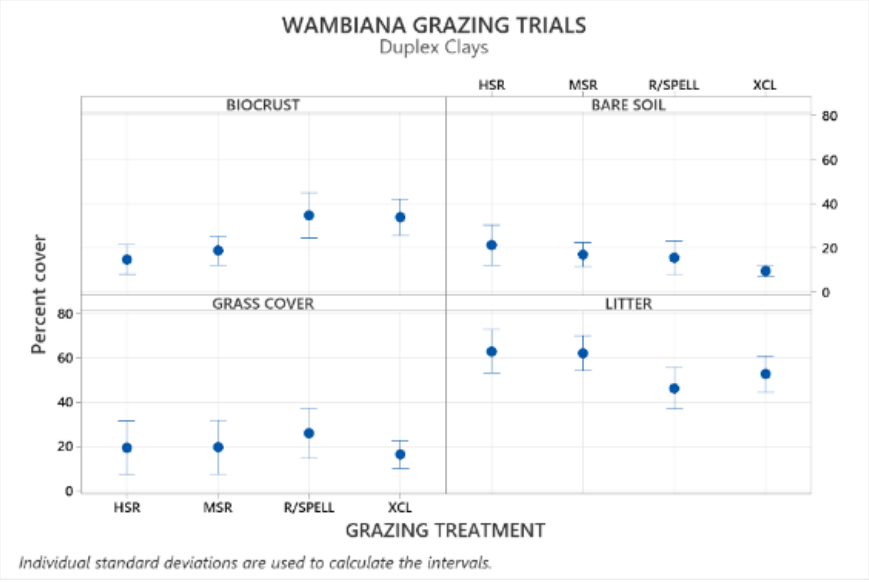
6.1.1 Biocrust hotspots in duplex soils

The biocrust cover was significantly higher in the exclosures (XCL), and the rotational spelled paddocks (R/Spell) compared to the heavily (HSR) and moderately (MSR) stocked paddocks ($p<0.001$). Biocrust cover across the XCL and R/Spell averaged ~34%, about double that of both MSR (18.7%) and HSR (14.6%) (Fig. 6.5.1).

In-paddock observations, followed by scanning electron microscopy, showed the well-developed and cyanobacterial dominated biocrusts in the XCL and R/Spell treatments compared to HSR treatments that were almost completely devoid of biocrust (refer to Fig. 6.5.1).

Bare ground cover was significantly lower in the exclosures (9.6%) compared with HSR ($p=0.03$, 21.3%) but were similar between XCL, MSR and R/Spell (9.6–16.9%). There were no significant differences between treatments for grass/shrub or litter cover (Fig. 6.5.1).

Figure 6.1.1 Duplex soils with comparisons of mean values ± SD for grass cover (included *Carissa sp.*), and ground cover: biocrusts, bare soil (no visible biocrust), litter and basal grass area that together make up 100% of the quadrats, at different stocking levels: High stocking rates (HSR), Moderate stocking rates (MSR), Rotational Spelling at Moderate stocking rates (R/Spell) and, No stock, Exclosures (XCL).



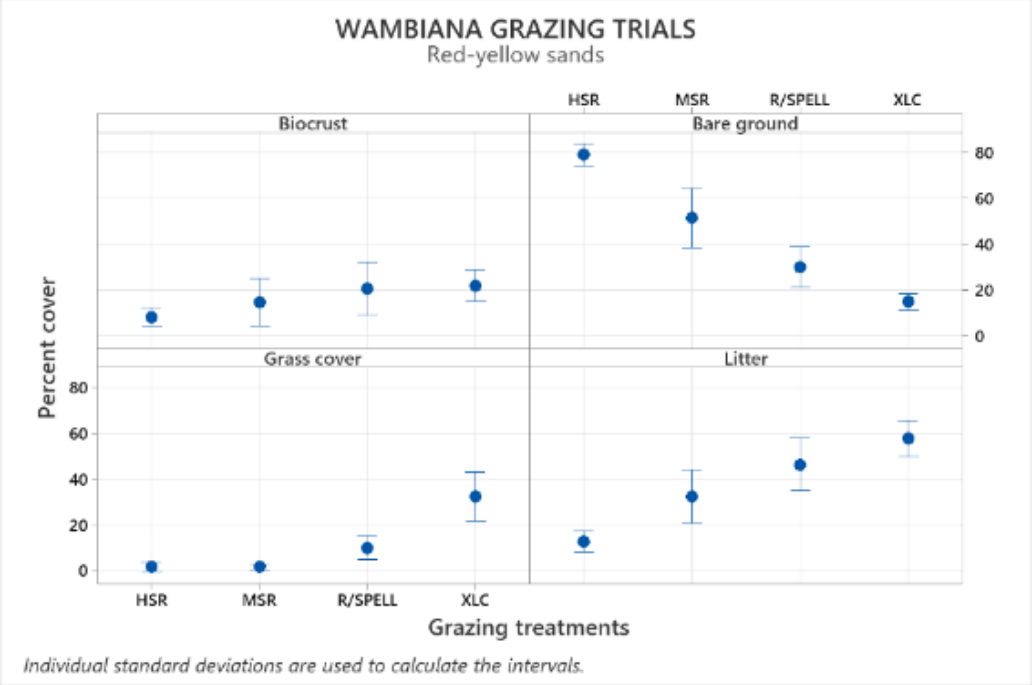
6.1.2 Biocrusts in red-yellow earths

The red-yellow earths (RY) did not significantly differ in their biocrust cover across grazing treatments; however, the bare ground in the heavily grazed paddocks was up to 2.5 times higher than the XCL, R/Spell and MSR ($p<0.001$). Overall, the various treatments were significantly different from each other where the bare ground cover (mean % ±SD) in the XCL was the lowest (14.8 ±11.75) and R/Spell (29.9 ±20.7) compared to the HSR (79 ±11.5) and MSR (51.4 ±30.9), (Fig. 6.5.2).

Observation in the paddock showed that the biocrusts on the RY soils were often thin and fragile and easily broken. We followed up with SEM and confirmed cyanobacterial dominated biocrusts in the XCL, and HSR were almost devoid of biocrust (Fig. 6.5.2).

Grass and litter cover were both significantly different across the grazing treatments $p<0.001$). Although grass cover (mean % ±SD) in the XCL was by far the highest (32.4 ±34.3), this was variable. However, HSR and MSR were similar (1.6 ±5.1 and 1.6 ±2.4% respectively) while R/Spell grass cover was 9.9 ±12.3%, highly variable and statistically similar to HSR and MSR. Litter cover ranged from 57.7% (XCL) to 12.7% (HSR), with a significant difference between the XCL and R/Spell ($p<0.001$), however, these were significantly different from MSR and HSR (Fig. 6.5.2).

Figure 6.1.2 Red-yellow earths with comparisons of mean values ± SD for grass cover, and ground cover: biocrusts, bare soil (no visible biocrust), litter and basal grass area that together make up 100% of the quadrats, at different stocking levels: High stocking rates (HSR), Moderate stocking rates (MSR), Rotational Spelling at Moderate stocking rates (R/Spell) and, No stock, Exclosures (XCL).



6.1.3 Landscape function across interspaces

Across the interspaces, all LFA indices were negatively affected by HSR management strategies, which had the lowest percentage indices for stability, infiltration, and nutrient cycling (Table 6.5.1). However, there were varied differences between the LFA indices across all treatments, particularly in the RY soil types (Fig. 6.5.2), especially in HSR that was dominated by >80% bare soil, and very low levels of biocrusts (Fig. 6.1.2). Although the Rotational Spelling (R/Spell) had the highest average levels of landscape function of all the grazed treatments, due to the high variance, especially in the RY soil type, there were no significant differences, and it was not included in the overall analysis (Table 6.5.1).

Table 6.1.1 LFA Indices across all treatments (Mean % ± SE). DC Duplex soil, RY, red-yellow soil. HSR heavy stocking rate, MSR moderate stocking rate, R/Spell rotational spelling (paddock resting during wet season) with MSR moderate stocking rate, and XCL exclosure.

Variable	Soil	HSR	MSR	R/SPELL	XCL
Stability	DC	65.0 ± 3.39	65.1 ± 1.64	65.1 ± 1.21	66.9 ± 1.24
	RY	53.8 ± 3.88	59.8 ± 1.94	62.1 ± 3.66	68.5 ± 2.24
Infiltration	DC	31.6 ± 0.76	34.8 ± 0.99	36.8 ± 0.82	37.1 ± 1.17
	RY	28.2 ± 1.93	32.3 ± 4.25	38.3 ± 3.89	40.6 ± 1.49
Nutrients	DC	31.8 ± 1.8	33.9 ± 3.38	35.6 ± 0.97	35.5 ± 1.86
	RY	19.4 ± 0.94	27.5 ± 4.11	33.8 ± 4.67	36.7 ± 1.55

6.1.4 Stability

The duplex soils (DC) and red-yellow earths (RY) that dominated the Box and Ironbark woodlands differed in their structure (Aspandiar et al., 2003), and the stability of the interspaces was significantly different ($p= 0.04$). In the DC soils, the exclosures (XCL) had significantly higher stability compared to the HSR paddocks ($p= 0.003$), and although not significant, XCL was somewhat different to MSR ($p= 0.06$). RY soil stability indices (mean %) had the widest ranges between 53.8% (RY, HSR), and 68.5% (XCL) (Table 6.5.1, Fig. 6.5.3).

6.1.5 Infiltration

DC and RY soils did not significantly differ in their infiltration indices ($p= 0.89$) (Fig. 6.5.3), however XCL had significantly higher infiltration compared to HSR ($p< 0.001$), and MSR ($p= 0.009$) (Fig. 6.5.4). RY infiltration indices (mean %) also showed the widest range between 28.2% for the HSR and 40.6% for the XCL (Table 6.5.1).

6.1.6 Nutrient cycling

Nutrient cycling across the interspaces was significantly different between the DC and RY soil types ($p= 0.05$) (Fig. 6.5.3). The XCL had significantly higher nutrient cycling levels than both for the HSR paddocks ($p< 0.001$), and for the MSR paddocks ($p= 0.03$) over both soil types. Yet, due to the high variability in DC soils (Table 6.5.1), HSR and MSR were not significantly different ($p= 0.13$). High litter levels in DC likely contributed to this (Fig. 6.1.3). Nutrient cycling indices in the RY soils also widely differed (mean %) from 19.4% (HSR) to 36.7% (XCL) (Table 6.5.1).

Figure 6.1.3 Overall results for LFA indices for heavy (HSR), moderate (MSR) rotational spelling (R/Spell) and exclosure (XCL) stock treatments at the paddock scale. Significant differences marked with * or NS for not significant.

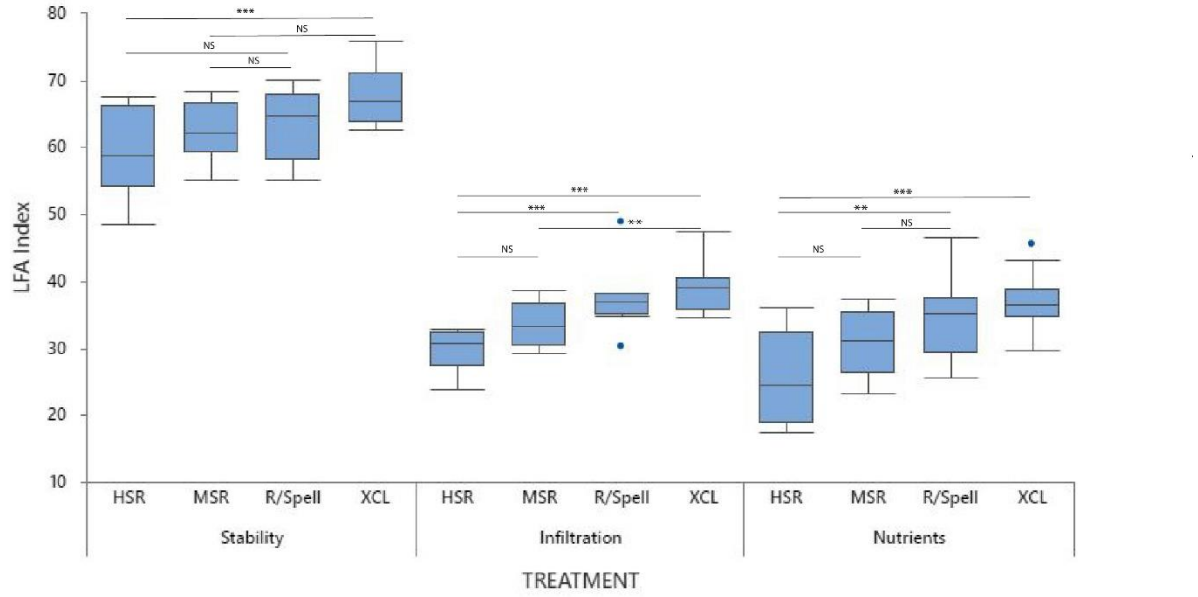
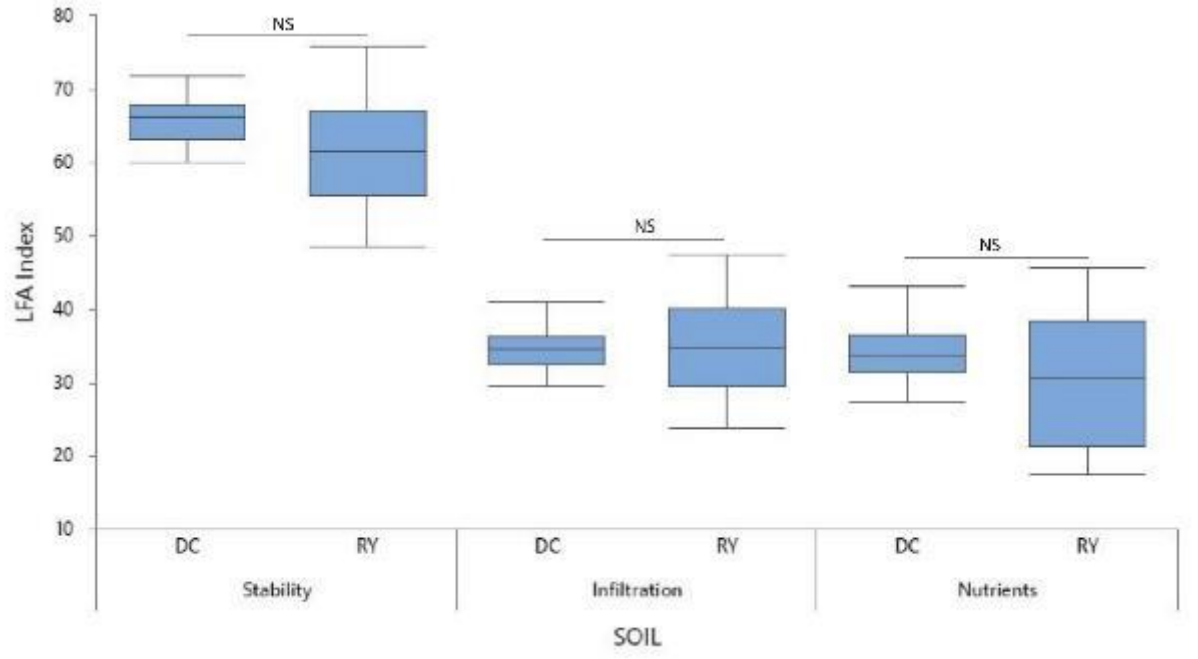


Figure 6.1.4 Overall results for LFA indices for both soil types, duplex clays and red yellow earth at the paddock scale. Significant differences marked with * or NS for not significant.

.



7 Metagenomic insights into biocrust function

7.0.1 Shotgun sequencing and gene-centric analysis of biocrust

Most studies limit microbial analyses to profiles of the relative abundances of taxa present. This is typically achieved by PCR amplifying and sequencing a 'barcode' gene (e.g., 16S) present in all members of the target group (e.g., bacteria). While this approach is useful for measuring biodiversity, its exclusive focus on the 'barcode' gene means that information about the other genes within the organisms present is ignored. New methods (i.e., metagenomics) now make it possible to sequence all of the genetic information present in a DNA sample, which means that we can generate not only profiles of who's there and at what abundance, but also which functions may be possible based on the genes present. For example, to assemble the nitrogenase enzyme complex, which mediates N fixation, microbes require a range of *nif*, *anf*, or *vnf* genes. Hence, if the relative abundance of these genes' changes in response to a treatment, this may indicate a shift in the capacity for the microbial communities to perform that function. For example, the presence of the *nif* genes indicates that a biocrust community, may fix nitrogen, i.e., convert atmospheric dinitrogen gas into ammonia, which is a plant-available form of nitrogen (Fig. 1.6.1). We can infer from the relative frequencies of such genes how the microbial community is able to fix nitrogen. We expect fewerof these genes in a degraded community and more useful genes in a healthy biocrust with, for example, more capacity to fix nitrogen. As microbes respond very quickly to environmental changes, we can detect changes in response to season and rangeland management.

Here, we performed shotgun sequencing of DNA using the Illumina NovaSeq platform. We used SingleM (Woodcroft et al., 2024) to generate profiles of the relative abundances of bacterial species present in each sample and customised GraftM (Boyd et al., 2018) packages generated in CI Dennis' lab for 57 nitrogen cycling genes and a range of other processes. (Fig 7.0.1).

We then analysed the relative frequencies of bacterial species and nitrogen cycling genes relative to the experimental treatments (Fig 7.0.2).

Genes for nitrogen fixation are only found in specialised diazotrophic bacteria which, in biocrusts, include photosynthesising cyanobacteria. Compared to other bacteria this is unique as they can fix both carbon and nitrogen, and are, like green plants, primary producers. Other N-fixing bacteria can live within the cyanosphere (the external layers of polysaccharides that envelope cyanobacterial

cells) or associate with other organisms (green plants, fungi, other) as available carbon will fuel their N fixation.

Figure 7.0.1 A visual explanation of a shotgun metagenomic sequencing workflow (untargeted).

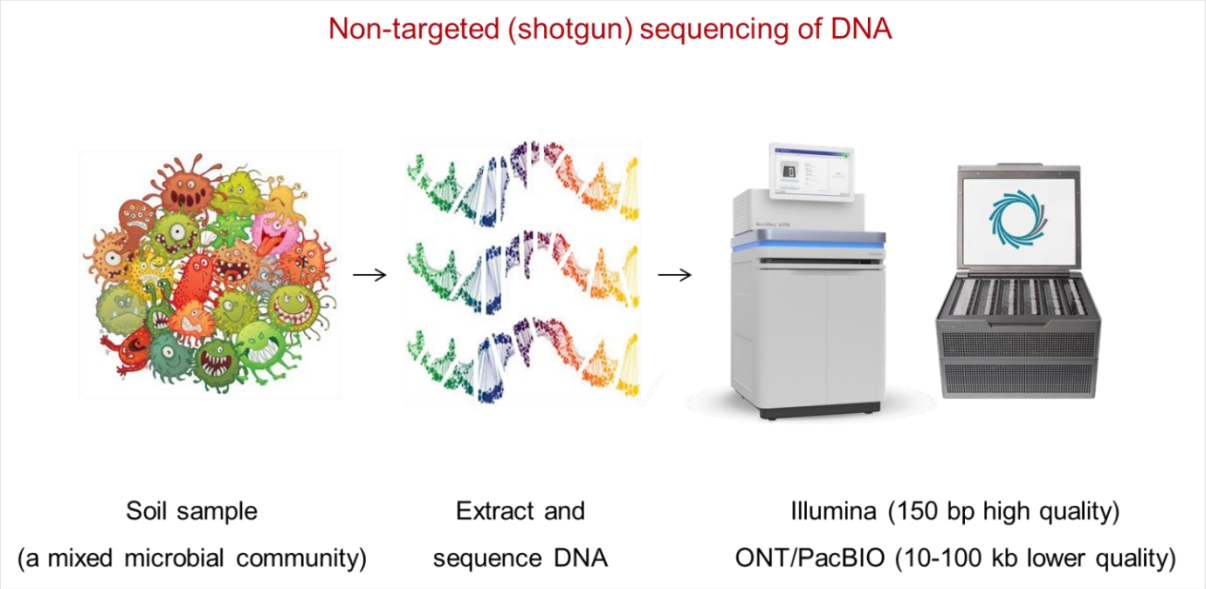
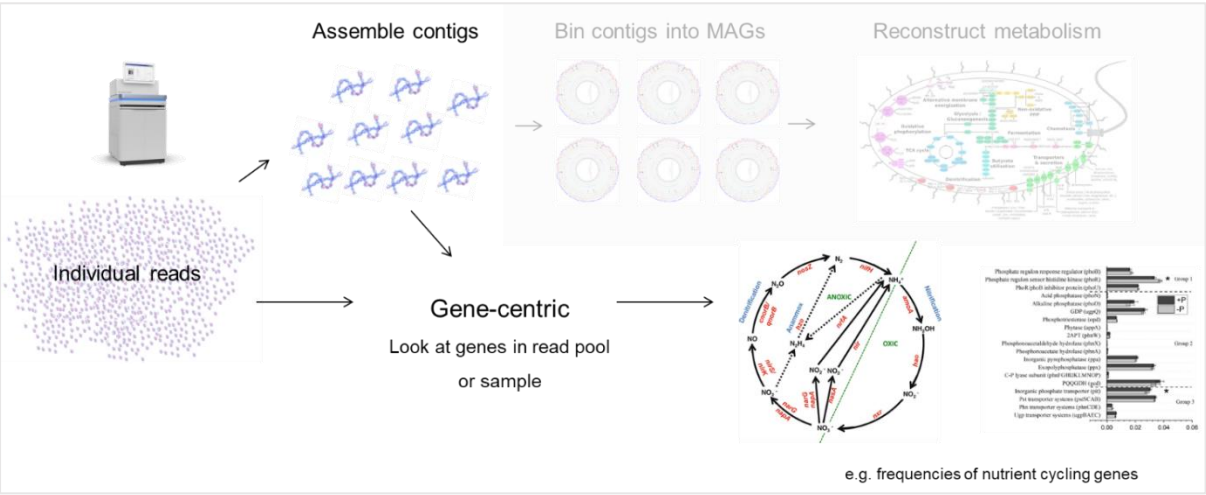


Figure 7.0.2 A visual explanation of a shotgun metagenomic sequencing workflow. This is called an untargeted approach as we obtain fragments or reads from all the DNA precent in a sample or microbial community (the rationale is explained in the paragraphs above), this way we can identify and quantify genes of interest, in our case, genes that are involved in the biogeochemical processes relevant to this research: nitrogen fixation, carbon fixation and metabolism of nitrogen.



7.0.2 Overall experimental design

Soil/biocrust samples were collected from the different microsites at the first centimetre of soil (Fig. 7.0.3). The microsites were identified as:

1) bare soil, no visible biocrust growth.

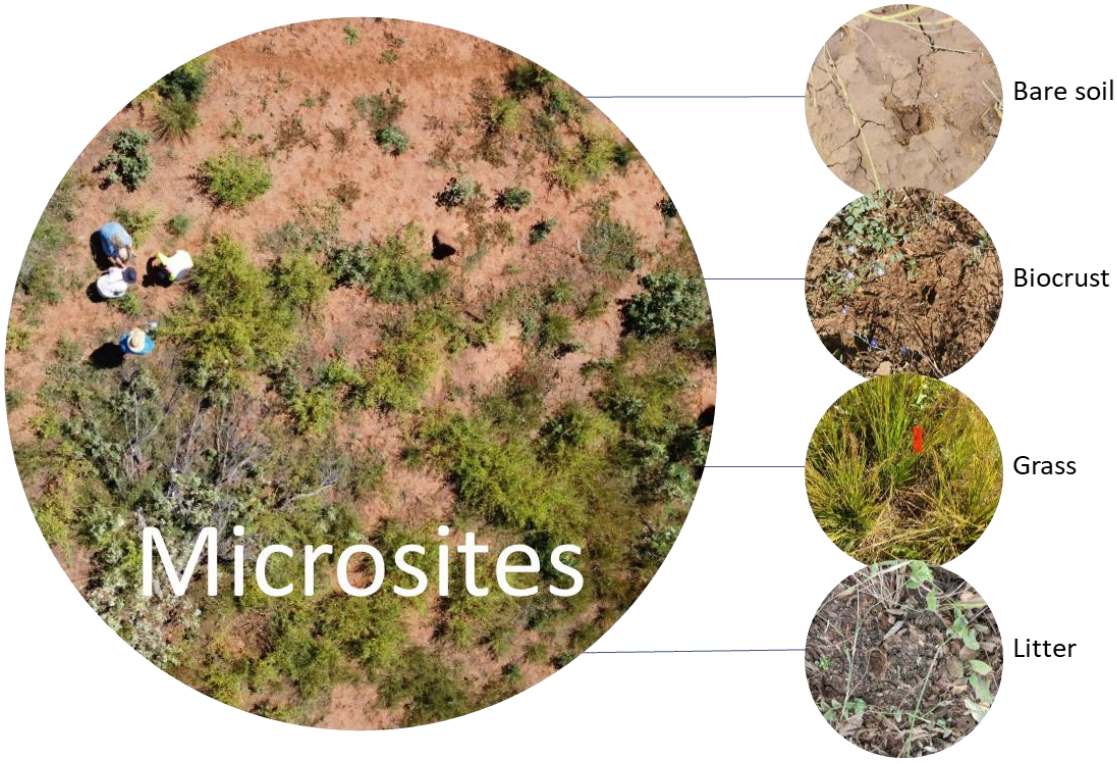
2) biocrust, visible biocrust growth in the interspaces.

3) grass, biocrust under grass canopy.

4) Litter, soil under perennial vegetation litter.

The first set of samples were collected in June 2021, in the dry season one-year post-fire and the second set of samples, in April 2022, late in the wet season. It should be noted that the wet season had extremely low rainfall and may have impacted some of the results.

Figure 7.0.3 Representative images of the landscape mosaic of microsites with bare soil, biocrust, grass and perennial vegetation litter. We identified these categories based on the landscape observation completed in this project as they enable proportioning a landscape into biocrust-relevant categories. Biocrust grows under litter and grass (and other herbaceous and woody vegetation) as well as in the open. In comparison with the bare soil, biocrust is supported by literature to reduce soil erosion in between other ecosystem services.



7.0.3 Biocrust function evaluation on fire and grazing trials

We used four different subsets of samples to understand possible changes in the microbial communities and their functions:

1) VRRS long-term fire regimes – Effects of fire regimes within biocrusts
   To understand the changes with fire: We used biocrust in the two main soil types, two seasons (dry and wet) and across the 7 fire regimes (treatments) at Kidman springs.

2) VRRS long-term fire regimes – Effects of microsites within Early 4-year fire regime
   To understand the changes in microsites: We used samples from a moderate fire regime, which is burnt early in the season at four yearly intervals (Early 4-years), to evaluate the effect of soil type, season (dry and wet) and microsites on nitrogen fixation genes.

3) Wambiana grazing trial - Effects of grazing intensities within biocrusts
   To understand the changes with grazing: We used biocrust in the two main soil types, two seasons (dry and wet) and across the four grazing intensities (treatments) at Wambiana.

4) Wambiana grazing trial – Effects of microsites within moderate grazing intensity with wet season spelling
   To understand the changes in microsites: We used samples from the current recommended grazing intensity or stocking rate, which is moderate stocking rate with wet season spelling, to evaluate the two main soil types, two seasons (dry and wet) and across the four microsites.

7.1 Results: Nitrogen processes within biocrusts

7.1.1 N cycle genes across microsites of the 'Early 4-year' fire treatment

Confirming the validity of our approach to analyse microsites rather than a 'whole of treatment site', we discovered that N cycle genes change significantly with soil, season, and microsites, with significant interactions between microsites and season (both sites, Table 7.1.1). In the vertosol,

microsite was the only significant determinant of N cycle genes, while in calcarosol, they are significantly influenced by season and microsite and the interactions.

Examining each of the functional groups of N cycle genes, we found that microsite within each soil type is the strongest determinant of gene presence (Table 7.1.1). In the vertosol biocrust, microsite was the only determinant of N cycle genes, whereas in calcarosol biocrust, season, microsite and their interactions were significant for all N cycle genes and highly significant for N fixation gene presence. This means that in calcarosol, the relative abundance of N fixation genes depends on season and microsites

Table 7.1.1 Results from PERMANOVA models summarising the main and interactive effects of soil (vertosol and calcarosol), microsites and season (dry and wet season) on the relative frequencies of genes associated with nitrogen cycle pathways (N fixation, Ammonification, Assimilation, Nitrification and Denitrification), as well as the effect of microsite and season within each soil separately.

Predictor	N cycle genes frequency			N fixation genes			Amonification genes			Assimilation genes			Nitrification genes			Denitrification genes		
	F value	R^2 value	*P* value	*F* value	R^2 value	*P* value	*F* value	R^2 value	*P* value	*F* value	R^2 value	*P* value	*F* value	R^2 value	*P* value	*F* value	R^2 value	*P* value
Both sites																		
Soil	11.859	0.165	0.001 ***	20.707	0.216	0.001 ***	0.608	0.013	0.591	6.266	0.085	0.005 **	25.466	0.277	0.001 ***	39.522	0.423	0.001 ***
Season	2.675	0.037	0.022 *	7.080	0.074	0.001 ***	0.799	0.017	0.443	2.590	0.035	0.109	3.757	0.041	0.032 *	1.236	0.013	0.297
Microsite	4.280	0.179	0.001 ***	5.684	0.178	0.001 ***	2.021	0.125	0.057 .	7.447	0.304	0.001 ***	3.733	0.122	0.003 **	2.518	0.081	0.027 *
Soil:Season	1.305	0.018	0.214	1.788	0.019	0.145	0.704	0.015	0.513	1.046	0.014	0.299	0.397	0.004	0.743	0.586	0.006	0.601
Soil:Microsite	0.939	0.039	0.543	1.621	0.051	0.115	0.531	0.033	0.849	0.601	0.025	0.695	1.820	0.059	0.106	1.377	0.044	0.220
Season:Microsite	1.823	0.076	0.022 *	3.041	0.095	0.005 **	1.301	0.081	0.267	1.597	0.065	0.173	3.129	0.102	0.012 *	1.980	0.064	0.083 .
Soil:Season:Microsite	0.966	0.040	0.471	1.124	0.035	0.341	0.895	0.056	0.471	0.853	0.035	0.487	1.383	0.045	0.206	0.816	0.026	0.583
Within vertosol																		
Season	1.28	0.042	0.242	3.25	0.091	0.053 .	0.30	0.011	0.919	1.58	0.047	0.192	1.19	0.033	0.322	0.51	0.018	0.723
Microsite	3.11	0.306	0.001 ***	3.88	0.325	0.003 **	1.95	0.219	0.057 .	4.78	0.429	0.008 **	3.17	0.263	0.007 **	2.73	0.293	0.005 **
Season:Microsite	1.29	0.127	0.194	1.65	0.138	0.153	1.51	0.170	0.144	0.50	0.045	0.796	3.15	0.261	0.012 *	1.07	0.115	0.433
Within calcarosol																		
Season	3.57	0.116	0.004 **	8.76	0.200	0.001 ***	0.91	0.040	0.422	2.11	0.062	0.148	4.48	0.154	0.010 **	1.74	0.063	0.150
Microsite	2.16	0.209	0.005 **	3.16	0.216	0.001 ***	1.04	0.137	0.387	3.52	0.314	0.015 *	2.07	0.213	0.053 .	1.22	0.133	0.268
Season:Microsite	1.62	0.157	0.048 *	3.21	0.219	0.001 ***	0.93	0.123	0.494	1.68	0.150	0.166	0.82	0.084	0.633	2.08	0.226	0.046 *

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Grouping the analyses of N cycling genes into the four microsites at the 'early 4-year fire treatment' demonstrates that the relative abundance of N fixing genes was largest in biocrust microsites for both soils in the wet season (Fig. 7.1.1). The bare soil microsite had the most variable relative abundance of N fixing genes, which may relate to the fact that 'bare' can span very little or considerable biocrust organisms present. Biocrusts under grasses had an intermediate relative abundance of N fixing genes, while under litter, biocrusts had the lowest relative abundance of N fixing genes, likely because of light limitation hindering energy inputs. The continuity of N fixing genes across the landscape underpins the importance of biocrusts in terms of a multifunctional suite of cyanobacteria, bacteria and others that is central to N-enrichment, especially over the course of the wet season (Table 7.1.2) (Williams et al., 2017).

These findings are highly relevant because they confirm that biocrusts have the capacity to generate N but that this capacity differs between microsites (Figure 7.1.2). Both, photosynthetic genes and N fixation genes point in the same direction: the more actively biocrusts fix carbon and generate the fuel for N fixation, the more N fixation occurs.

Figure 7.1.1 Soil type (vertosol, calcarosol) and microsites are key in providing ecosystem services to the plants through the benefits of the bacterial genetic function of the biocrust. The darker squares show increased N-fixation during the wet season associated with the biocrust sampled from the top 1 cm of the soil surface. This heatmap has been row-scaled to show the relative abundance of each respective gene across microsites. White-blue shading represents the frequency per million bacterial reads as the sum of the square mean gene frequency values across metabolic pathways.

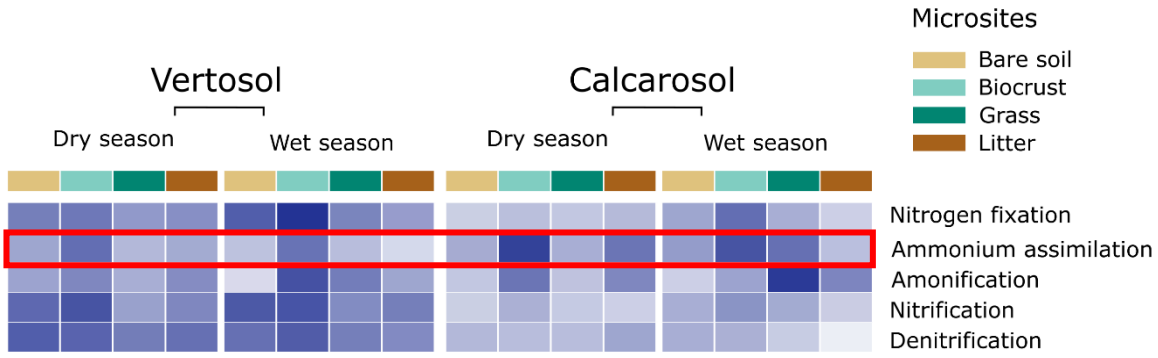
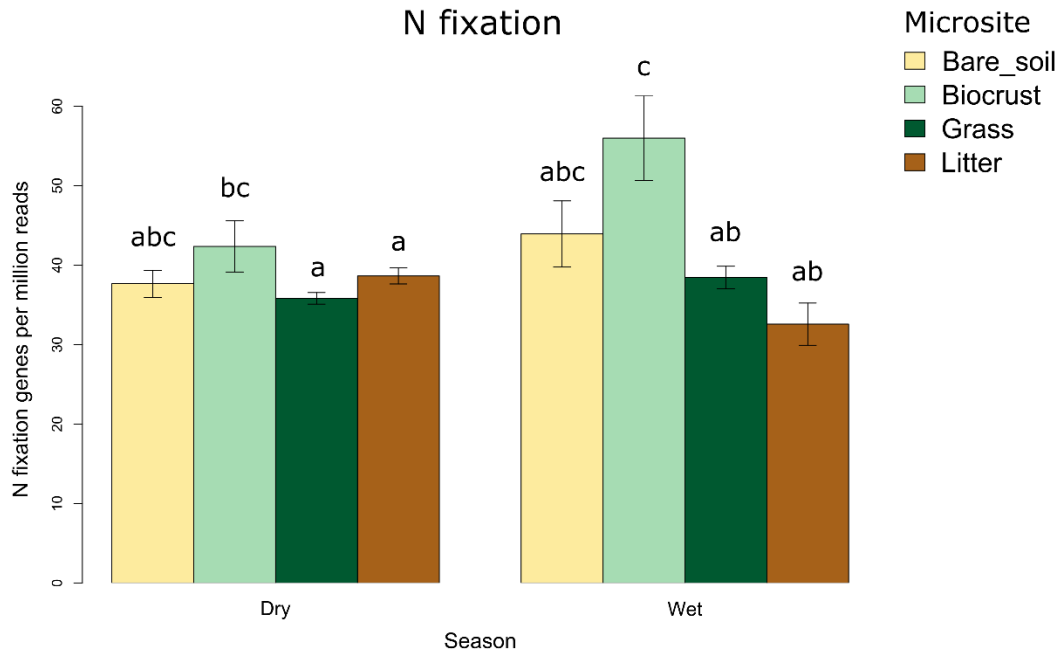


Figure 7.1.2 Barplot summarises the microsite and season effect on nitrogen fixation for the four-yearly early burnt treatment. Different letters mean significant values ($P < 0.05$).



7.1.2 Bacterial community profile: Fire treatment, soil and season effects at VRRS

With an understanding of the roles of the biocrust forming cyanobacteria present, the lack of cyanobacteria found in the dry season in the vertosol soils reflects the cracking clay soils where ephemeral biocrusts exist (Fig. 7.1.3). In contrast, the calcarosol soils are more stable and there is a more persistent biocrust present across both the wet and dry season. Leptolyngbya is referred to as a disturbance specialist that may represent many species. This is consistent with where on the calcarosol it was present on the 2 yearly late and 4 yearly early burnt treatments (Fig. 7.1.3). But in contrast on the vertosol it was found in greater frequency at the least disturbed sites in the 6 yearly early burnt and unburnt control. At VRSS, a full detailed description of the biocrusts in the 60-year CSIRO exclosure and the adjacent grazed paddocks has been published in Ecological Indicators and provided here in Appendix 4 (Vega-Cofre et al., 2023).

Figure 7.1.3 Heatmap summarising the frequencies of bacterial OTUs present at ≥0.5% mean relative abundance grouped at family level within any treatment group. Relative abundances are Hellinger transformed.

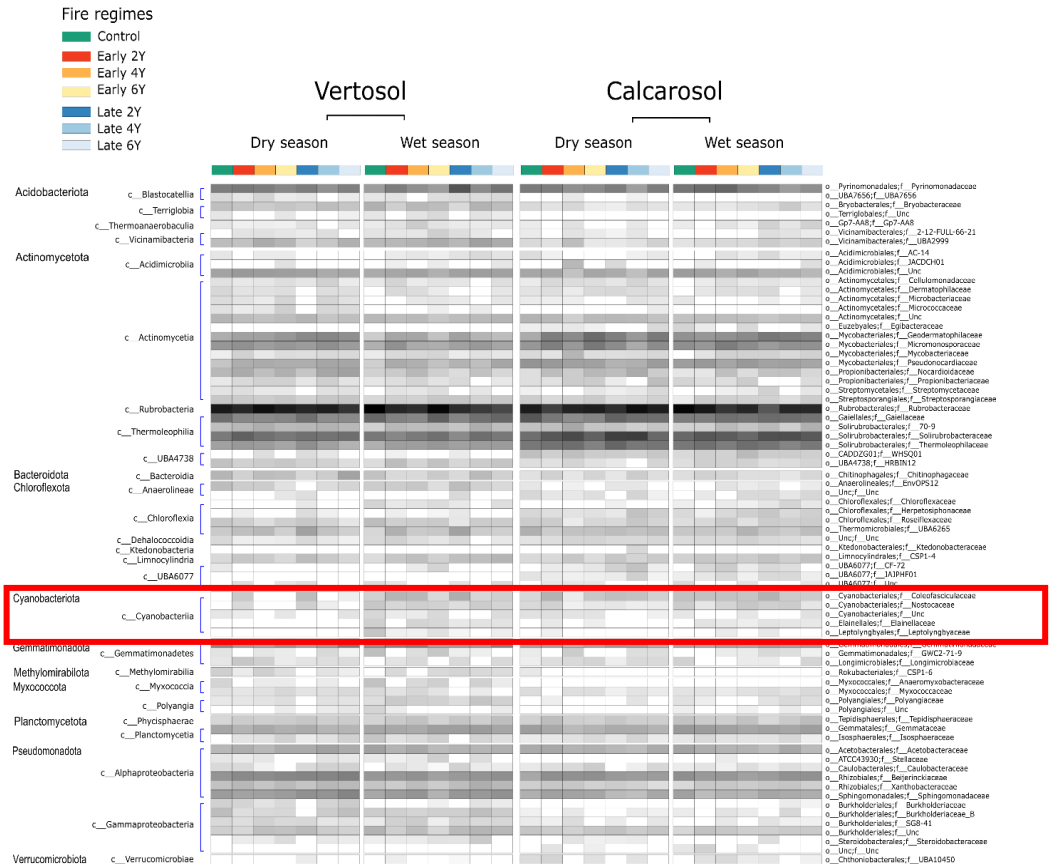


Table 7.1.2 Indicator species analysis on nitrogen cycling genes. Showing genes identified as significant seasonal indicators ($P < 0.05$, indicator analysis) for the vertosol.

Season	*P* value	Gene	Function
Dry	0.030	nirBD	Denitrification pathway
	0.040	ureJ	Ammonification pathway
Wet	0.001	nifHDK	Nitrogen fixation
	0.003	anfHGDK	Nitrogen fixation
	0.002	vnfHGDK	Nitrogen fixation

7.1.3 Explanation of results in terms of land management implications.

Observations of N fixing gene abundance in biocrusts	**Implication for managing grazing lands**
Biocrusts growing between grasses have the greatest relative potential for N fixation, particularly in the wet season (Williams et al., 2017)	Ensure sufficient suitable space is available for biocrust habitat because 'between grasses' is the most beneficial conditions for biocrust growth and N fixation. Likely reasons include that grasses shelter biocrusts environmental extremes including excessive light, drying out and air movement.
Biocrusts growing under grasses have the second highest relative potential for N fixation in the wet season.	Good grass cover is obviously a priority for producers and will also benefit biocrusts. It is possible that different grass species creating various light, moisture and nutrient environments influence biocrust growth and N fixation, but this was not investigated here.
Biocrust microbiomes growing under leaf litter have a relatively low potential for N fixation.	Shading by leaf litter results in low biocrust photosynthesis and N fixation. This is more pronounced in calcarosol than in vertosol, likely because vertosol is self-mulching and less leaf litter accumulates on the soil surface compared to calcarosol where N fixing gene relative abundance was lowest in both seasons. A management strategy worth testing is if regular fire reducing leaf litter boosts N fixation.
Biocrusts in 'bare' microsites vary in their relative N fixation capacity with higher levels in vertosol than calcarosol.	Bare soil is the most wide-ranging microsite as it includes soil spanning from very little to more biocrust (even if not visible as organisms can live in the upper centimetre of soil rather than surface). Bare soil is undesirable because it is prone to erosion. The variable N fixation potential however may indicate the crucial role of biocrust in restoring soil fertility by replenishing N. Vertosol biocrusts have comparatively high relative N fixation capacity compared to calcarosol

7.1.4 Effects of microsites and grazing on N at Wambiana grazing trial

At Wambiana, the relative frequencies of nitrogen cycle genes differed significantly between soils (Table 7.1.3). In the red-yellow soils, the relative frequencies of nitrogen cycle genes differed significantly between microsites, while in the duplex-clay soils, they were significantly influenced by season (Table 7.1.3). Examining the nitrogen pathways separately, there was an effect of microsite in nitrogen fixation, ammonification, and ammonium assimilation genes in the red-yellow and a strong influence of season in the relative abundances of N fixation genes, an effect of microsite in ammonium assimilation and an impact of the season in denitrification genes in the duplex-clay (Table 7.1.4).

Table 7.1.3 ANOVA and PERMANOVA results summarising the main and interactive effects of soil (red-yellow and duplex-clay), season (dry and wet season), and grazing treatment, on Shannon's Diversity Index (alpha diversity) and community composition (beta diversity), respectively.

Predictor	Shannon index		Community composition		
	F value	*P* value	*F* value	R^2 (%)	*P* value
Both sites					
Soil	0.859	0.361	5.524	9.893	0.001 ***
Season	0.808	0.375	1.853	3.318	0.021 *
Treatment	0.071	0.975	1.235	6.633	0.107
Soil:Season	0.903	0.349	1.057	1.894	0.333
Soil:Treatment	1.877	0.153	1.740	9.346	0.003 **
Season:Treatment	1.053	0.383	0.993	5.333	0.461
Soil:Season:Treatment	0.355	0.786	1.169	6.278	0.167
Within Red-Yellow					
Season	1.436	0.248	1.47	5.647	0.068 .
Treatment	0.726	0.551	1.68	19.339	0.003 **
Season:Treatment	1.005	0.416	1.17	13.509	0.180
Within Duplex-Clay					
Season	0.002	0.969	1.44	5.945	0.050 *
Treatment	1.338	0.297	1.28	15.853	0.039 *
Season:Treatment	0.261	0.852	0.98	12.157	0.520

P < 0.001***, *P* < 0.01**, *P* < 0.05*, *P* < 0.1 '.'

The heatmap represents the relative frequency of nitrogen cycle bacterial genes found in the moderate stocking rate with wet season spelling, and how they change with soil type between seasons and the different microsites. The heatmap shows the mean values of three sample repetitions, and the genes are grouped by pathway or function. In the red-yellow soil, all pathways are more frequent in the biocrust during the wet season, except denitrification, which is higher in the bare soil (Fig. 7.1.4).

The heatmap shows that in the duplex-clay, the ammonification genes are relatively more frequent in the biocrust and under the grasses in the dry season while in the wet season they are relatively more frequent in the litter. The N fixation genes are relatively more frequent in the biocrust and litter within the dry season, nitrification genes are relatively more frequent in the bare soil with the dry season, the ammonium assimilation associated genes were relatively higher in the biocrust with the dry season, and denitrification genes were relatively more frequent in the bare soil in the wet season (Fig. 7.1.4).

Figure 7.1.4 Relative frequency heatmap of nitrogen cycling gene frequency by microsites. Genes are in rows grouped by metabolic pathway and samples are in columns ordered by soil, season, and microsite. White-black shading represents the frequency per million bacterial reads while the white-blue shaded shows the sum of the square mean gene frequency across metabolic pathways. This heatmap has been row-scaled to show the relative abundance of each respective gene across microsites.

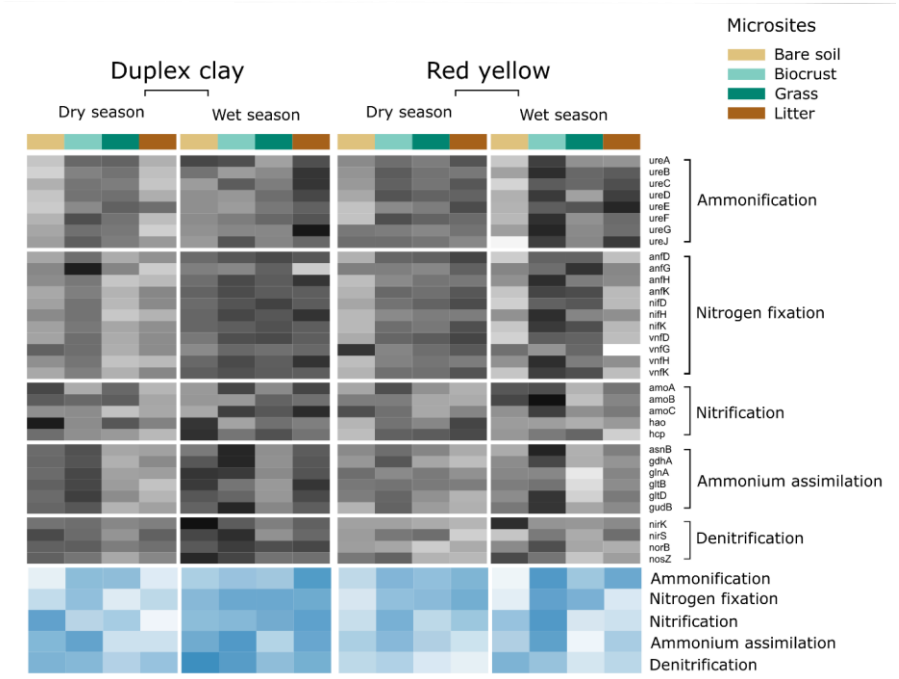


Fig. 7.1.5 Distance-based Redundancy Analysis (db-RDA) ordination plots highlighting differences in the composition of bacterial communities between grazing treatments (coloured ellipses), and seasons (empty ellipses). The IDs of the most discriminating metagenomic OTUs are shown in square brackets in red and are consistent with those shown in heatmap (Fig. 7.1.4). In both db-RDAs, the response was a Hellinger transformed OTU table, and the constraints (predictors) reflected the significant predictors identified using PERMANOVA. Hence, for the Red-yellow soil, the constraint was Grazing treatment, while for the Duplex soil, the constraints were the main effects of Grazing treatment and Season.

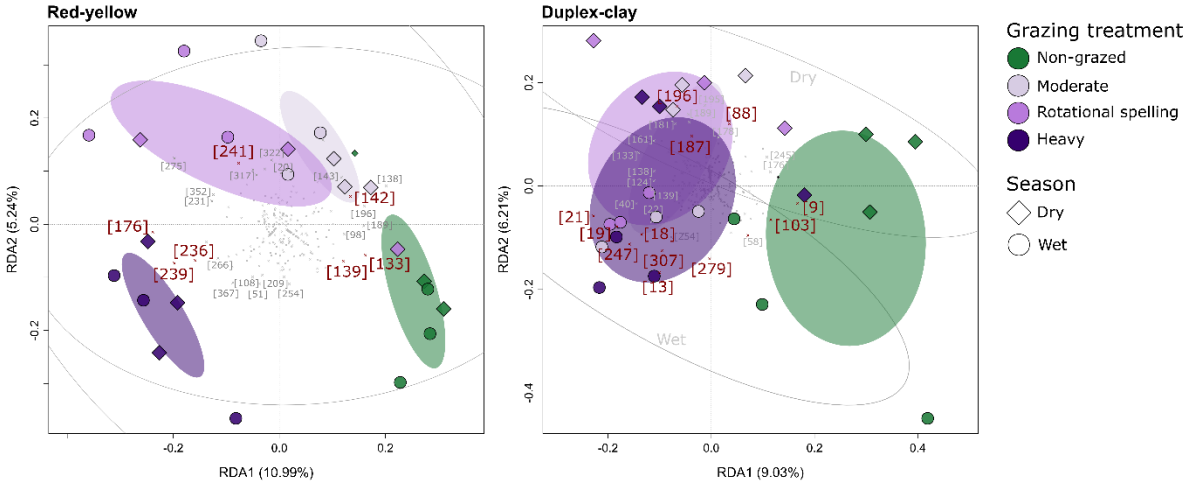
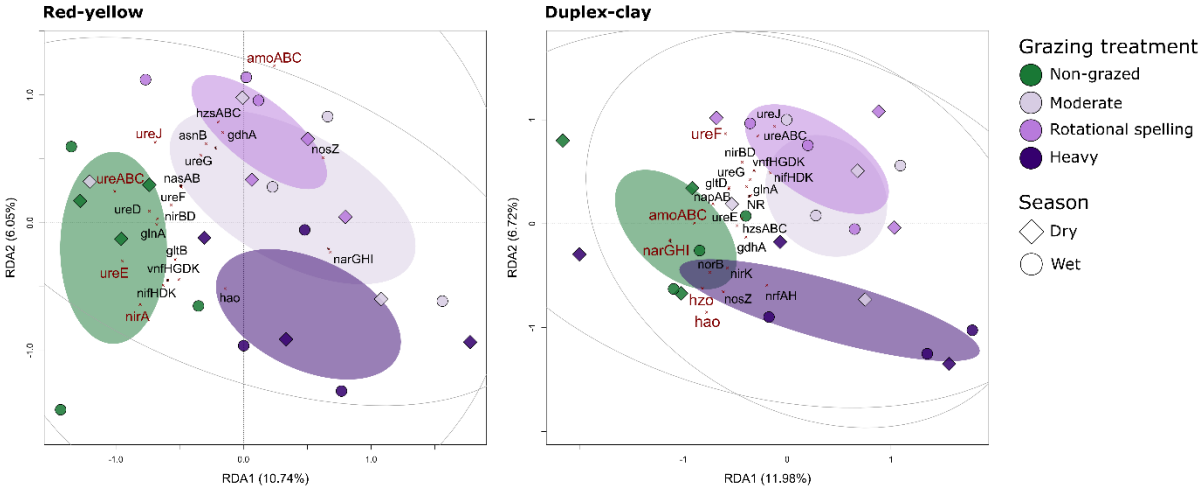


Table 7.1.4 Results from PERMANOVA models summarising the main and interactive effects of soil (red-yellow and duplex-clay), microsite and season (dry and wet season) on the relative frequencies of genes associated with nitrogen cycle genes, as well as the effect of microsite and season within each soil separately.

Predictor	**N cycle genes frequency**		
	F value	R^2 value	*P* value
Both sites			
Soil	2.235	0.039	0.029 *
Season	2.693	0.047	0.009 **
Microsite	2.749	0.144	0.001 ***
Soil:Season	2.027	0.035	0.045 *
Soil:Microsite	0.919	0.048	0.562
Season:Microsite	1.333	0.070	0.116
Soil:Season:Microsite	1.090	0.057	0.300
Within Red-Yellow			
Season	1.25	0.044	0.207
Microsite	2.39	0.251	0.001 ***
Season:Microsite	1.37	0.144	0.088 .
Within Duplex-Clay			
Season	3.49	0.129	0.006 **
Microsite	1.39	0.154	0.126
Season:Microsite	1.14	0.126	0.318

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Figure 7.1.7 Distance-based Redundancy Analysis (db-RDA) ordination plots highlighting differences in the relative frequencies of nitrogen cycling genes between grazing treatments (coloured ellipses), and seasons (empty ellipses). The **most discriminating nitrogen cycle genes** are shown **in red** and are consistent with those shown in the indicators analyses. In both db-RDAs, the constraint (predictor) was grazing treatment as identified using PERMANOVA. Relative gene frequence are log2 transformed and standardized.



7.2 Quantifying biological nitrogen fixation of biocrusts

We commenced this core activity of the project by testing the most used quantification methods for Biological Nitrogen Fixation (BNF) (Barger et al., 2016; Belnap, 2003). We analysed biocrust and associated topsoil samples, collected from red and black soils across all fire treatments at VRRS (Wet Season 2020). In this first step, 84 biocrust samples (upper 0.5 cm of soil) were tested.

7.2.1 Acetylene reduction

The acetylene reduction assay (ARA), when BNF occurs, generates ethylene by cleaving the triple bond between carbon atoms. Acetylene mimics the triple bond of N_2 which the nitrogenase enzymes that are responsible for BNF can use as an alternative substrate. Ethylene is then quantified via gas chromatography. ARA is the most widely used BNF assay with the advantage that many samples can be analysed in a cost-effective manner. However, ARA does not allow quantifying BNF unless the relationship between ARA and true N_2 fixation has been established (see below). ARA's strength is relative comparisons between different types of biocrusts, in relation to fire or grazing in our case, or differences of biocrusts from subsites (i.e. inter-patches between vegetation, patches near vegetation).

Ethylene production is calculated as a rate, based on measures of biocrust; this can include:

- Weight of biocrust and upper soil (certain cyanobacteria are motile and move between the soil surface and shallow soil depth to optimise their environment. We calculated: ethylene produced per unit weight of biocrust + soil * time
- Biomass of biocrust gauged using the proxy of chlorophyll *a*; as all cyanobacteria have this pigment, and if cyanobacteria dominate biocrust, chlorophyll a will be a useful basis for ARA. We calculated: ethylene produced per unit chlorophyll a (in a given biocrust sample) * time.

Note: results not shown.

7.2.2 Stable isotope $^{15}N_2$

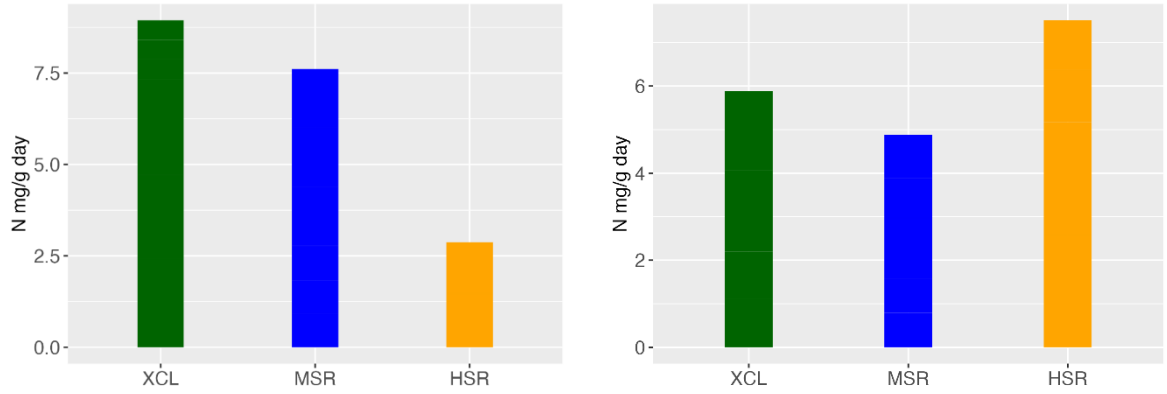
Using the stable isotope $^{15}N_2$-labelling assay, we inject isotopically heavy nitrogen gas into biocrust vials. In the first test, we replaced 10% of air in the vials with 99.6% labelled nitrogen gas. Enrichment above natural abundance of 15N allows to quantify the rate of BNF. This assay generates an accurate measure of BNF and is used to calibrate ARA. A benefit of isotope labelling is that it can be performed in the field, which we are planning for the wet season 2021. A drawback is high cost ($4/injection, $20/analysis) and that it cannot be used if the isotope enrichment is below the detection limit. The BNF rate has to be sufficiently high to enrich the existing N in biocrusts above natural abundance levels. Longer term incubation is possible but the air in the vials changes over time, compromising the assay. We submitted samples for mass spectrometry (DES laboratory, Ecoscience precinct).

Since running these assays, we discovered that other researchers had found the labelled gas was contaminated with N. This meant we could not ascertain whether the results were valid or necessarily use the data to upscale. We have since processed a portion of the samples (results shown) and consulted widely about validation for any future work.

7.3 Interim Results: Quantifying N fixation at Wambiana grazing trial

Interim results are for rates of N fixation across two grazing trials (moderate and heavy), and an ungrazed control for the two soil types at Wambiana. Note that N-fixation will not occur unless moisture is present to drive photosynthesis however cyanobacteria can fix N at very low light levels although at a much slower rate. These results indicated that to some extent livestock presence does affect the rate of N fixation. In the red-yellow soil there was a clear downward trend the more disturbance the less capacity the biocrust had to recover and fix N. Contrary to expectations in the duplex clay soil the heaviest disturbance had the highest rates of N-fixation. This goes somewhat to explaining why there were higher levels of both available and total N found in this treatment. In this case it would be anticipated that microbial activity went into overdrive producing a nutrient rich soup that was not able to be taken up fast enough by plants because these areas were more or less bare and denuded of grass. Bacteria and cyanobacteria have the capabilities of reproducing fast in a high-nutrient environment that includes excess dung and urine. A well-known example of this is in waterways that are nutrient enriched resulting in an algal bloom.

Figure 7.3.1 Daily rates of N fixation in the Red-Yellow soil and (b) in the duplex clay.



(a) (b)

Special Note – The 15N labelled gas used for these tests may have been contaminated and further examination of the results and consultation with chemists is underway. The trends in these results appear feasible so they are presented here as a general guide.

7.4 Bioavailability of nitrogen from biocrusts

7.4.1 Ion exchange resin methodology

Measurements of soil nitrogen within grazing land has been explored using ion exchange resin (Qian and Schoenau 2002; Cain et al. 1999; Subler et al. 1995). In this case the resin captures the nitrogen that has been either biologically fixed (by cyanobacteria) or transformed by the bacteria that also live in the biocrust (Staal, 2003). These principles can be applied to measure the nitrogen bioavailability that is released from biocrusts into the plant root zone, available for immediate uptake. Resin bags were made by sewing 8 cm x 8 cm squares of nylon mesh (300 µm). 5 g of ion exchange resin (Superco 13687-U mixed anion/cation) was poured into the bag (Fig. 7.4.1). The bags were placed 1-2 cm under the soil surface (Fig. 7.4.1).

7.4.2 In situ fire and grazing wet trial on calcarosol at VRRS

In March 2023, 33 resin bags were deployed along a 39 m transect at VRRS, Kidman Springs, spanning over an existing cattle pad and into an area with grass and biocrust cover (Fig. 7.4.1). The

transect was split into 3 categories the degraded area (D), grass and biocrust cover (C) and areas where the biocrust was recovering on the edge of the degraded area (E) (Fig. 7.4.1, Table 7.4.1). Following 50.2 mm of rain the resin bags were removed in early May and sent for analysis. After each resin bag had been retrieved the nitrate and ammonium absorbency of the samples were measured using Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) measured at 540 nm for NO_3^- and 625nm for NH_4^+.

Table 7.4.1 Experimental layout of in-situ ion exchange resin at Kidman Springs

D = Degraded, C = grass and biocrust cover and E = edge of degraded area (recovery zone)

Treatment	Number of bags deployed	Percent of transect
D	11	34
C	9	27
E	13	39
Total	33	100

Figure 7.4.1 Transect line where 33 resin bags were deployed 1 m apart.

7.5 Results: Bioavailability of nitrogen from biocrusts at VRRS

The mean ug NO_3^- -N g^{-1} resin extracted after 34 days and 50.2mm of rainfall was the highest in the transition area along the transect (41.6 ± 26.1 ug NO_3^- -N g^{-1} resin). The annuals and biocrust cover had the lowest mean of 27.8 ± 26.3 ug NO_3^- -N g^{-1} resin. The degraded area was in between with 40.7 ± 27.1 ug NO_3^- -N g^{-1} resin (Fig. 7.5.1).

The degraded area had the lowest mean of ug NH_4^+ -N g^{-1} resin extracted (1.2 ± 0.8 ug NH_4^+ -N g^{-1} resin). Followed by the transition area (9.8 ± 7.4 ug NH_4^+ -N g^{-1} resin) and annuals and biocrust cover (20.9 ± 26.2 ug NH_4^+ -N g^{-1} resin). (Fig. 7.6.2).

Figure 7.5.1 Mean ug NO_3^- -N g^{-1} resin extracted from in-situ resin bags at Kidman Springs, Fire Graze site ($p>0.05$). Error bars denote standard deviation.

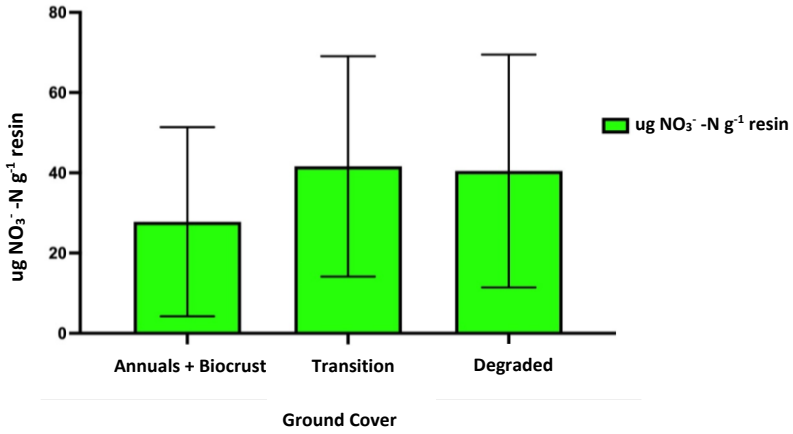
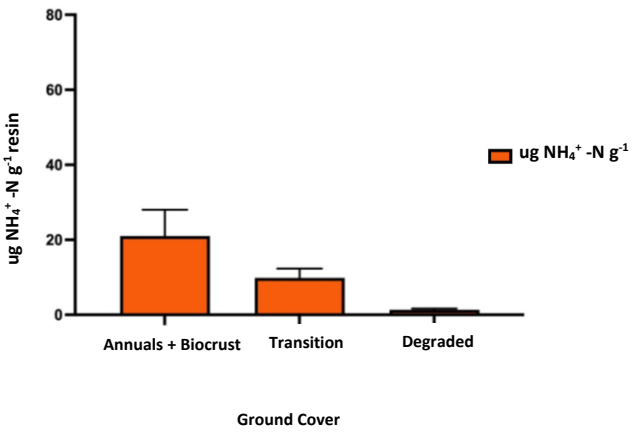


Figure 7.5.2. Mean ug NH_4^+ -N g^{-1} resin extracted from in-situ resin bags at Kidman Springs, Fire Graze site ($p>0.05$). Error bars denote standard deviation.



7.6 In situ wet season study at Wambiana grazing trial

In August 2023, prior to the start of the wet season 66 resin bags were made and placed in-situ across a similar range of grazing treatments at Wambiana (Fig. 7.6.1) with the intention of removing the bags after the early wet season rains (Table 7.6.1). Due to potential interference by predators in the field environment and the high chances of disturbances by cattle and other critters in unprotected sites, extra bags were placed in pre-existing exclosure sites to increase the chances of successful retrieval.

Figure 7.6.1 Example of an ion exchange resin bag and how it sits under the soil surface.

Table 7.6.1 Experimental layout of in-situ ion exchange resin methodology at Wambiana grazing trial. HSR= heavy stocking rate, MSR= moderate stocking rate, WSS = wet season spelling.

Paddock number	Soil type	Treatment	Number of bags
4	Duplex Clay	HSR	7
5	Duplex Clay	MSR	7
5	Duplex Clay	WSS	7
8	Duplex Clay	Exclosure	18
5	Red-Yellow earth	Exclosure	7
5	Red-Yellow earth	MSR	7
2	Red-Yellow earth	WSS+MSR	6
9	Red-Yellow earth	HSR	7

7.7 Results: Wambiana grazing trial

7.7.1 Duplex clays

Within the duplex clays the highest average ammonium level extracted over 99 days and following 118.9 mm of rainfall was from the wet season spelled paddock with the ungrazed exclosures second highest (Fig. 7.7.1).

The wet season spelled paddocks (WSS/MSR) also had the highest extraction of ug NO_x -N g^{-1} (48 ± 25.2) and the heavily stocked paddocks (HSR) had the lowest (29 ± 16.5 ug NO_x -N g^{-1}). Moderately stocked paddocks (MSR) extracted 37 ug NOx -N g-1 resin over the 99 days (± 10.8) and the exclosure sites once again had the second highest extraction with 44 ug NOx -N g-1 (± 12.7), not been included in graph (Fig. 7.7.2).

Figure 7.7.1. Mean ammonium extracted from in-situ resin bags in the duplex clays at Wambiana grazing trial (p>0.05). Error bars denote standard error.

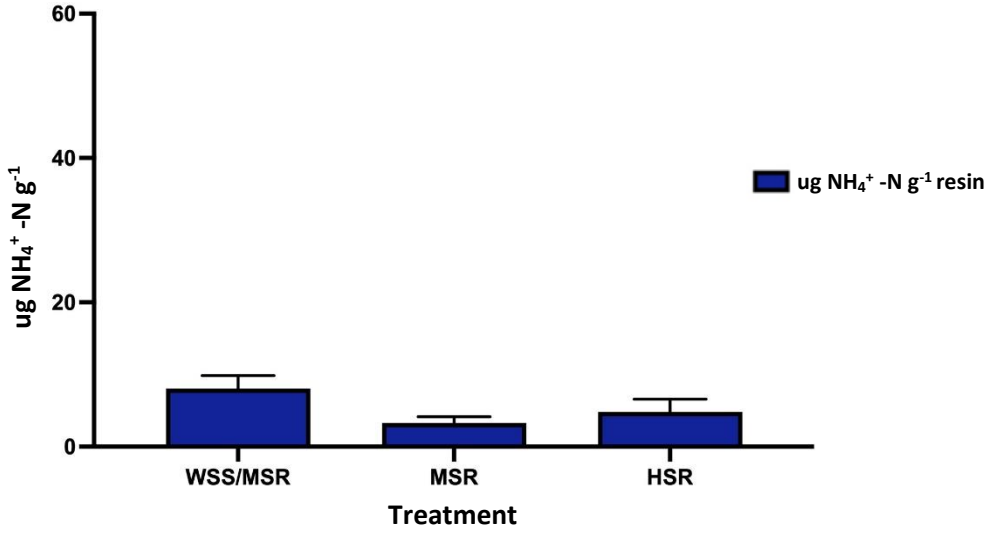
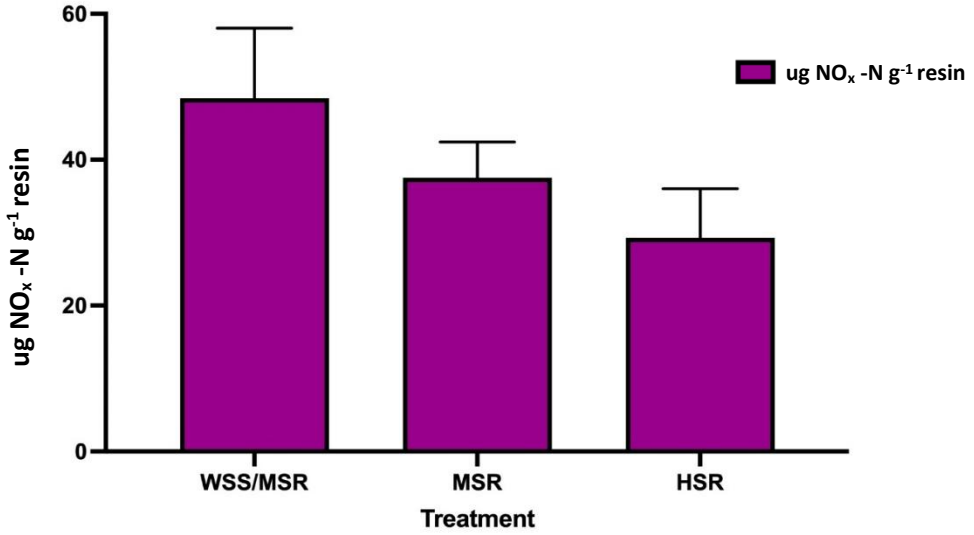


Figure 7.7.2 Mean ug NO_x -N g-1 resin extracted from in-situ resin bags in the duplex clays at Wambiana grazing trial (p>0.05). Error bars denote standard error.



7.7.2 Red-yellow earths

For the red-yellow earths, the moderately stocked paddocks had the highest extraction of NH_4^+ -N g^{-1} over the 99 days (15 ± 9.8 ug NH_4^+ -N g^{-1} resin). Followed by the exclosure site (EXC) (9 ± 5.7 ug NH_4^+ -N g^{-1} resin) and the wet season spelled paddock (8 ± 7.7 ug NH_4^+ -N g^{-1} resin) (Fig. 7.7.3).

The results from the heavily stocked paddock have been excluded from the graph due to abnormally high average extraction of NOx (2758 ± 1969.6 ug NOx -N g-1 resin) (75 x the combined average of other treatments). The exclosure site had the second highest average extraction 39 ug NOx -N g-1 resin (± 13.5), the moderately stocked paddock extracted 34 ug NOx -N g-1 resin ± 5.6. The wet season spelling paddock followed (29 ± 5.1 ug NOx -N g-1 resin) (Fig. 7.7.4).

Figure 7.7.3 Mean ug NH_4^+ -N g^{-1} resin extracted from in-situ resin bags in the red-yellow earths at Wambiana grazing trial (p>0.05). Error bars denote standard error.

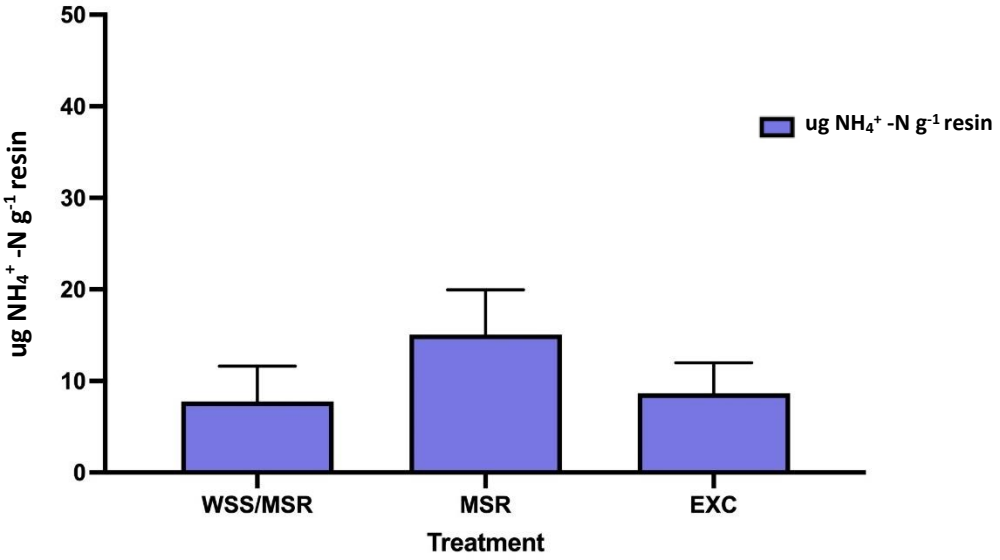
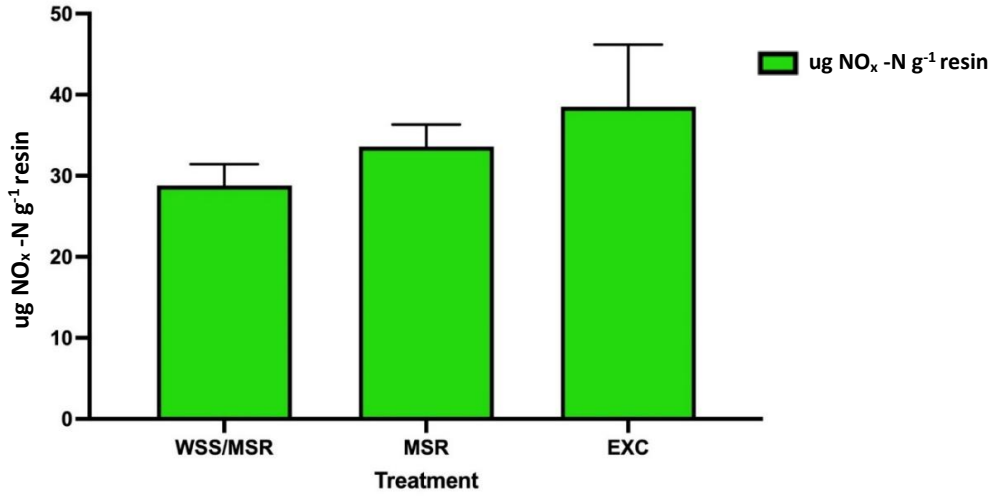


Figure 7.7.4 Mean ug NO_x -N g-1 resin extracted from in-situ resin bags in the red-yellow earths at Wambiana grazing trial (p>0.05). Error bars denote standard error.



8 Nitrogen and carbon dynamics: fire and grazing

8.1.1 VRRS Sampling Program

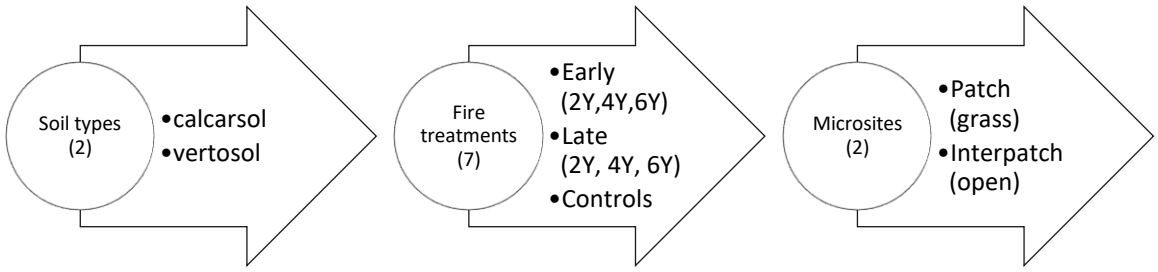
The field work and sampling program was based on the fire trial plots (Fig. 8.1.1). The vertosol (1–16) and calcarosol (17–32) soil trials consist of a total of 16 plots each with six fire treatments (two replicate plots per treatment) and four control plots, where each plot is 160 x 160 m (Fig. 8.1.2).

Figure 8.1.1 Grid layout of fire plots and treatments. Numbers are plot numbers. Fire codes – Control 0 - no burns, 2, 4, 6 - burnt every two, four, or six years; E - early in the dry season (June cooler fires), or L - late in the dry season (October hotter fires).

1 L6	2 E4	3 E4	4 E6
5 E6	6 L4	7 L4	8 0
9 0	10 L2	11 L6	12 E2
13 0	14 E2	15 L2	16 0

17 L6	18 0	19 E6	20 L4
21 0	22 E4	23 E2	24 L2
25 E6	26 0	27 E4	28 E2
29 0	30 L4	31 L2	32 L6

Figure 8.1.2 The soil sampling strategy incorporated two soil types (calcarosol, vertosol) x seven treatments x two microsites as illustrated below.



Across 14 plots (two plot replicates per treatment including two controls) the following methods were carried out:

1) For each fire treatment and controls (no fire) we established two x 30 m transects. These were positioned central to existing permanent transects two and three.
2) Adjacent to each transect three patch (grassy) and interpatch (open) sites were identified and marked out.
3) Using a corer three samples each from under grass plants, litter, biocrust and bare ground were excavated at 0-1cm, 1-5 cm depths, (2 soil types x 14 treatment plots x 12 microsite reps x 2 depths) (n=672).
4) Six contiguous 1 m2 quadrats were assessed for biocrust cover, bare ground, basal grass cover, litter, and foliage projection cover.
5) Biocrust samples (Petri dishes) were collected under the grass and in the open for species ID and N-fixation tests (12 reps per treatment) (n=672).
6) Samples were collected for DNA sequencing and biomass analysis (3 reps x 4 microsites, n=336).

Due to COVID-19 we undertook the post wet season field work from 19th July to 2nd August (2020) the following year after all fire treatments had been burnt in June or October 2019.

A total of 2,018 biocrust and soil samples were collected as detailed below:

- 672 soil samples (at 2 depths) for C, N and bio-available N
- 672 biocrust samples (for ID and N-fixation analysis)
- 336 biocrust samples (for 16sRNA sequencing)
- 336 biocrust biomass samples (chlorophyll *a* extraction)
- Landscape Function Analysis (28 plots)
- 1008 quadrats ground cover (% biocrust, grass litter, bare, 28 plots)

Figure 8.1.3 Kidman Springs field trip team (July 2019) collecting biocrusts (top right) and quadrat measurements (bottom right).




8.1.2 Statistical Methods

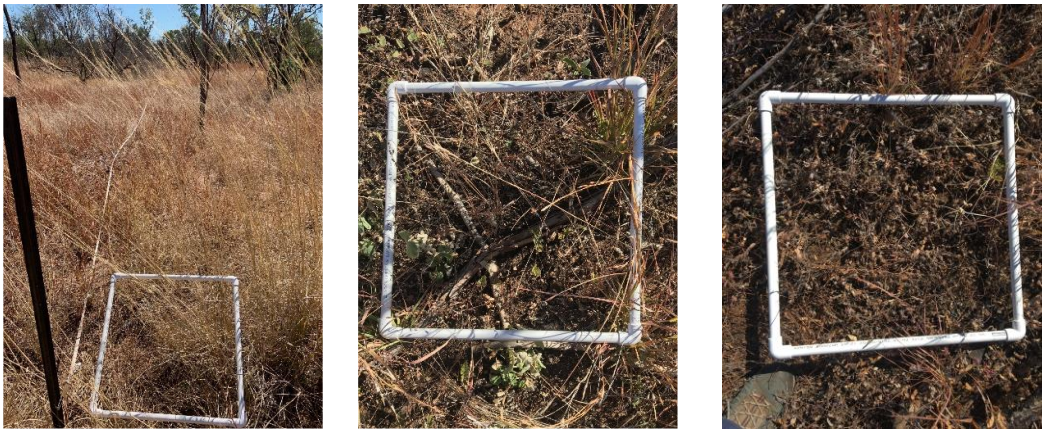
All statistical analyses for nitrogen, carbon, and carbon nitrogen ratio for Calcarosol and Vertosol at Victoria River Research station were conducted using two-way ANOVA analysis in GraphPad Prism version 10.2.2, GraphPad Software, Boston, Massachusetts USA, www.graphpad.com. Both bar graphs and heat maps were also created in GraphPad Prism.

8.1.3 Preliminary results and field notes

This survey took place in top x cm of soil microsites post-fire, post-wet season. about three months after the end of the wet season in 2019/20 and 12 months after the early fires. To aid interpretation of the results the average microsite cover has been included for early and late 6 yearly plots (Fig. 8.1.4). It should be noted that basal area of perennial grass plants (1.4%) is used to complete the area of the one-metre quadrats however for this plot the grass cover (including canopy spread over biocrusts, bare ground and litter) was 28% or less than one third of overall ground cover. One of the features recorded during sampling was the good condition of the biocrust following six years without fire (Fig. 8.1.6).

Figure 8.1.4 Average microsite cover for early and late 6-year fire plots demonstrating the disproportionate levels of litter and bare ground.

Figure 8.1.5 (a) Early 6-year plot with a high level of grass and biocrust cover and less bare ground (compared to late 6-year), measurements taken post-fire, post wet season, no grazing (2020), (b) sample quadrat with grass where the biocrust is visible as a dark colour underneath and (c) a area covered with annuals with biocrust.



(a) (b) (c)

Figure 8.1.6 (a) Late 6-year plot with remains of Acacia saplings and typical understorey (2020), (b) sample quadrat with grass where the biocrust is visible as a dark colour underneath and (c) a bare area with some remnants of biocrust still visible. Following the fires in 2019, there was a large litter fall when the Acacia was burnt.

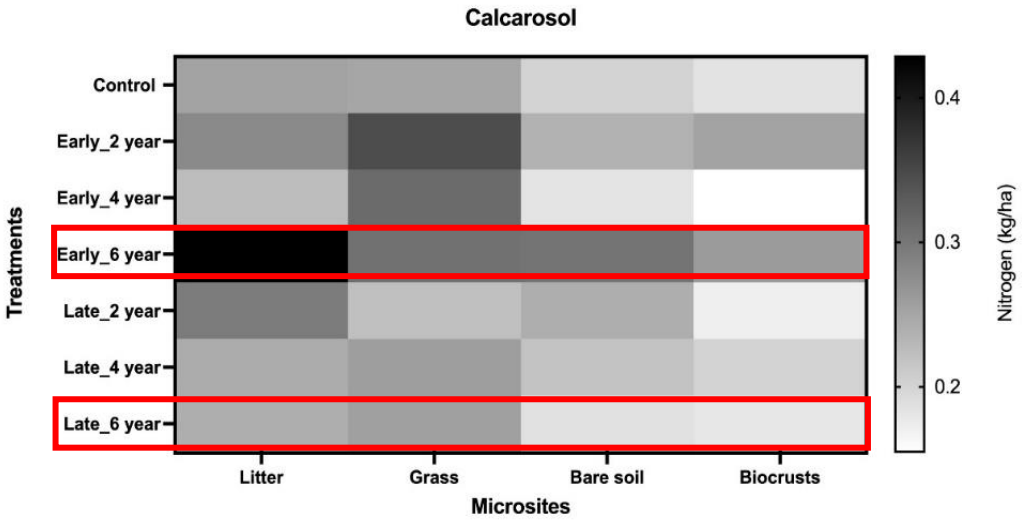
(a) (b) (c)

8.2 Results: Nitrogen and carbon dynamics at VRRS (2020)

8.2.1 Calcarosol N and C stocks (kg/ha)

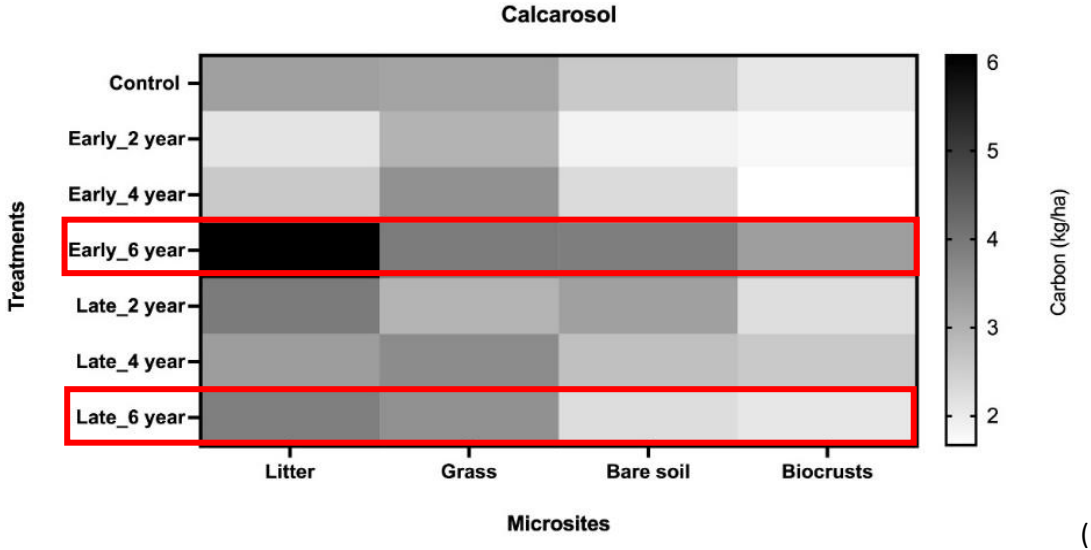
We detected statistically significant differences between treatments and microsites in soil N and C stocks of calcarosol, however these differences did not necessarily follow a simple pattern with fire regimes. Rather the differences were associated with microsites (Fig. 8.2.1–8.2.3). We have looked at the calcarosol soil 6-year fire plots in detail as an example.

Figure 8.2.1 N across all microsites and treatments in top x cm in calcarosol



(a)

Figure 8.2.2 C across all microsites and treatments top x cm in calcarosol

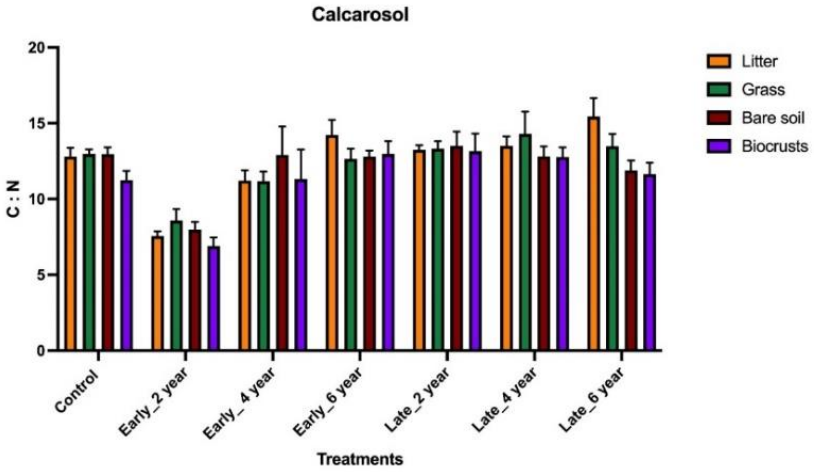


(b)

Compared to most other treatments, the early 6-year fire treatment had the highest C and N stocks across all microsites; this treatment had a higher level of biocrust cover and heavier cover of grass and annuals which is most likely to have contributed N input (Fig. 7.7.1–7.7.3). This affected the results across all microsites (i.e. increased stocks of N/C, highlighted with red outline) however does not mean that this was a preferred fire regime.

Across all treatments, grass microsites had the most uniform C and N stocks which is expected as soil associated with grass would be the most fertile - being vegetated and protected from erosion. Bare and biocrust microsites had the lowest stocks as also expected because the extent of biocrust development and their recovery of eroded, vegetation free sites will result in overall lower and variable C and N stocks compared to vegetated or litter covered microsites.

Figure 8.2.3 C/N ratio for all treatments and microsites in top x cm.



The C/N ratio was mostly conserved across sites (an exception was the early 2-year fire where N was relatively higher, perhaps legacy of recent manure deposition.

In summary for the calcarosol the early/late 4-year was the most consistent in N/C stocks across all microsites however, the climatic conditions would influence management decisions as C/N ratio in this season (2020) favoured the early 2-year fire. This was perhaps influenced by two years of low lead up rainfall and a poorer season overall. Nevertheless, burning every two years is not generally recommended for pasture management (Cowley et al., 2021).

8.3.1 Vertosol N and C stocks (kg/ha)

In the vertosol late fires appeared to have greater stocks of N and C across all microsites. Some of these results can be explained by the increased presence of the cyanobacterium Nostoc, known to be instrumental in higher levels of N-fixation (Williams et al., 2017). In the vertosol soils there were large depressions that favoured highly protected sites with more water pooling for longer. These were covered by tall grasses and had thick cyanobacterial biocrusts present. DNA results showed that N-fixation and biocrust photosynthesis occurred in the wet-season. In this case, we sampled during the dry season that explains the lower remnant values of N/C stocks in the biocrusts and bare areas.

Figure 8.3.1 N across all microsites and treatments in top x cm in vertosol

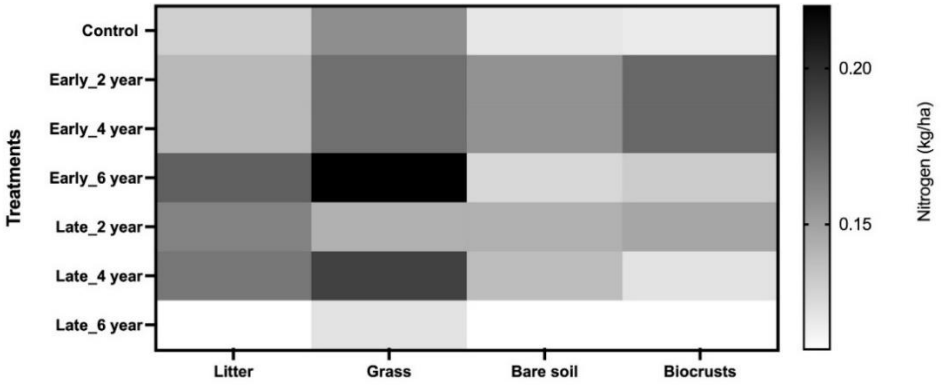
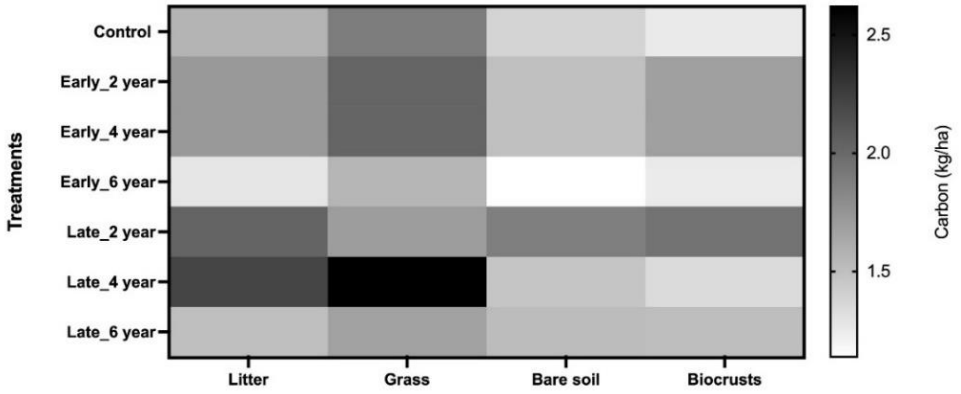
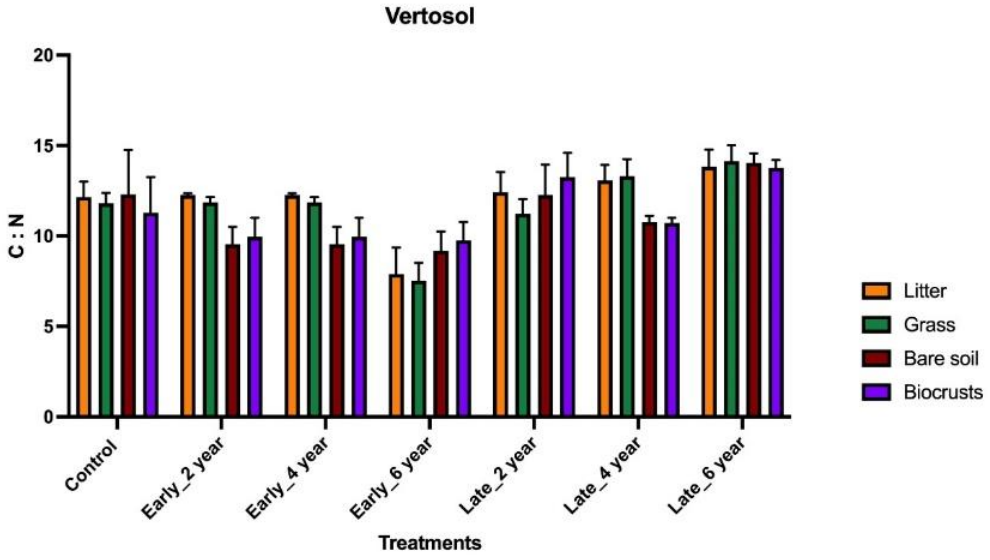


Figure 8.3.2 C across all microsites and treatments in top x cm in vertosol



In vertosol, no statistically significant differences in N or C stocks occurred between treatments or microsites. C/N ratios ranged from 8–12, the expected value for soil and biocrusts. Overall, C and N stocks were lower than in calcarosol. Vertosols are self-mulching soils which therefore have less stratification than calcarosol so that biocrusts and their associated nutrients are redistributed into the deeper soil rather than being present in the top cm of soil. The trend was that late 4-year burning had the best response for C stocks (litter, grass microsites), while early 6-year burning had higher N stocks, mirroring what was observed for calcarosol.

Figure 8.3.3 C/N ratio across all microsites and treatments in top x cm in vertosol.



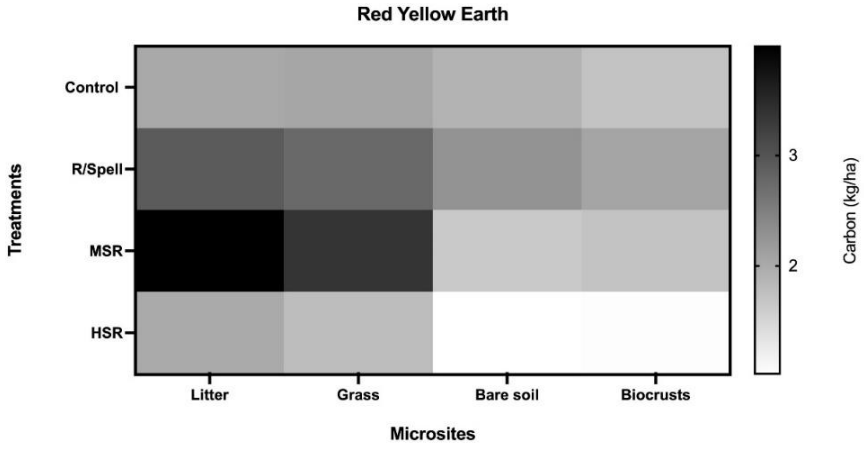
8.4 Results: Nitrogen and Carbon dynamics at Wambiana grazing trial 2020

8.4.1 Red yellow earth N and C stocks kg/ha

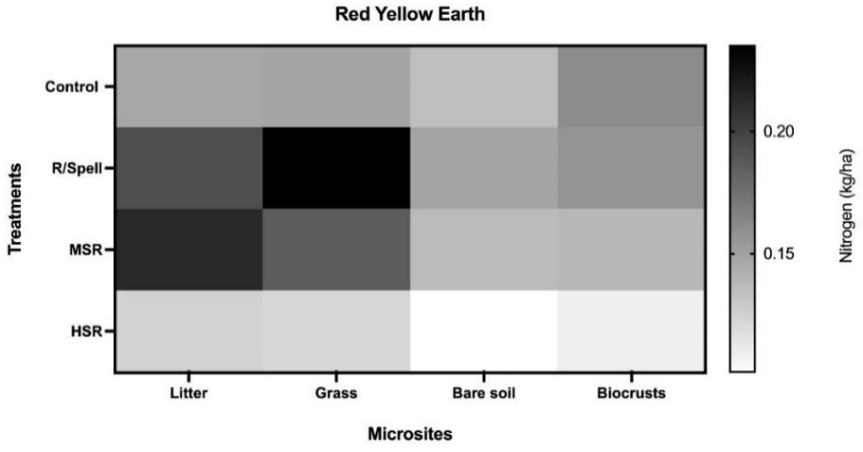
In red-yellow soil paddocks that are rotationally spelled and moderately stocked, N and C stocks in litter and grass microsites were significantly higher than in heavy stocking and control sites (Fig. 8.4.1). Heavy stocking had a deleterious effect on biocrusts, and the resulting bare ground had extremely low N and C stocks indicative of severe degradation. Such high level of disturbance and lack of biocrust regeneration mean that a tipping point of degradation has been reached and no evidence of for example subsurface cyanobacteria providing stability for recovery phases. The

rotationally spelled paddocks had higher C and N stocks than moderately stocked paddocks, confirming that wet season spelling enhances biocrust benefitting soil fertility.

Figure 8.4.1 (a) C and (b) N across all microsites and treatments in red yellow earth for top 1 x cm at Wambiana

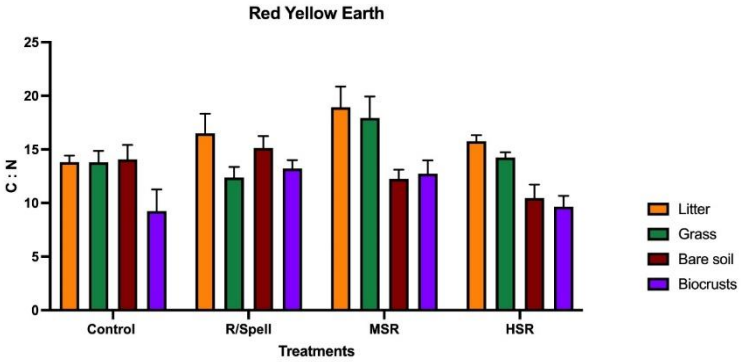


(a)



(b)

Figure 8.4.2 C/N ratio for red yellow earth across all microsites and treatments.



In summary C/N ratios across microsites and soil types ranged from 8–20 with lower values in biocrusts, similar to previous studies in the Australian rangelands (Waters et al., 2015).

8.4.2 Duplex clay N and C stocks (kg/ha)

In the duplex clay the overall C/N ratio was higher than the red yellow earth (Fig. 8.4.2). This is indicative of the microbial activity across the various microsites in breaking down nutrients into plant-available forms. The higher values of TN and TC across all HSR treatments is more likely due to the increase in Conkerberry N inputs through litter, or no grasses to use the N??

Fig. 8.4.2 Duplex clay N and C stocks (kg/ha) in top x cm of soil microsites post-fire, post-wet season at Wambiana (2020)

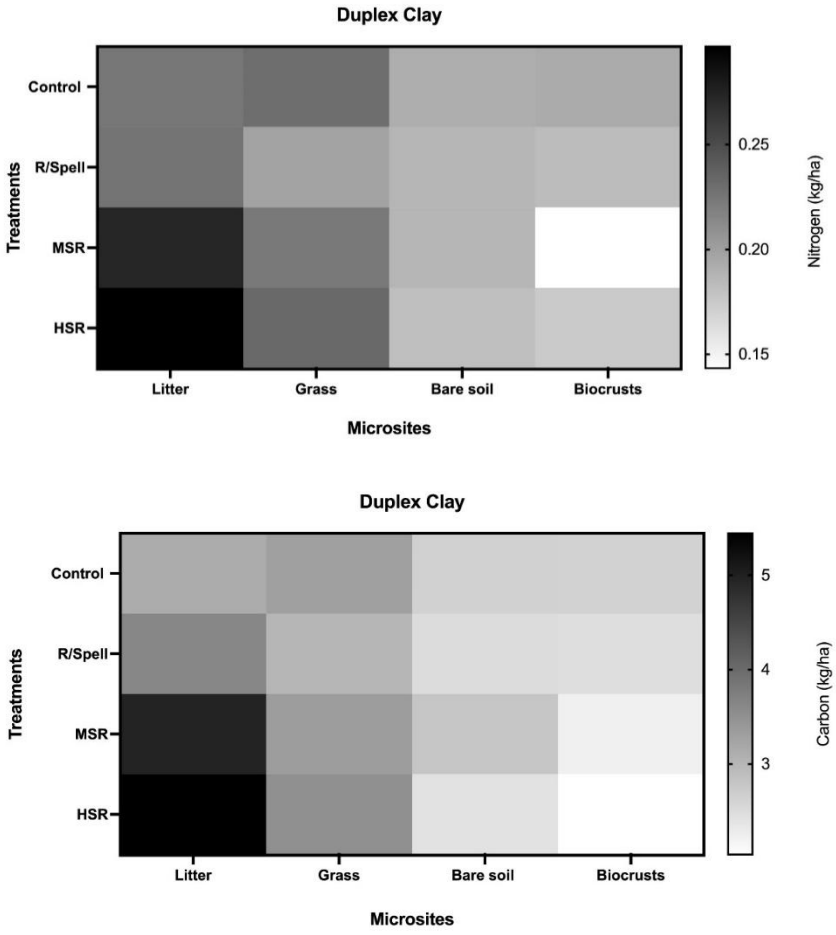
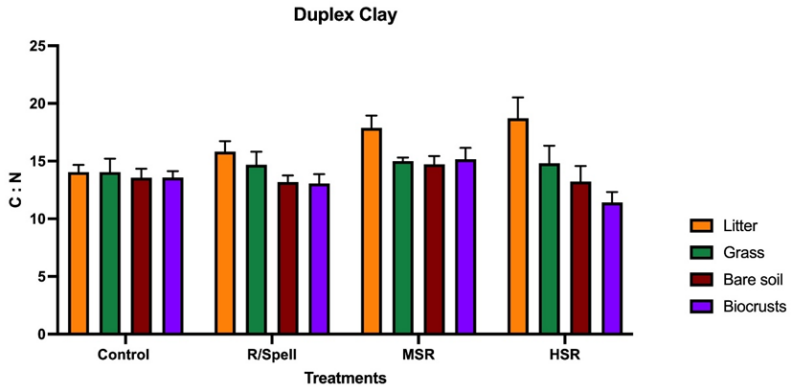


Figure 8.4.3 C/N ratio for duplex clay across all microsites and treatments.



Microsites under litter had highest stocks of C and N in heavy and moderately stocked paddocks. This could be a result of drought conditions with leaching of nutrients and high microbial activity

during small rain events that characterised the sampling year so that topsoil had increased C and N levels as vegetation did not grow sufficiently to absorb N. Biocrust microsites were the worst affected in the heavy and moderately stocked paddocks leaving ground exposed and prone to degradation. Over all treatments, C and N stocks of biocrusts and bare soil microsites were lower than the other microsites however this is expected because biocrusts cycle their own C and fix nitrogen (Waters et al., 2015). C:N ratios of microsites were relatively uniform (~12-15) across treatments. Exceptions were biocrust microsites with heavy stocking rate (C/N 9) which is indicative of loss of soil C. Heavy and moderate stocking treatments had high litter C/N ratios of 18 which are likely to be caused by vegetation adapted to low N conditions and high C/N leaves and resulting litter.

9 Fire, biocrusts and seed germination at VRRS

9.1 Seed germination and fire

This research focused on how fire management practices such as early dry season and late dry season fires could impact the selection of seeds that might germinate in areas of biocrusts as opposed to bare soil. The overall aims for this project were to investigate the relationships between fire, biocrusts and seed germination in two different soils. Seeds used are listed in Table 9.1.1.

We hypothesized that the number of plants germinating in the biocrust samples would be greater in the plant patches rather than the interspaces since the seeds may fall nearby. The number of germinants was also hypothesized to be greater post fire as we anticipated the fire would play a role in breaking dormancy.

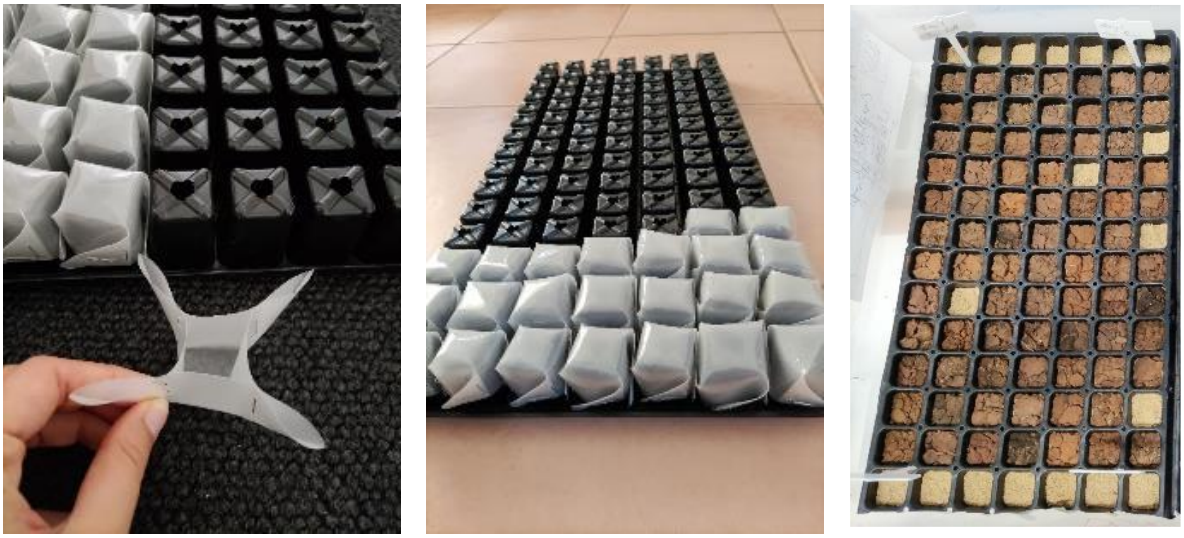
9.1.1 Greenhouse seedbank germination

Eight small pot trays were prepared to fit into four plastic boxes. One row at each of the long ends was cut off to leave seven by fourteen pots remaining on each tray. Plastic laminate sheet offcuts were used to make folded bases for each individual pot as they each had a hole in the base. A 10 cm x 10 cm piece of laminate was folded and stapled at the top of each of the four folded sides to slide onto a pot (Fig. 9.1.1).

Table 9.1.1 A list of species used in the experiment and their known dormancies. Some seeds had no published information on germination.

Species	Common name	Embryo	Germination stimulants
***Dichanthium fecundum* S.T.Blake**	Curly bluegrass		Smoke
Heteropogon contortus L. Beauv	Black Spear grass	Immature – require after ripening for 12-15 months	Nitrate, smoke, gibberellin
Dichanthium sericeum A. Camus	Silky bluegrass	After ripening for 2-24 month	Fluctuating temperature, smoke
Mnesithea formosa R. de Koning & M.S.M. Sosef	Silky top		
Sehima nervosum Stapf	Rat-tail/ White grass		
Eriachne obtuse R.Br.	Northern Wanderrie / Wire grass	After ripening for ~12 months	
Brachyachne convergens F. Muell	Native Couch		
Chrysopogon fallax S. T. Blake	Ribbon grass		
Enneapogon avenaceus C. E. Hubb	Bottle Washer		
	Cane grass		
Waltheria sp.			
Eulalia sp.			

Figure 9.1.1 Images of preparation of pot covers and example showing Box 1 containing the biocrust samples of the Calcarosol soil (reps 1-3).



Once every pot base was covered the trays were placed into the clear plastic boxes. Each pot was filled with sand up to 2 cm from the top. The biocrust samples were placed on top of the sand in each pot to cover it entirely. They were arranged randomly within blocks of replicates. The first and last row of each tray was left as sand only pots. The first four rows then contained the first replicate of each treatment, the next four rows the second replicate of each treatment, and the next four

rows the third replicate of each treatment in the first box for each soil. The second box had two rows on each end left as sand only pots as well as two in the middle as there was only a block of the fourth and one of the fifth replicate of each sample left for these trays (Fig 9.1.2) Within each of the replicate blocks there were also four sand only pots.

The pots were then watered with 20 ml of DI water each, which saturated the biocrust samples. The boxes were then transported onto a glasshouse bench at the University of Queensland, St Lucia. The pots were checked every day for seedling emergence, which was initially recorded by placing a coloured toothpick next to the emerged plant. The pots were watered on alternate days or as required. After the cooling system had failed to work on a hot day and most plants died, subsequent seedlings were removed after being recorded as having emerged. The pots were last watered after 15 days and left to dry subsequently to be stored back in the laboratory. Biocrust cover was observed and given a number from 1 to 4 where cover was estimated to be: 1 = 0-25%, 2 = 25-50%, 3 = 50-75%, and 4 = 75-100% biocrust cover in each pot (Fig. 9.1.3).

Figure 9.1.2 Example of biocrust cover. Biocrust cover was estimated from 1-4 for the pots from left to right.

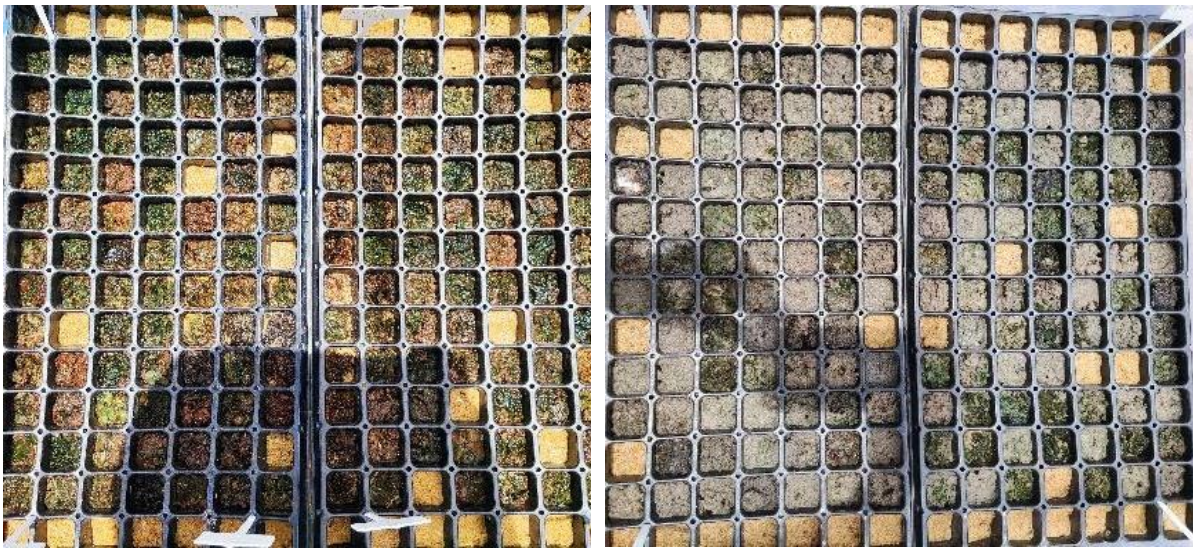
9.1.2 Statistics

The zero-inflated Poisson model was used to analyse the data. All analyses were conducted using R version 4.0.4 (R Core Team, 2021).

9.2 Results: Germination before and after fire with biocrusts

The microcosms successfully grew easily distinguishable biocrusts and germinated seeds across both soil types while there was no indication of contamination in the bare sand controls (Fig. 9.2.1).

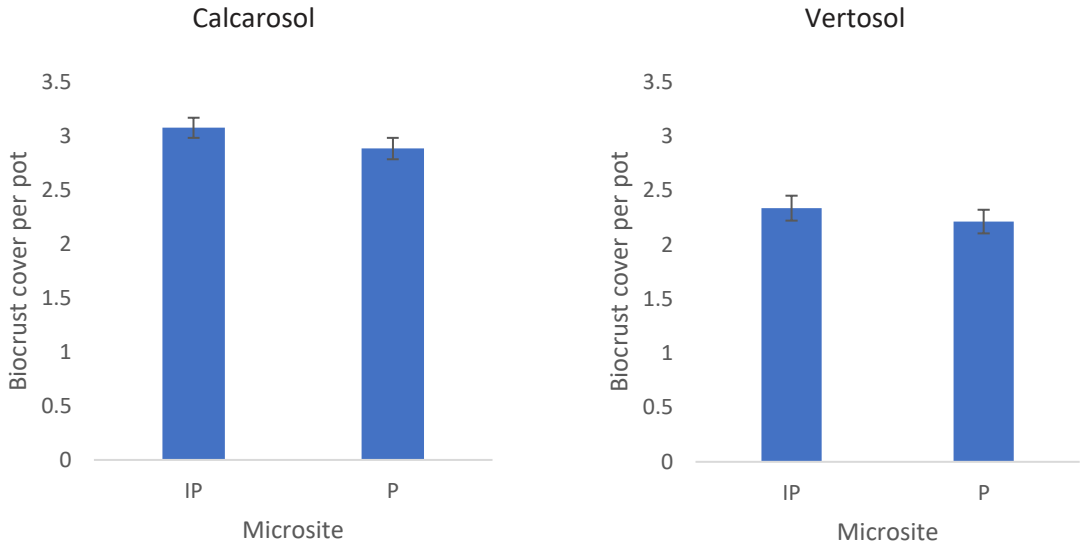
Figure 9.2.1 Replicates containing the biocrust samples of the Calcarosol soils (left) and Vertosol soils (right)



9.2.1 Biocrust cover and seed germination

The estimated mean biocrust cover per pot did not vary between the interspaces (IP - interpatches) and the grass patches (P) in both soils (see Figs. 9.2.2 a,b). Nevertheless, mean biocrust cover was slightly higher on the interspaces compared to the grass patches on the calcarosols (IP mean = 3.08 ± 0.09, $p > 0.05$, and P mean = 2.89 ± 0.10, $p > 0.05$) and vertosols (IP mean = 2.34 ± 0.12, $p > 0.05$, and P mean = 2.22 ± 0.11, $p > 0.05$). Biocrust cover was less overall on the vertosol.

Figure 9.2.2 (a) Mean biocrust cover in the interspaces and patches (p > 0.05) on the Calcarosol. Error bars denote standard errors. (b) Mean biocrust cover in the interspaces and patches, (p> 0.05) on the Vertosol. Error bars denote standard errors.

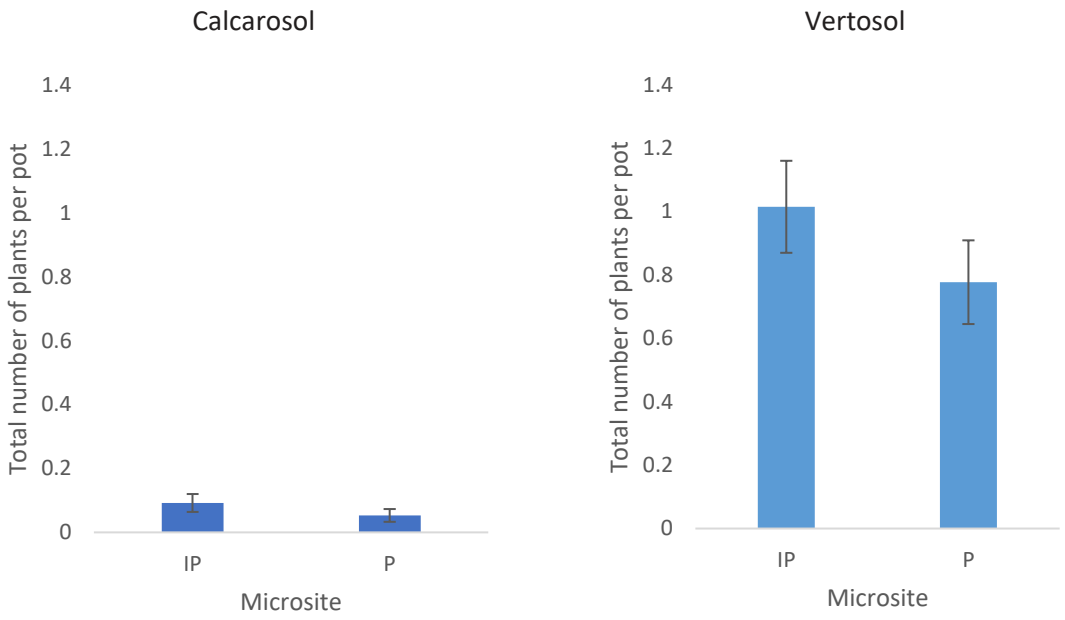


(a) (b)

9.2.2 Microsite germination

The mean number of plants germinated per pot was significantly greater in the interspaces, for the calcarosol samples (0.09 ± 0.03 and 0.05 ± 0.02 for interspaces and patches respectively p = 0.013) (Figs. 9.2.3 a,b), but not in the vertosols (1.02 ± 0.15 for the interspaces and 0.78 ± 0.13 for the patches, p > 0.05). The calcarosols had a much fewer plants germinate compared to the vertosols.

Figure 9.2.3 (a) The mean number of germinants in the interspaces were significantly higher than the patches (p = 0.013) on the Calcarosol. Error bars denote standard errors. (b) The mean number of germinants in the interspaces was not significantly different from the patches, (p > 0.05) on the Vertosol. Error bars denote standard errors.

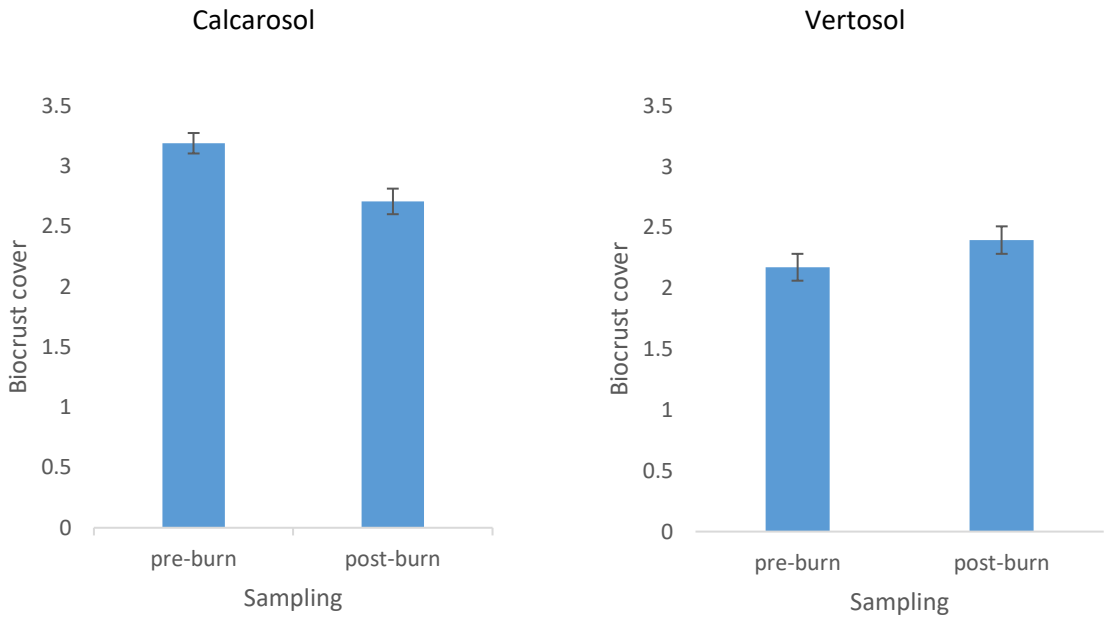


(a) (b)

9.2.3 Biocrust cover in pre and post fire samples

The estimated mean biocrust cover did not differ significantly between samples collected before the burn in 2019 and samples collected after that fire (Figs. 9.2.4 a,b). It was slightly higher for pre-burn samples in the calcarosol (post-burn mean = 2.71 ± 1.11, pre-burn mean = 3.20 ± 0.09, p > 0.05) but slightly lower for those samples in the Vertosol (post-burn mean = 2.40 ± 0.11, pre-burn mean = 2.17 ± 0.11, p > 0.05).

Figure 9.2.4 (a) The mean biocrust cover did not significantly differ between fire treatments ($p > 0.05$) on the Calcarosol. Error bars denote standard errors. **(b)** The mean biocrust cover did not significantly differ between fire treatments ($p > 0.05$) on the Vertosol. Error bars denote standard errors.

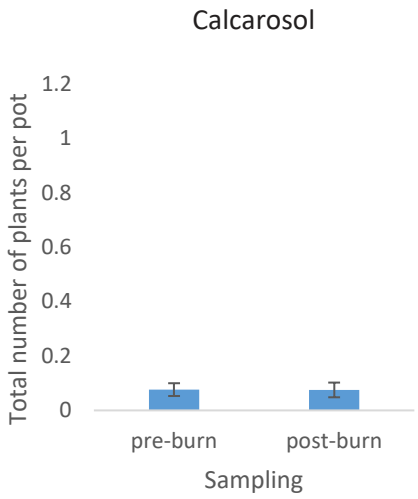


(a) (b)

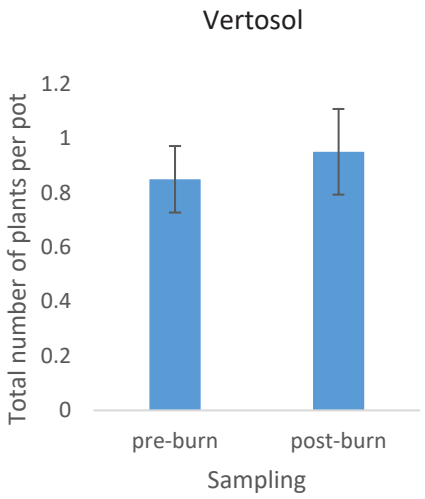
9.2.4 Germination pre and post fire

There was no significant difference in germination for the Calcarosol samples, whereas in the Vertosol the mean number of plants germinated per pot was slightly but significantly greater for samples collected post burn than the ones collected before the fire (Figs. 9.2.5 a,b). The Calcarosol had many fewer plants germinate (post-burn mean = 0.08 ± 0.03, pre-burn mean = 0.08 ± 0.02, $p > 0.05$) than the Vertosol (post-burn mean = 0.95 ± 0.16, pre-burn mean = 0.85 ± 0.12, $p = 0.01$).

Figure 9.2.5 (a). Mean number of germinants pre- and post-fire in the Calcarosol were not significantly different (p> 0.05). Error bars denote standard errors. (b) The mean number of germinants post-fire were significantly higher than before burning (p= 0.010) on the Vertosol. Error bars denote standard errors.



(a)



(b)

10 Rapid assessment of ground cover with mobile phones at VRRS

This work has been published and the methods are presented as an abbreviated version.

(Swe et al., 2023; Appendix 5).

10.1 Field measurements

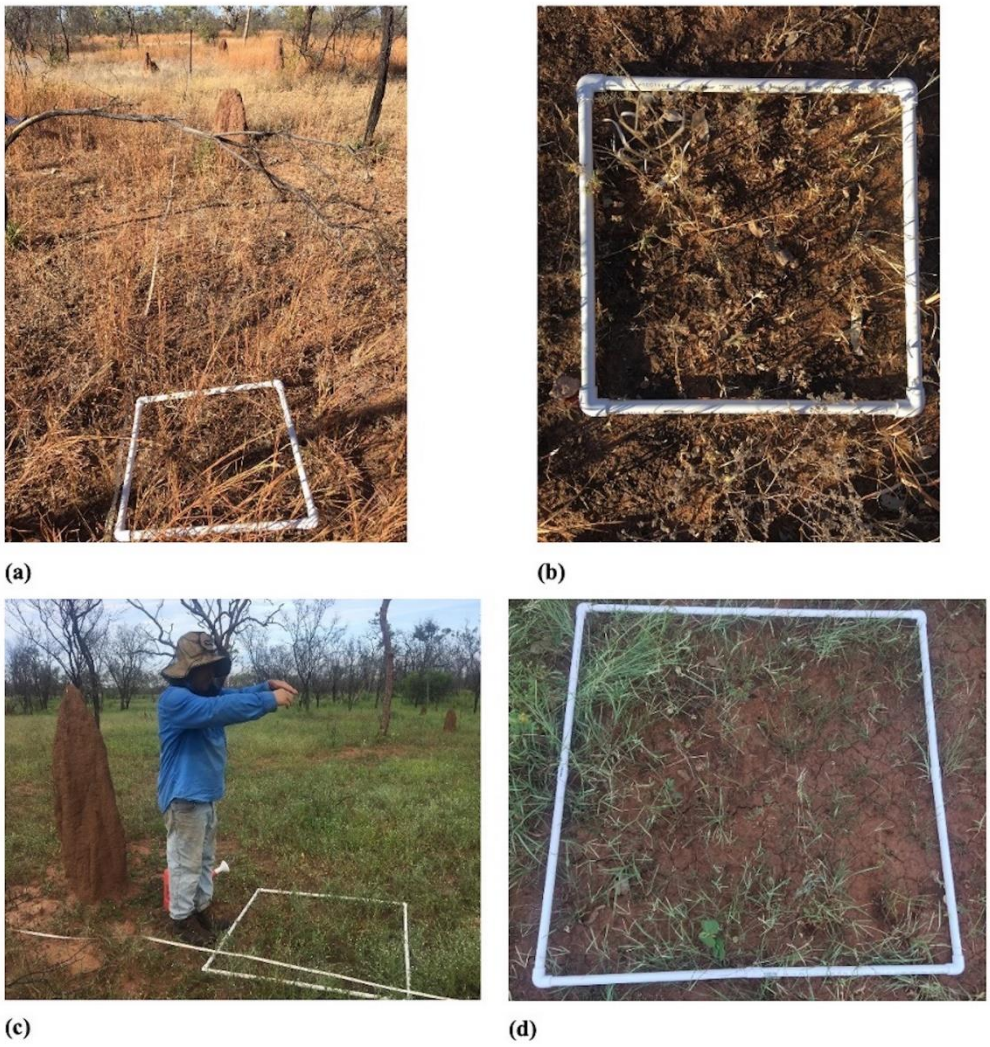
10.1.1 Biocrusts and ground cover

To assess ground cover, two 30 m transects were set up at all fire treatment sites (early and late fire season with 2, 4, and 6-years interval) and unburned control plots. Sites were sampled mid wet season in February 2020 and in the dry season in July 2020 after all treatments were burnt in June or October 2019. Due to limited access in the mid-wet season, measurements were carried out for one plot per treatment (n=7), while two plots per treatment were sampled in the dry season (n=14, full season recovery). At each treatment plot, one square meter quadrats were placed at five-meter intervals alongside the 30 m transect. Overall cover of grass and small shrubs were visually estimated for each quadrat (Fig. 10.1.1); ground cover, including under grass and shrubs was also measured. Biocrust, bare soil, litter and basal area of the grass cover were visually estimated and recorded as a percentage relative to total cover. The four measurements made up 100% of the ground cover whereas the overall grass cover was measured separately. Field observers were trained over several quadrats to assess cover attributes and data calibrations were carried out by experienced biocrust researchers. Note that this assessment was only done for the calcarosol soil as there was a greater colour contrast between the red soil and black coloured crust.

10.1.2 Biocrust field collections and pigment analysis

Three micro-cores (3.56 cm^2 x 1 cm depth) of biocrusts (either visible or bare) were collected from all treatment microsites (biocrust, grass, bare ground). Biocrust under litter was only collected during the dry season (July 2020) as there was not enough litter mid-wet season as this had recently followed burning. The samples were dried and individually stored in sealed Falcon tubes and analysis was undertaken within two weeks of sample collection. Prior to extraction, the samples were moistened with equal amounts of water to facilitate cell rehydration. Biocrust pigment content, based on chlorophyll concentrations (a, b +c) were carried out with a two-hour dark extraction with a 1:5 ratio of di-methyl-sulfoxide (DMSO) (Barnes et al., 1992) and calculated using Wellburn's (1994) equations.

Figure 10.1.1 (a-d) 1 m^2 quadrats used for visual and digital estimation of ground cover attributes.



10.1.3 Taking RGB photos in the field.

RGB photos of each quadrat were collected for use in EasyPCCr to determine ground cover attributes. In each plot, photos for the one square meter quadrat were taken at about 1.5m height using a mobile phone (iPhone 11 pro) (Fig 10.1.1 c, d).

10.1.4 Quantifying ground cover using decision tree-based segmentation model (DTSM)

The photos were processed to obtain the cover of grass, biocrusts, litter and bare soil, using a decision tree-based segmentation model (DTSM). The model uses a Classification and Regression Tree (Barnetson et al. 2019) algorithm to create a series of nodes to stepwise discriminate the photo

elements under different light conditions. Variables of colour in this model includes red (R), green (G), blue (B), hue (H), saturation (S), value (V), lightness (L*), red/green intensity (a*) and yellow/blue intensity (b*). It covers the differences in colour space and texture information and works well in situations with relatively simple background classes. The study used the EasyPCCr tool, which provides a graphical user interface (GUI) for implementing the model from creating sample data, model training and generating classification maps (Guo et al. 2017).

Table 10.1.1 Sampling fire treatments used for digital analysis.

Sample Timing	Soil Type	Fire Interval (years)	Plots (treatments)	Fire season	Total quadrats treatment (m^2)	Total number analysed
Mid wet season (Feb 2020)	Calcarosol	2,4,6, unburnt (control)	7	Early (cool), Late (hot) (n=2)	12	7 x 2 x12 = 168
Dry Season (July 2020)	Calcarosol	2,4,6, unburnt (control)	7	Early (cool), Late (hot) (n=2)	24	7 x 2 x 24 = 336

10.1.5 Preparation of photos and defining Region of Interest (ROI)

For each RGB image, the entire frame was used for locating the training pixel and then creating a training dataset for each cover type. The image was then cropped to the quadrat frame to be fed into the model for comparisons across photos. This was done using ROI tool in EasyPCCr. The training dataset was generated for individual photos due to different light conditions and the DTSM segmentation was also applied to each image.

10.1.6 DTSM application and accuracy evaluation

Acquiring good training data is a crucial part of the analysis. The cover classes in the photos were selected for training data by selecting the relevant pixels in individual photos (Fig. 10.1.2). The vegetation cover included green leaves and stems of annual and perennial plants. The identification of litter was carried out for debris including stones, dry branches, and dead grasses. Biocrusts were identifiable by their dark colour (pigment) compared to the natural red colour of the soil. Only visible biocrusts from the photos were used in this study. Biocrust located underneath dense grass cover

could not be determined in the RGB image analysis as it was obscured. Bare soil were areas that had no distinguishable biocrust on the soil surface.

The DTSM algorithm was adopted to create the decision tree, and the noise reduction filter (size 100) was applied to reduce the misclassification of the training photos. This step is needed to enhance photo quality and thus increase accuracy of the classification and segmentation of individual classes generated by the model. The analysis was carried out in the EasyPCCr tool that showed the output of percent cover (%Cov) of individual classes in each photo (Fig 10.1.2). DTSM generates individual class data as cover portions that total 100% and are generated as surface cover for each training photo.

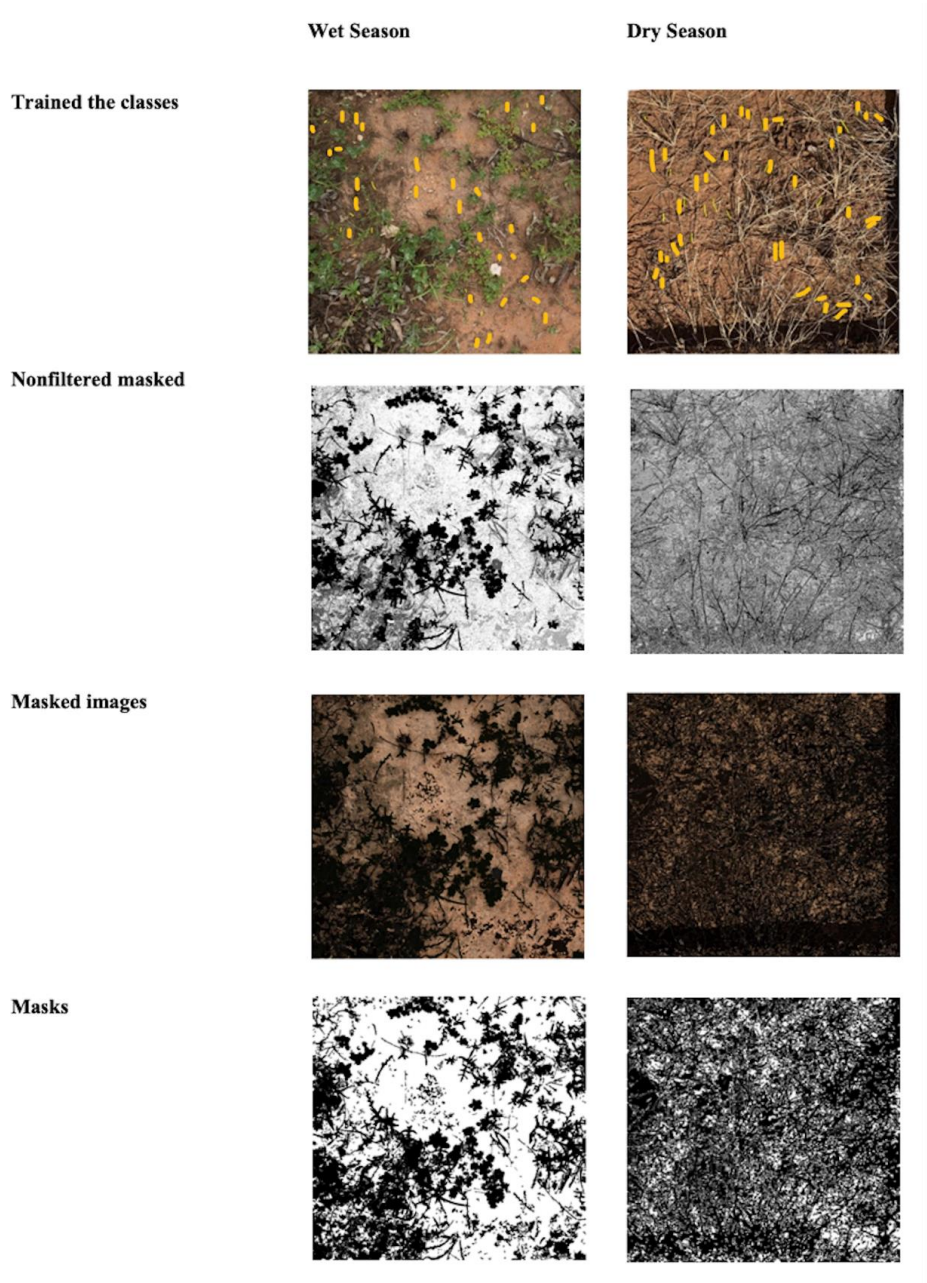
The verification process is an important step that determines the accuracy of an algorithm model or machine learning tool. In this study, verification of the model was implemented by comparing statistical analysis data between DTSM generated ground cover and the field collected ground cover. The DTSM step in EasyPCCr generated the four ground cover classes: grass cover, litter, visible biocrust and bare soil, which were identified in training photos. This information provides the means to analyse the effects of fire treatments and how the landscape has recovered.

10.1.7 Statistical analysis

For each grass, litter, visible biocrust, bare soil measurement, a single value was generated for each plot by averaging values from all quadrats for wet and dry seasons derived from individual experimental plots. This provided two values (mid-wet season and dry season) per treatment. The research data were analysed using R-studio statistic software (Su et al. 2021). We used DTSM to analyse the grass and litter combinations within one-metre square and the field observed biocrust percentage cover (%Cov ± SE) for both mid-wet season and dry season. In the case of the field observations grass and litter cover were more than 100% because litter could occur under the grass canopy. The grass and litter combined cover was then analysed to determine the difference between DTSM and field measurements. Visible biocrust detected in training photos in DTSM was assigned by comparing the biocrust in the photos with field data. Biocrust %Cov was analysed in R statistics software. The combination of grass and litter data was analysed using three-way analysis of variance and Tukey's honestly significant difference post hoc test (HSD) to identify the significant differences. A simple linear regression analysis was applied to explore the relationships between the various

ground covers in both seasons. Biocrust pigment content (Chla) for microsites was calculated using one-way analysis of variance and Tukey's HSD and the heap maps.

Figure 10.1.2 Training quadrats for different ground cover attributes with the lines representing the identification of classes and the non-filtered masked and masks for images illustrating the outcomes.



10.2 Results: Modelled seasonal cover

In the wet season, there were no significant differences between the modelled cover and the field measurements ($P > 0.05$). There was, on average, a difference of 8.4%Cov between modelled (48%Cov ± 2.54) and field measurements (56%Cov ± 3.53; Fig. 10.2.1). Across the early season burning treatments, the average difference in modelled grass cover (49.4%Cov ± 8.81) was 8.2% lower than field observations (57.6%Cov ± 8.81). In the wet season, modelled grass cover in the late season burning treatments was lower than the field observations. In the unburnt control plots, the modelled grass cover was lower than that in the field data.

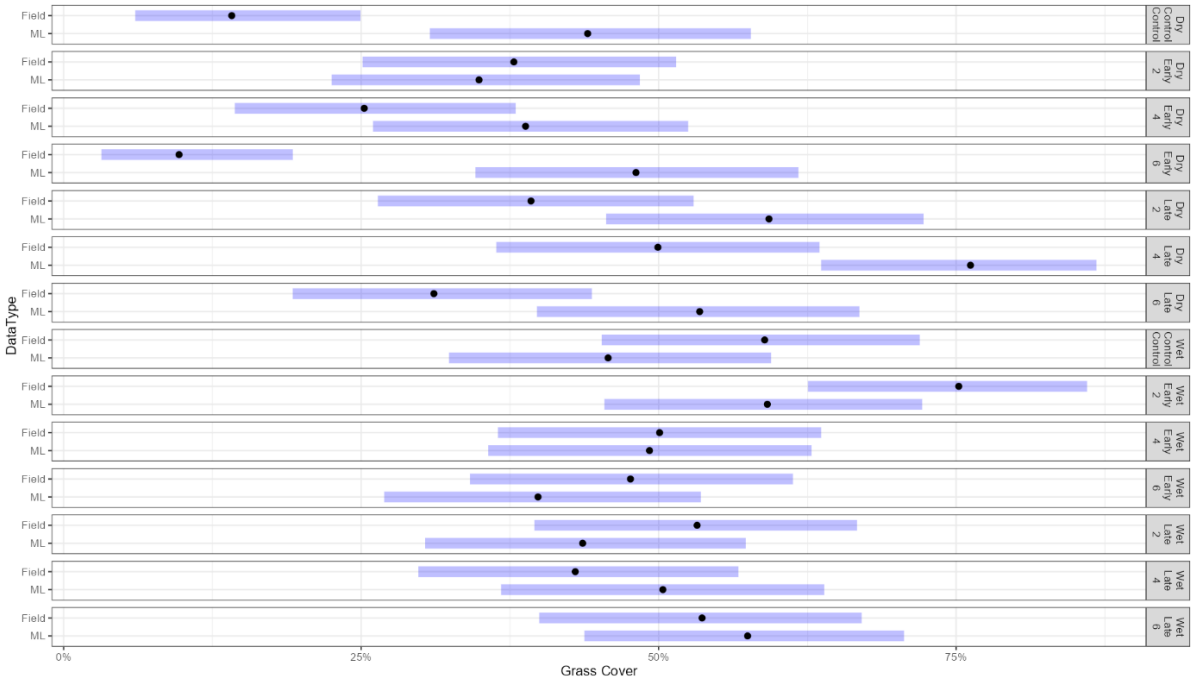
In the dry season, there were significant differences between modelled and field methods in estimated grass cover. Modelled grass cover was higher than field observation across fire treatments and control plots ($P < 0.01$). For example, in the 4-year early burn plots, grass cover (modelled) was 38.8%Cov, with an average difference of 13.5%, compared to field data. In contrast, modelled grass cover for the 2-year early burn plots (34.9%Cov ± 2.52) was a near match to field observations (37.8%Cov ± 6.48). However, there were no significant differences ($P = 0.07$) between modelled and field data across the early burning treatments, except for the early 6-year burnt plots ($P < 0.001$). In the late-burn plots, the modelled grass cover for the late 2-year and late 4-year plots was 59.3%Cov (±1.8) and 76.2%Cov (±7.61) respectively, and significantly ($P < 0.001$) different from the field observations of late burn every 2-year plots, 39.3%Cov ± 6.6, and late burn every 4-year plots 49.9%Cov ± 7.15, (Fig. 10.2.1). Field observations of grass cover in the wet season (56% ± 3.53) were higher than those in the dry season (30% ± 5.41), whereas modelled grass cover in the wet season (48% ± 2.54) was lower than that in the dry season (51% ± 5.29).

10.2.2 Modelled grass and litter cover compared to field measurements

When grass and litter cover were estimated together, there was greater similarity between methodology estimates. For the wet season, grass and litter %Cov derived from photographs averaged 8.8% (±2.6) across all treatments and was comparable to field measurements, with an average of 1% difference between them (Fig. 10.2.2). We also measured grass and litter cover across fire-plot treatments in July 2020 during the dry season (rain ceased in May). Modelled grass and litter %Cov across all treatments was 20.9% (±0.6), compared to field observations of 22.21% (±0.32). Furthermore, no significant differences were observed between fire treatments and the unburnt control plots in either wet or dry seasons ($P = 0.99$; Fig. 10.2.2) where there was an average

cover difference of 1%. Both field observations and modelled grass cover in the wet season were slightly lower than those in the dry season.

Figure 10.2.1 Comparisons of mean grass cover between field data and machine learning in February 2020 and July 2020. Bars that do not overlap are significantly different.



10.2.3 Relationships between microsites

In both seasons, when grass and litter cover were analysed in combination, as their cover increased, the modelled biocrust cover decreased (Fig. 10.2.3). There was a strong inverse relationship between grass and litter cover compared with visible biocrust cover in both the wet (R2 = 0.95) and the dry seasons (R2 = 0.95; Fig. 10.2.3). Additionally, biocrust from the field observation decreased when the combined grass and litter coverage in the plot increased, especially in the dry season (R2 = 0.80). DTSM can also distinguish between biocrust and bare soil in both seasons (wet, R2 = 0.15; dry, R2 = 0.09). Yet, the relationship between bare and biocrust from field observations in the wet season showed that as bare cover increased, the biocrust cover decreased (R2 = 0.80).

Figure 10.1.2 Comparisons of grass and litter combined as mean cover between field data and machine learning (ML) in the mid-wet season (February 2020) and dry season (July 2020). Bars that do not overlap are significantly different.

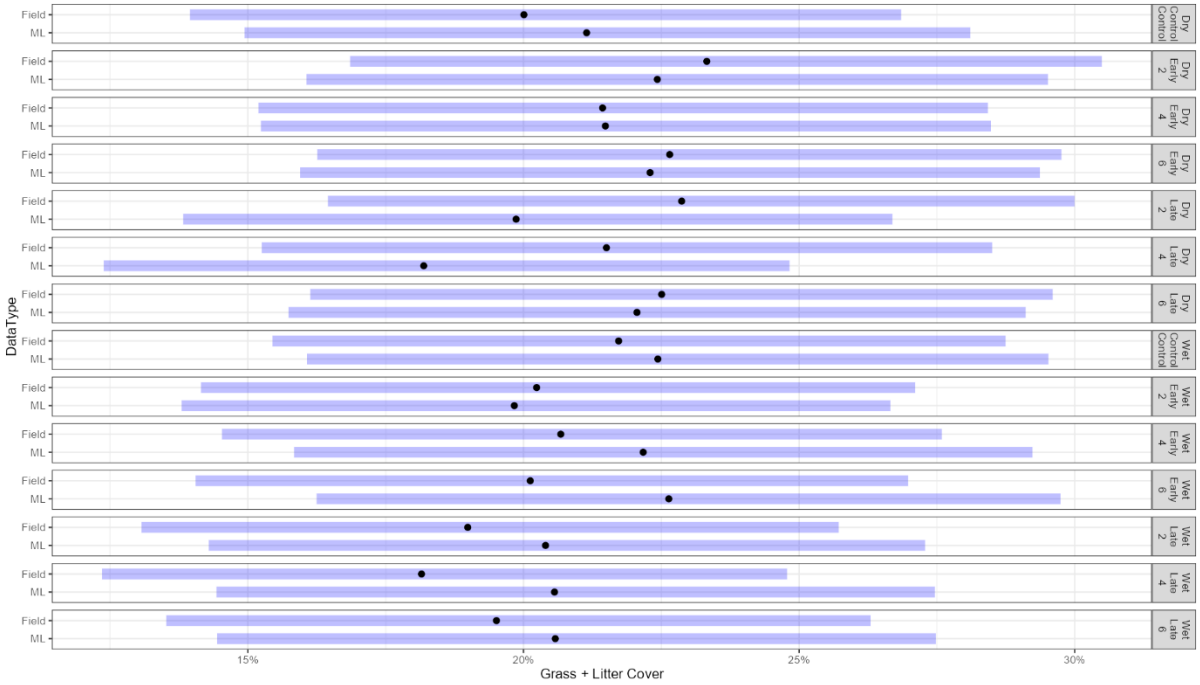
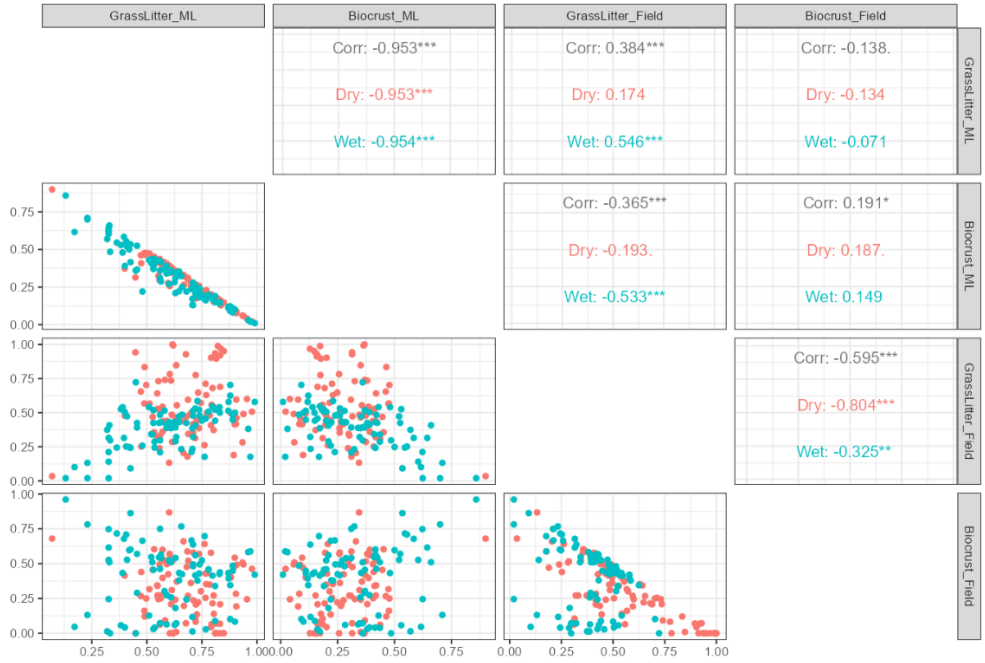


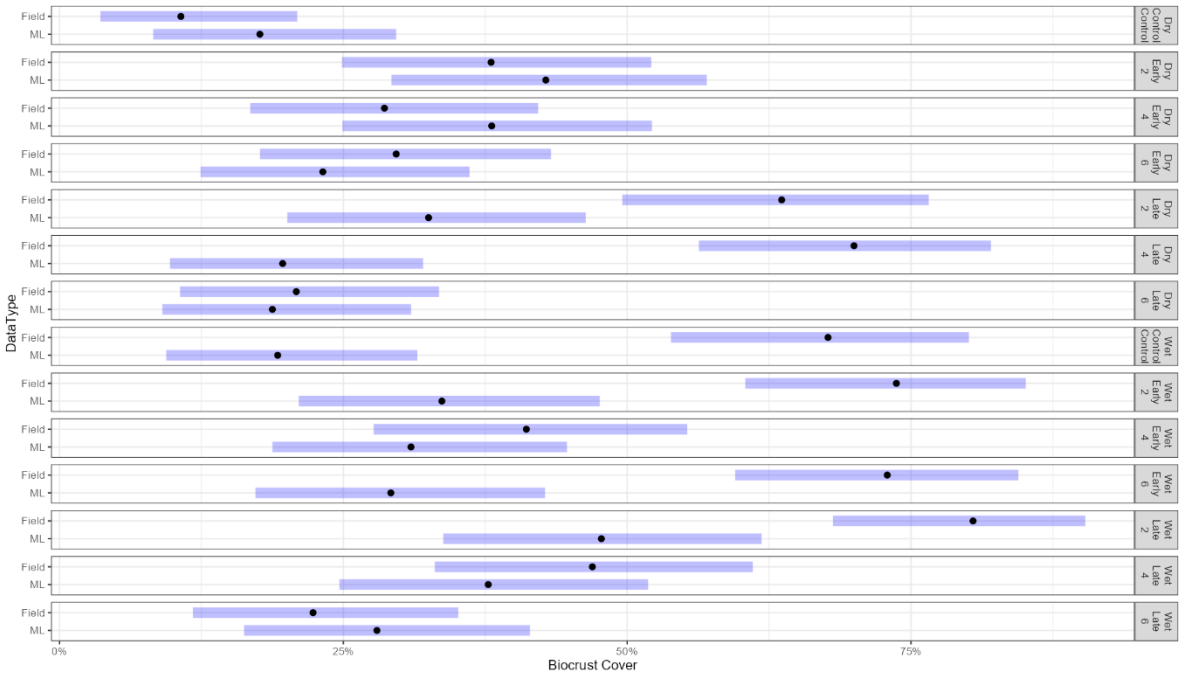
Figure 10.2.3 Relationship between ML and field observations for grass, litter, and visible biocrust in February 2020 and July 2020, postfire recovery. *Asterisks represent the level of significance. ML, machine learning.



10.2.4 Modelled biocrust cover compared to field measurements

In the wet season, modelled biocrust cover (34%Cov ± 2.0) differed significantly from field measurements when averaged across all treatments (39%Cov ± 3.2; P = 0.04; Fig. 10.2.4). These differences were more obvious in early burning treatments, such as the 6-yearly early burning treatment (P < 0.01) where the modelled cover was, on average, 20% lower than the observed field result (Fig. 10.2.4). Furthermore, in the unburnt control plots, detected biocrust cover (20%Cov ± 2.1) was also significantly lower than field observations (57%Cov ± 5.8) (P < 0.01). In contrast, modelled biocrust cover (33%Cov ± 3.3) was comparable with field observations (42%Cov ± 6.2) in late-burning treatments (P = 0.21; Fig. 10.2.4). In the dry season, modelled biocrust cover across all burnt and unburnt treatments was comparable to field observations (Fig. 10.2.4). Biocrust cover in early burning plots averaged 30%, with no significant differences between DTSM-modelled cover and field observations. Similar data were generated for modelled biocrust cover on control plots (18%Cov ± 2.8) and field observations (15%Cov ± 5.6), although DTSM-modelled biocrust cover in late-burn plots (25%Cov ± 2.7) differed significantly from field observations (53%Cov ± 4.4). However, there were no overall differences between modelled biocrust cover and field observations in either fire treatments and control plots in the dry season (P = 0.06), with the exception of the late 4-year burning treatment (Fig. 10.2.4).

Figure 10.2.4 Comparison of biocrust recovery cover collected with trained field observers versus machine learning derived from phone images in the mid-wet season (February 2020) and dry season (July 2020). Bars that do not overlap are significantly different.

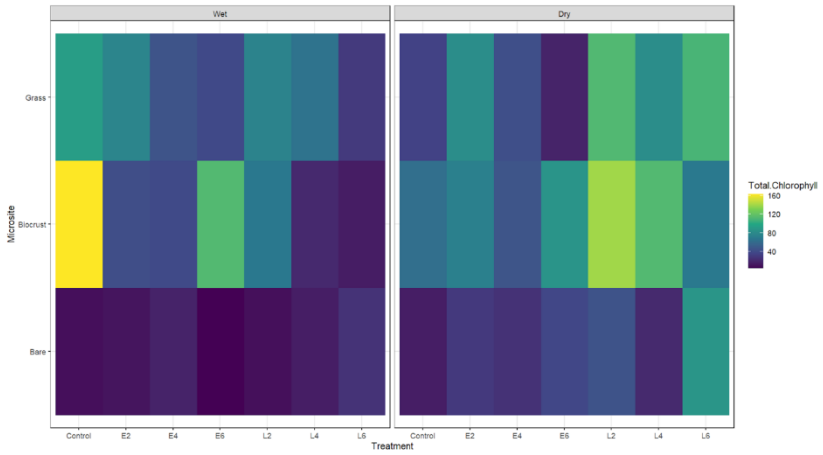


10.2.5 Biocrust pigment content across microsites

Biocrust pigment content based on chlorophyll concentration (Chl) was analysed across three microsites, i.e. biocrust, grass and bare soil in both wet and dry seasons (Fig. 10.2.5). Overall, bare soil microsites had the lowest and biocrust microsites the highest pigment concentrations. Biocrust pigment was recorded under litter but only in the dry season. In the wet season, the highest average pigment concentration was detected in biocrust microsites in the control plots (162 ± 35 mg Chl/m2 ; $P < 0.01$). In the burnt treatments, the early season 6-yearly (E6) burn plot was not significantly different from the unburnt controls (112 ± 12 mg Chl/m2 , $P = 0.35$) whereas L6 (14 ± 1 mg Chl/m2) and L4 (20 ± 3 mg Chl/m2) had significantly lower pigment concentration compared to the other treatments ($P < 0.01$), in contrast to E6 which had high concentrations in the biocrust microsites. Furthermore, in the wet season, microsites under grass canopy also had the highest pigment concentrations in the unburnt control plots (92 ± 19 mg Chl/m2) and E2 (75 ± 5 mg Chl/m2), whereas L6 (29 ± 1 mg Chl/m2) had significantly ($P < 0.01$) lower pigment concentrations in grass microsites. Microsites under grass in E6 (38 ± 4 mg Chl/m2) and E4 (45 ± 1 mg Chl/m2) had also

significantly lower pigment concentrations than did the unburnt control (P < 0.01). In the wet season, although low, L6 bare microsites had significantly (P < 0.05) higher pigment concentrations (26 ± 5 mg Chl/m2) than did other burnt bare microsites, including E2 (10 ± 2 mg Chl/m2), L2 (8 ± 2 mg Chl/m2), unburnt control (8 ± 2 mg Chl/m2) and E6 (2 ± 2 mg Chl/m2 ; P < 0.01). Pigment concentration in bare microsites E4 (17 ± 4 mg Chl/m2) and L4 (14 ± 1 mg Chl/m2) was significantly higher than E6 (P < 0.04). In the dry season, L6 had the highest pigment concentrations for all burnt treatments and in the unburnt control plots; however, they were not significantly (P = 0.16) different. The highest pigment in biocrust microsites ranged between (L6) and 128 ± 35 mg Chl/m2 (L2). The lowest pigment recorded was in E4 (45 ± 11 mg Chl/m2) and the unburnt control (61 ± 31 mg Chl/m2). Litter microsites in the control had the highest pigment (236 ± 48 mg Chl/m2) together with E4 (190 ± 116 mg Chl/m2), but both showing the large variability between sample points. L6 also had the highest pigment concentration under the grass canopy (108 ± 13 mg Chl/m2), whereas E6 had the lowest pigment concentration (17 ± 2 mg Chl/m2 ; P < 0.01). Similarly, the control had low pigment (34 ± 7 mg Chl/m2), significantly different from L2 (86 ± 5 mg Chl/m2 ; P < 0.01), L4 (104 ± 7 mg Chl/m2) and E2 (81 ± 17 mg Chl/m2 ; P < 0.03). E4 (42 ± 7 mg Chl/m2) was significantly (P < 0.01) lower than L6 and L2. Similarly, L6 had the highest pigment concentration in dry-season bare microsites (86 ± 38 mg Chl/m2) and the lowest in the unburnt control plots (14 ± 3 mg Chl/m2), although differences were not significant (P = 0.06). For example, E6 (37 ± 3 mg Chl/m2), L2 (35 ± 3 mg Chl/m2), E2 (29 ± 9 mg Chl/m2) and E4 and L4 were both 25 ± 3 mg Chl /m2 .

Figure 10.2.5 Pigment concentrations (mg chlorophyll/m2) for calcarosols at three microsites (biocrust, grass, bare soil) in the wet season (February 2020) and dry season (July 2020) for unburnt controls and early season (E) and late-season (L) fires implemented in 2-, 4-, and 6-yearly intervals.



11 Detecting biocrusts using remote sensing before and after fire

11.1 Large scale fire and grazing demonstration at VRRS

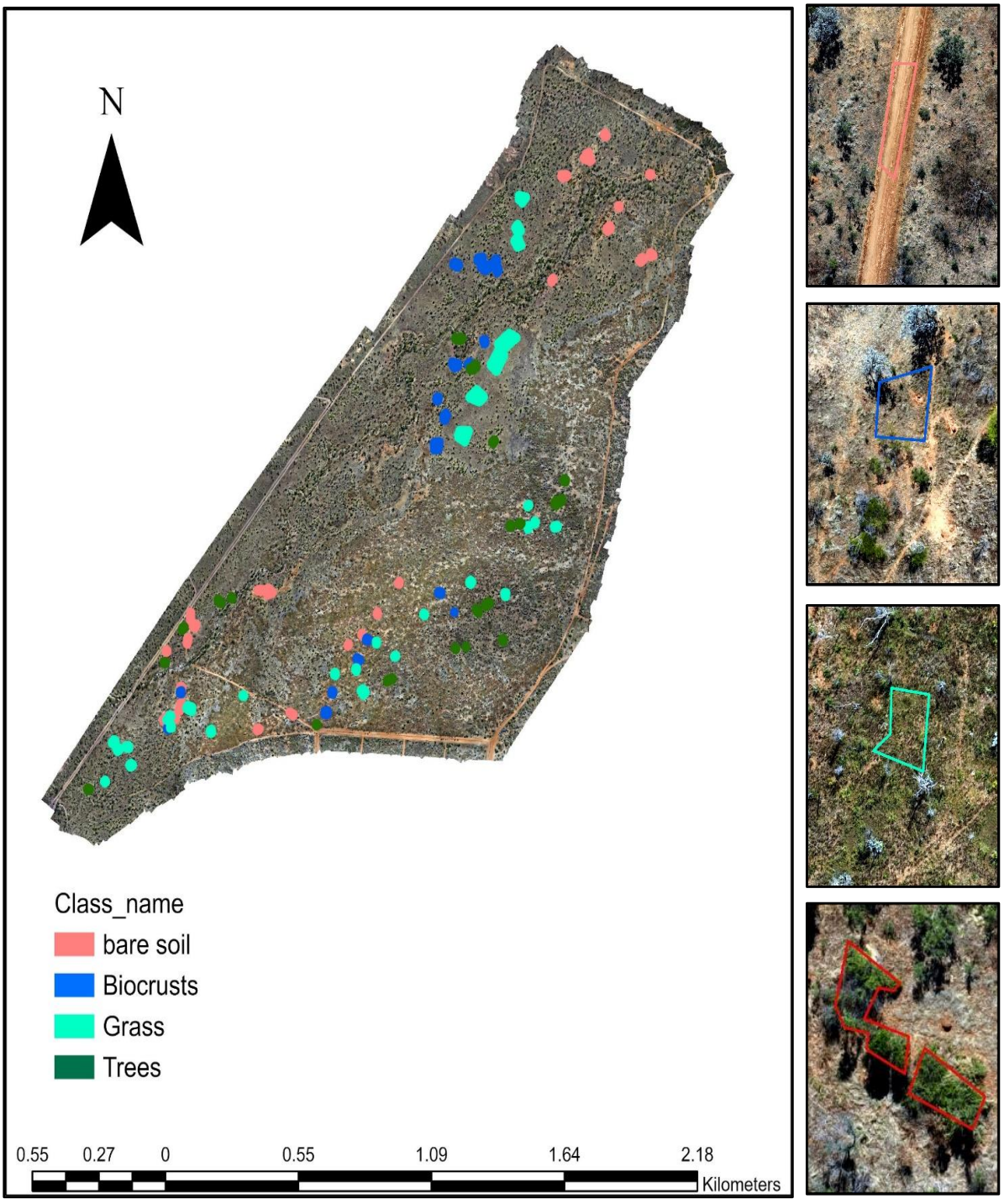
This research explored changes in land cover by analysing the spectral responses of different land cover classes both before and after fire at Victoria River Research Station (VRRS). We assessed an area of approximately 2.6 km^2 where there were many examples of bare degraded areas caused by cattle trampling and camping together with areas of pure biocrust and biocrust with grasses (Fig. 11.1.1). In October 2022 it was burnt to encourage cattle to use parts of the paddock they normally avoided and effectively spell other areas they normally preferred in an effort to manage degraded areas. This site was used as a reference site with the aim to understand the changes in land cover including biocrusts, before and after fire and after the following wet season (Fig. 11.1.2).

Figure 11.1.1 (a) A cattle camp area to be used as a reference site for bare soil without biocrusts, (b) a small area of high cover of biocrusts illustrating the erosion of the soil from the site leaving unprotected and unproductive surfaces.

(a)

(b)

Figure 11.1.2 Land classification map of large-scale fire demonstration site depicting various reference points and the four classes of land cover (bare soil, biocrusts, grasses and trees including shrubs).



Biocrusts photosynthesise the same way as plants do and contain chlorophyll, so it is possible to detect them with satellite imagery. However, they reflect specific bands that are unique but fall within the same spectrum as plants. As a result, various researchers globally have experimented and developed an algorithm to extract the exact bands that represent biocrusts called the Crust Index (CI). Nevertheless, it is necessary to check that this is relevant in our specific environment. Here we examine the changes in the spectral reflectance of biocrusts, vegetation and bare soil before and after fire using a range of vegetation indices as described below.

11.2 Data Collection, Preparation, and Processing

In the process of land cover classification, the vegetation indices, including the Normalized Difference Vegetation Index (NDVI), Optimized Soil-Adjusted Vegetation Index (OSAVI), and Enhanced Vegetation Index (EVI), were extracted from Planet Scope imagery, offering a spatial resolution of 3 meters (Fig. 11.1.2). Additionally, the Crust Index (CI) was applied to elucidate the spectral responses of biocrust in relation to the observed shifts in land cover dynamics.

11.3 Training Data Collection

To enable precise classification of land cover—specifically bare soil, biocrust, and grass—within our study area, we conducted systematic training data collection. This process involved the meticulous traversal of cross-diagonal transects in the field. To ensure high accuracy, we employed a high-precision GPS mobile phone application called Avenza Maps, which provides horizontal accuracy to within 2 meters and incorporates its own compass functionality (Design, n.d.). The GPS coordinates obtained were subsequently utilized to define training polygons for each distinct land cover class within the ArcGIS Pro geographical information system.

11.4 Preparation of Planet Scope Data

To monitor and assess grass, soil, and biocrust cover, we utilised the capabilities of Planet Scope imagery. The Planet Scope dataset offered an extensive array of eight spectral bands, which includes Coastal Blue, Blue, Green 1, Green, Yellow, Red Edge, and Near Infrared. The acquisition of Planet Scope imagery was facilitated through the use of Planet Explorer for the pre-fire event in September 2022 and post-fire event in January 2023, which was also after the commencement of the wet season.

11.5 Data Processing

In this study, we conducted an analysis of land cover classes' spectral responses within a Fire Grazed area, utilizing high-resolution (3m) Planet satellite imagery for both before and after fire events. Our data processing workflow was conducted with the following procedures.

Training Polygon Creation: We initiated the process by generating thirty training polygons that were created using GPS points and carried out within ArcGIS Pro (Version 3.0.0).

11.5.1 Sample Point Allocation

Within each of the thirty training polygons, we randomly distributed twenty sample points, resulting in a total of six hundred strategically placed sample points across the study area. This approach ensured a comprehensive representation of the land cover diversity within the Fire-grazed area.

11.5.2 Spectral Value Extraction

To obtain spectral values for the land cover classes and facilitate the calculation of three distinct vegetation indices, as well as the crust index, we employed two key geospatial tools: the "spatial join" and the "extracted multi-value to points." These tools allowed for the precise extraction of spectral information associated with each sample point, ensuring the accuracy of subsequent calculations.

11.5.3 Calculation of Vegetation and Crust Indices

To quantitatively assess vegetation and land cover changes, the following formulas were applied:

Normalised Difference Vegetation Index (NDVI) (Tucker, 1979)

$$\text{NDVI} = \frac{NIR-Red}{NIR+Red}$$

Optimised soil adjustment vegetation index (OSAVI) (Rondeaux et al., 1996)

$$\text{OSAVI} = \frac{NIR-Red}{NIR+Red+0.16}$$

Enhancement Vegetation Index (Rodríguez-Caballero et al., 2015)

$$EVI = 2.5 * \left(\frac{NIR - Red}{(NIR + 6 * Red - 7.5 * Blue + L)}\right)$$

Crust Index (CI) (Karnieli, 1997)

$$\text{Crust Index} = 1 - \frac{Red - Blue}{Red + Blue}$$

11.6 Statistical analysis

The mean value of vegetation indices, NDVI, OSAVI and EVI, and crust index, CI, were analysed to identify the overall trend of chlorophyll signal changes from September 2022 to January 2023. The research data were analysed using R-studio statistic software. The spectral value of indices was calculated using analysis of variance ANOVA and Tukey's honestly significant difference post hoc test (HSD) to examine the significance of individual indices spectral profile changes.

Note: UAV images were obtained for all plots and used for further extraction of reference sites and supervised classification for Planet Scope images (Fig. 6.1.3).

Figure 11.6.1 The VRSS drone mission was undertaken in 2022 before and after fire. All fire plots and the large-scale fire trial were mapped at 6 cm resolution.

12 Detecting biocrusts using remote sensing before and after fire.

The foundation of the spectral analysis lies in the assessment of land cover changes using vegetation and crust indices. This investigation has highlighted the changes in the spectral signature of land cover classes both before and after the fire event in the Fire Grazed area. In this research, our findings underscore the discriminative capabilities of the Enhanced Vegetation Index and Crust Index in capturing the spectral response differences among individual classes.

12.1.1 Spectral response of land cover classes before the fire event

In the vegetation indices and the Crust Index, both grass and biocrust exhibited significantly higher spectral values compared to bare soil ($p<0.0001$). Moreover, when analysing the Crust Index and Enhanced Vegetation Index, the spectral value of grass significantly exceeded that of biocrust ($p<0.001$). However, the spectral values of grass and biocrust were statistically indistinguishable in the Normalized Difference Vegetation Index and Optimum Soil Adjusted Vegetation Index ($P>0.05$), as illustrated (Fig. 12.1.1).

Figure 12.1.1 Composite images of fire, bare biocrust areas and green date with new grass growth amongst recovering biocrusts.

Figure 12.1.2 Comparative analysis of spectral values for Bare Soil, Biocrust, and Grass using Normalized Difference Vegetation Index (NDVI), Optimized Soil Adjusted Vegetation Index (OSAVI), Enhanced Vegetation Index (EVI), and Crust Index (CI_Crust Index) before burning.

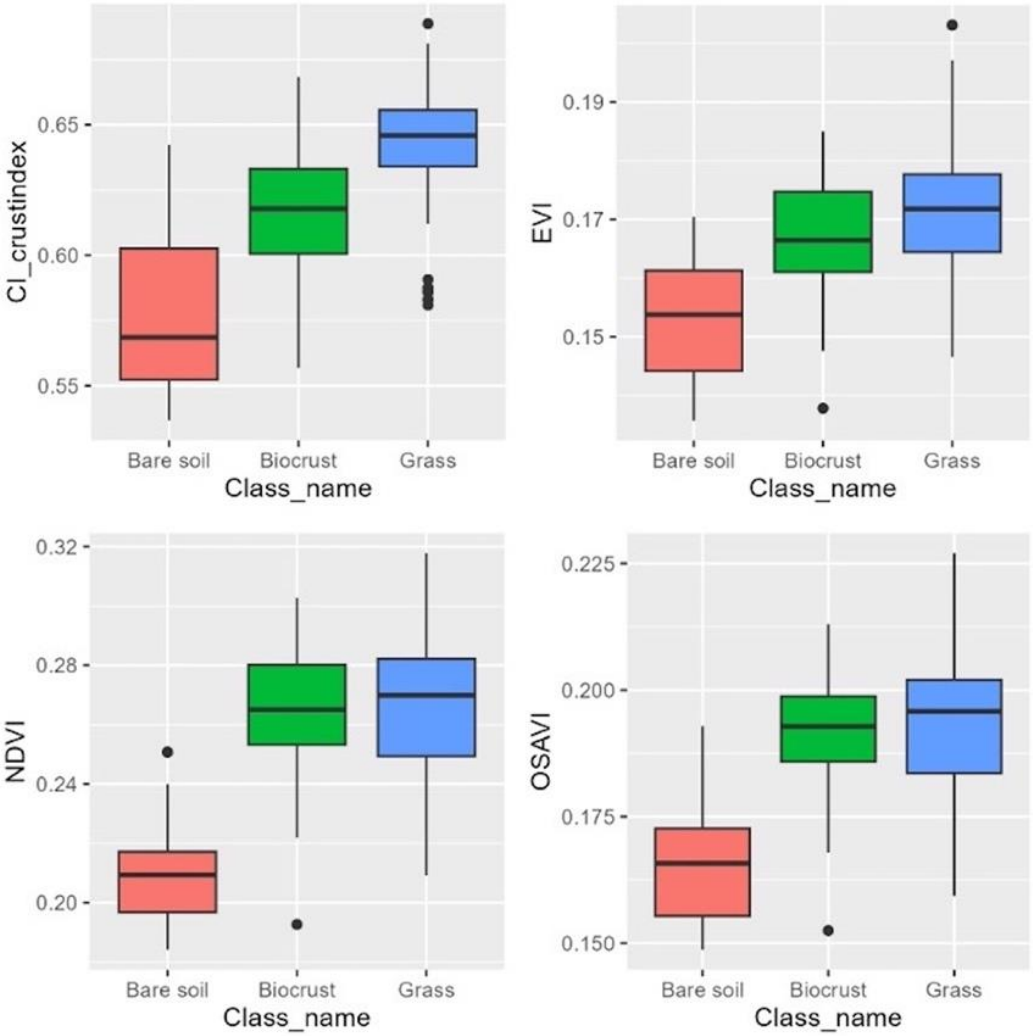
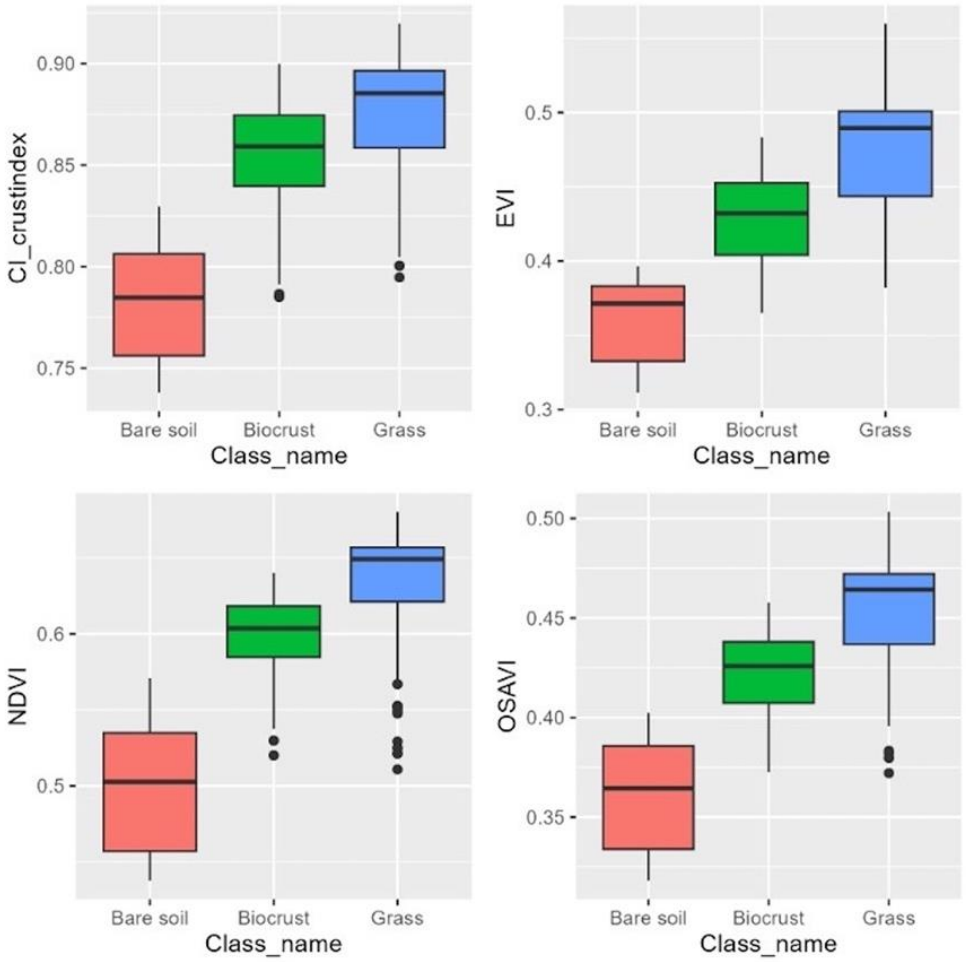


Figure 12.1.3 Comparative analysis of spectral values for Bare Soil, Biocrust, and Grass using Normalized Difference Vegetation Index (NDVI), Optimized Soil Adjusted Vegetation Index (OSAVI), Enhanced Vegetation Index (EVI), and Crust Index (CI_Crust Index) after burning.



Applying the range of spectral formulas, we determined that biocrust was clearly discernible from other ground cover when there was no grass, shrub or tree cover concealing it from view. To understand the extent of biocrust cover at the Fire-graze site, reference points were used to estimate cover underneath the grasses as well as in open areas. Furthermore, the roadways were used as a reference in the comparison of bare ground to open degraded ground. This illustrated a clear distinction between bare soil and all other landcover (Fig. 12.1.2, 12.1.3).

13 Large scale fire and grazing demonstration at VRRS

After burning and subsequent rainfall in the Fire-Graze area, both the vegetation indices and the Crust Index exhibited discriminatory capabilities among the three land cover classes: grass, biocrust, and bare soil (Fig. 13.0.1). Notably, the spectral response of grass was the most pronounced and significantly differed from that of biocrust and bare soil ($p<0.0001$) across all vegetation indices and the Crust Index. Conversely, bare soil consistently displayed the lowest spectral values across all indices and demonstrated the capacity to be differentiated from biocrust ($p<0.0001$). trees and shrubs?

Figure 13.0.1 Planet Scope satellite imagery of Fire-Graze site showing land cover attributes at 3 m resolution in September 2022 during the dry season and before fire. The biocrust (dark blue) is clearly visible during the dry season.

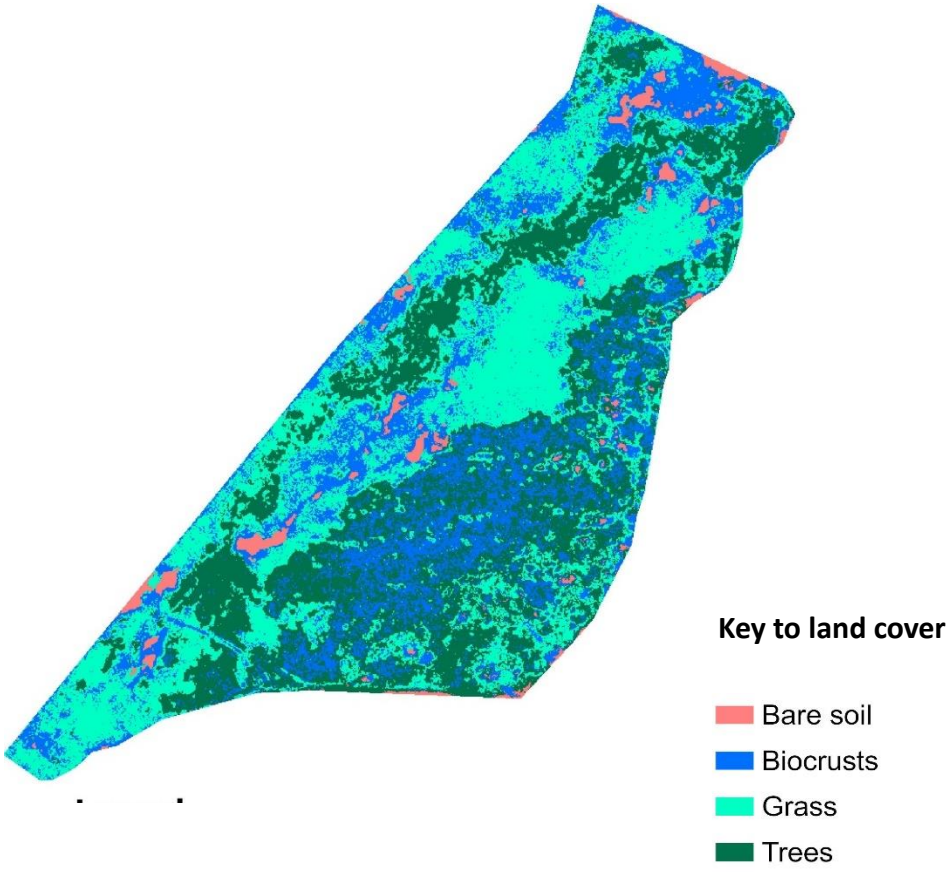


Figure 13.0.2 Planet Scope satellite imagery of Fire-Graze site showing land cover attributes at 3 m resolution in February 2023 during the dry season and after rain and June 2023 after the wet season. The biocrust (dark blue) is still visible during the early wet season. By then end of the wet season, the biocrusts have become less visible due to the grass canopy but still showing up as speckles underneath and in between grass plants.

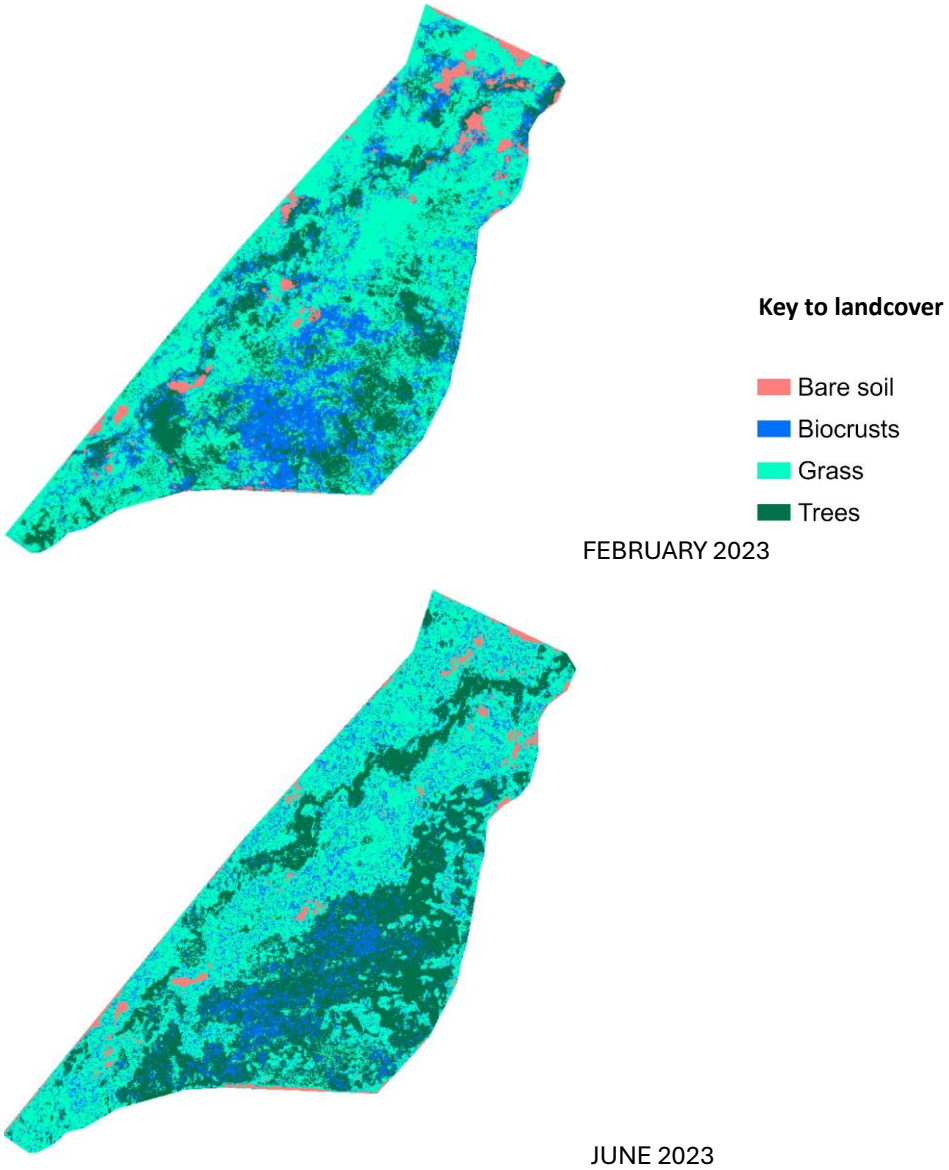


Figure 13.0.3 Seasonal changes in land cover attributes showing the wet season increases in grasses covering the biocrusts to the overhead view from the Planet-Scope images.

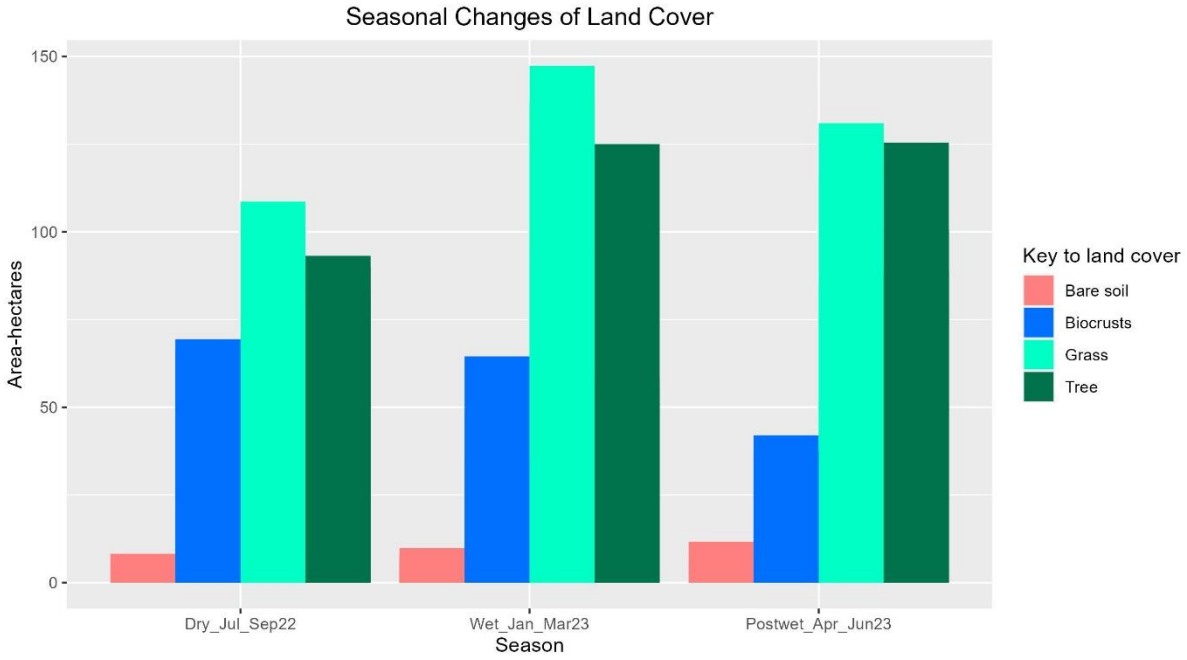
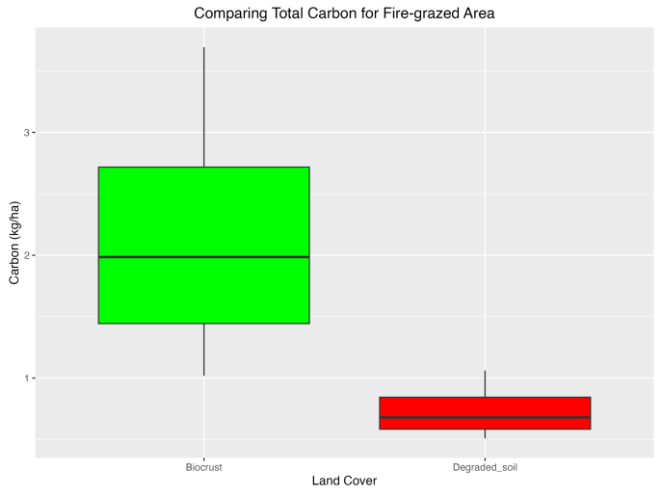


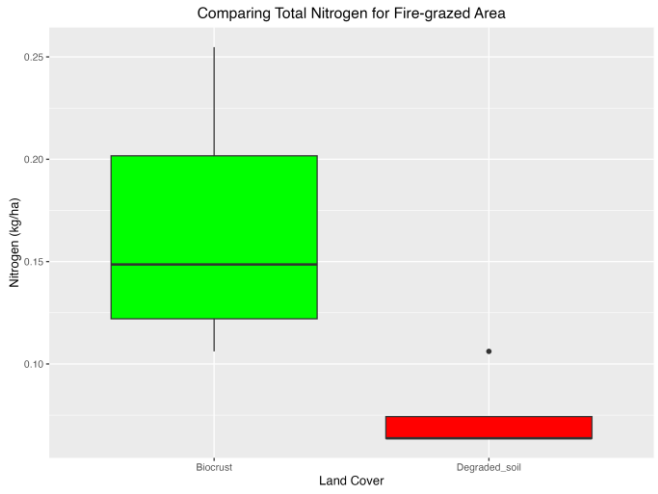
Table 13.0.1 Dry season before fire for top 1 cm: Total Carbon (TC), Total Organic Carbon (TOC) and Total Nitrogen (TN) across 2.6 km^2 Fire-Graze site (Aug 2022).

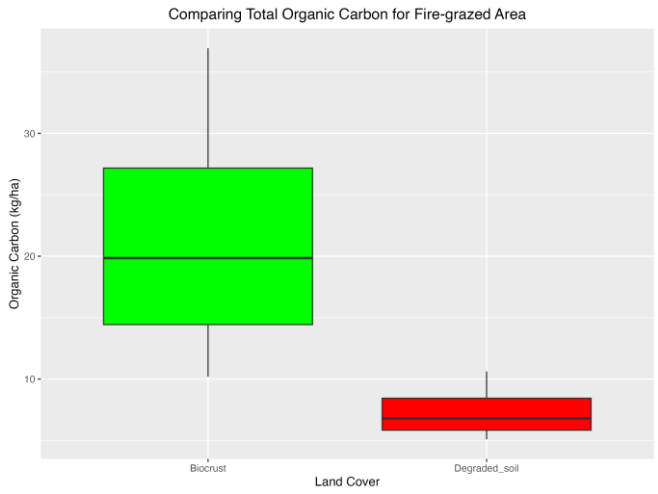
Landcover type	Total area - ha	Ave TC kg/ha	TC kg/area	Ave TN kg/ha	TN kg/area	Ave TOC kg/ha	TOC kg/area
Bare soil	7.22	0.72	5.2	0.07	0.51	7.24	52.27
Biocrust	66.25	2.11	139.79	0.17	11.26	21.11	1398.54

With the bare ground there was a highly significant loss of key soil nutrients (carbon and nitrogen) which represented an average loss of 1-5 cm biocrust and soil (Fig. 13.0.3). This resulted in a reduction loss of around two thirds of TC and TOC, and 60% of the TN (Table 13.0.1). Biocrust ± grass included biocrust cover not visible under areas with grass cover averaged for these sites. The presence of biocrust under grasses was verified at a site level.

Figure 13.0.4 Comparison of Total Carbon (TC), Total Nitrogen (TN) and Total Organic Carbon (TOC) for Fire-graze sites *before burning* showing the significant differences between biocrust and bare degraded ground.







.

Figure 13.0.5 Typical degraded area with upper centimetres of biocrust and soil removed.

Although the total area of bare and degraded ground was a fraction of the total area covered with biocrusts, the area of these degraded regions increased over the course of the wet season (Table 13.01), which would have resulted in a net loss of nutrients. It was noted (with game camera monitoring) that the cattle continued to camp and traverse in the same areas that had previously been degraded. In addition, cattle tracking (collars) showed an increase in grazing of burnt areas post fire once pastures started regrowing following rain. Based on the seasonal importance that the biocrust plays in N-fixation and N-cycling (Section 7) we suggest the practice of wet season spelling post-fire is essential to ensure biocrust recovery including the benefits biocrust provide with increased nutrients, stabilisation and a niche for seed germination.

14 Communications

14.1 Video production

for producer and advisor training “Biocrusts: the living skin of the rangelands” (Appendix 1). This video is now a part of the rangelands management courses run by DITT (NT), Desert Channels QLD, Southern Gulf Catchments QLD and Southern Queensland Landscapes. Education and training is underway in Northern Territory (Appendix 2). Conferences included the Australian Rangelands Society meetings in Longreach (https://www.youtube.com/watch?v=aWO5nvSTIhs 2021) and Broome (2023) and four international conferences. Furthermore, we have:

1. Engaged with the NT Department of Industry Tourism and Trade (DITT) to establish a commercial size burn at VRRS that demonstrates the value of wet season spelling to facilitate the recovery of degraded sites. We will use satellite imagery to track the recovery of biocrusts (see Section 8).
2. Partnered with Territory Natural Resources and Management in the ‘Rain Ready Rangelands’ (RRR) in their demonstration of wet season recovery of degraded Mitchell grass plains, Mulga Lands in the Barkly Tablelands, Mulga Park and Mt Denison (NT). Here we will highlight the role of biocrusts in these processes (drone imagery and temporal sampling).
3. Set up a small-scale biocrust recovery demonstration at Wambiana Grazing Trial to illustrate how biocrusts could be used to facilitate and speed up the recovery of heavily degraded soils. This would serve as a pilot program to develop biocrust for rapid recovery of degraded land, e.g., by distributing propagules via drone.
4. Presented project findings in person at relevant meetings, four on-farm days (QLD), two on-farm days (NT), five soil health workshops (NT, QLD), and one Beefup shruburn day at VRRS (NT). Several radio interviews have been given at various times, deepening communication from our initial ‘raising awareness of biocrusts in industry’ to delivering detailed information on biocrust in the context of grazing land management, with the assistance of MLA.
5. *Articles (https://www.mla.com.au/news-and-events/industry-news/biocrusts-offer-natural-solution/ and https://industry.nt.gov.au/publications/primary-industry-publications/newsletters/regional-newsletters/rural-review/nt-rural-review-november-2022/vrrs-beefup-and-field-day).*

(a) Summary of on-farm communications

Year	Location	Organisation	Producers
2024	Lara Downs	Southern Gulf Catchments	20
2024	Begonia QLD	Southern QLD Landscapes	28
2024	Claravale Station QLD	Southern QLD Landscapes	16
2023	Various	Livestock Industries NT Rangelands management courses	30+
2022	Grantham Station QLD	Southern QLD Landscapes	12
2022	Darwin, Katherine, Alice Springs NT	Territory NRM	30+
2022	Longreach QLD	Landcare & local producer	15
2022	VRSS NT	Beefup MLA	25+

(b) Trained post or graduate students (Honours, Masters, PhD)

Name	UQ School	Research level	Date commenced	Finish date	Completed	Topic
Maria Vega	SE	PhD	Jan-22	Dec-24	No	Effects of rangeland management on soil and biocrust microbiomes
Than Myint Swe	AGFS	PhD	Jan-23	Dec-26	No	Development of a framework for the inclusion of biocrusts in rangeland management integrating proximal and remote sensing
Nicole Parker	AGFS	Masters	Jan-24	Nov-24	No	**Restoration**
Than Myint Swe	AGFS	Masters	Feb-20	Jul-22	Yes	Using smart phone images for the rapid assessment of ground cover of a grazed Australian savanna under different fire regimes
Jaidyn Eastaughffe	AGFS	Honours	Jan-22	Dec-22	Yes	Bioavailability of nitrogen in biocrusts
Henry Baskerville	AGFS	Honours	Jan-22	Dec-22	Yes	Northern Australian rangeland pastures mediated by biocrusts
Sara Waak	AGFS	Honours	Jan-22	Dec-22	Yes	Impact of fire on biocrust and seed germination of native grasses in a northern Australian subtropical savanna
Madailein Dooley	AGFS	Summer project	Dec-20	Jan-21	Yes	Biocrust recovery from fire
Harry Cosgrove	AGFS	Summer project	Dec-20	Jan-21	Yes	Biocrust recovery from fire

15 Discussion and Conclusions

The project has raised awareness of the central role of biocrusts for ongoing pasture productivity, as biocrusts were not on the radar. As stated by a producer: '*when you first see them, you think it is all sediment that dries up after the wet season and flakes up*'. The industry has been receptive to the 'good news story' that biocrusts present and the knowledge generated by the project that has been communicated.

The project findings can guide producers on how to accommodate biocrusts in their management decisions. It would be interesting to hear from producers whether their most productive paddocks have good biocrust cover, and what management has enabled this. Such information will be valuable for future work that broadens the project from analysing long-term research stations to commercial enterprises across a broader range of locations. We have identified suitable methodology, spanning cutting-edge quantification of nitrogen fixing genes to satellite imaging. This range of methods has provided a powerful and integrated approach to address the central question.

We find that biocrusts are prolific in tropical savannas that are carefully managed for vegetation cover. The capacity of biocrusts to regenerate nitrogen and carbon contributes significantly to replenishing and maintaining pastures. Importantly, the project presents evidence why wet season spelling delivers for pastures with undisturbed biocrusts can maximise nitrogen inputs.

A confident estimate is that well-developed biocrusts - accessing sufficient moisture and protected from erosion and trampling during peak nitrogen fixation in the wet season - can generate annually 5 kg nitrogen per hectare which equates to 500 kg N per square km. The conservative estimate means that biocrusts provide sufficient nitrogen for 25-50 tonnes of pasture (1-2% N in dry matter) per square km.

16 Key Findings

To manage pastures for biocrusts, consideration should include:

Optimising stocking rates in line with climatic conditions and industry recommendations that ensures sufficient ground cover to protect soil from erosion and fosters the presence and function of biocrusts.

Optimised wet season spelling as informed by paddock condition and climate, i.e., more frequent, or longer wet spelling in more degraded pastures. Future work should explore biocrust responses to wet season spelling intervals and length and intensity of the wet season across biophysical conditions to inform planning at the property level to ensure it is not overgrazed.

Ongoing work following the **landscape level impacts of post fire grazing**. The cattle tracking data at the VRRS commercial size 'Fire-graze trial' suggests post fire grazing has contributed to a 20% increase in degraded land where land condition declined from B to C during the 2023 wet season.

Future work must explore how often and **how long pastures should be spelled** in a wet season with spelling frequency and length adjusted to landscape condition and soil type, extent of the wet season, and prior disturbances such as fire, drought, and flooding. Such work should combine UAV and satellite imagery with other information.

Optimised fire regimes at VRRS Climate should influence burning decisions, for example, four-yearly early fire is recommended following successive good wet seasons.

17 Future Research and Recommendations

Modelling the commercial impact of degraded soils that includes the loss of biocrusts is an important goal. The GRASP model indicates biocrusts have an important role in increasing pasture quality. Modelling needs to be stepped up to CLEM so that additional climatic and seasonal effects can be taken into account. There will be a further section added to this report to include current progress with modelling at a property level. This research is still underway.

Future work should consider expanding fire management options. The burning regimes investigated here were comparatively hot dry season fires. Fire after first rains or during the wet season are cooler which may be advantageous for biocrust formation and nitrogen fixation. Burning after first rains was discussed at the 2023 Annual Rangelands Society conference (Broome) where a WA producer found such burning regime benefitting his pasture although it was not reported if biocrusts had a role in this.

Considering climate, soil and biocrust types (e.g. cyanobacteria or lichen dominated) because biocrusts are more vulnerable in sandier soils than clay soils, and degraded sandy soils take longer to recover biocrusts. Future work should explore other types of biocrust (e.g., lichen crusts in southern regions that are highly vulnerable to damage by trampling (Belnap and Eldridge, 2001).

18 Benefits to industry

In summary, rangelands support an extensive grazing industry worth billions of dollars and employing many Australians. To keep pastures healthy and ensure long-term productivity, nitrogen must be replenished. Declining soil fertility is caused by net nitrogen removal which depletes soil organic matter and carbon.

Increasingly, land degradation is exacerbated by natural disasters with frequent droughts, floods, fires, and cyclones. This costs Australia's grazing industry dearly as eroding landscapes have less capacity to support pastures. It also costs society as dust storms from by unprotected soils impacts citizens and infrastructure, and soil (sediment) loss deteriorates waterways and Great Barrier Reef. The decline of soil as natural capital and production base makes Australia's grazing industry vulnerable to climate change and criticism from regulators and consumers.

Pasture management that considers nitrogen input from biocrusts will position the industry for future markets that demand proof of sustainable practices (e.g., ESG certification). This should not be difficult if producers make wise decision that consider biocrusts as agents for protecting and regenerating soil nitrogen and carbon. The biocrusts studied here harbour cyanobacteria and bacteria adapted to Australia's hot and dry climates, which means nature provides what is needed. Globally, beneficial soil bacteria, fungi and other soil organisms are increasingly put to work for

sustainable production. The methodology and insights generated in this project will translate to opportunities by fine-tuning decisions of grazing and fire management.

Producers are seeking better ways of managing their land which includes short duration high intensity grazing practices, intensively grazing and trampling paddocks for short intervals before returning them to rest. How biocrusts respond to such treatments has not been investigated. Studying biocrusts and nitrogen flows in these systems can guide practices to maximise input and retention of nitrogen.

Further to managing biocrust to boost soil fertility, they can be used to regenerate severely degraded pastures that do not recover naturally from extreme events. After prolonged flooding in recent years in north Queensland, pastures that had suffered long droughts remained unproductive for a long time. Future work should explore the use of biocrusts for active restoration.

The project has advanced understanding and awareness of biocrust and identified several critical drivers that promote biocrusts and nitrogen fixation. As expected, after four years of research, questions remain that next steps research, guided by industry needs, can address.

18.1 Acknowledgements

We acknowledge and thank The University of Queensland Team for their hard work and enthusiasm for their contributions to various parts of this project (Table x and Section 14). We also greatly appreciate the support of Queensland Department of Agriculture and Fisheries and Northern Territory Department of Industry, Tourism and Trade. We also thank Felice Driver from MLA for expert support and assistance throughout the project.

Table 18.1 Roles and research of main contributors to the project.

Prof Susanne Schmidt	Project leader	University of Queensland
Prof Paul Dennis	Project Co-leader	University of Queensland
A/Prof Andries Potgieter	Remote Sensing Specialist	QAAFI University of Qld
Dr Wendy Williams	Project Co-leader	University of Queensland
Dr Nicole Robinson	Project co-ordinator	University of Queensland
Dr Yan Zhao	Remote Sensing specialist	QAAFI University of Qld
Dr Robyn Cowley	Shrubburn lead and advisor	Kidman Springs, NT
Dr Peter O'Reagain	Wambiana Grazing Trial advisor	Charters Towers, Qld
Ms Maria Vega	PhD Candidate	DNA analysis
Mr Than Mynit Swe	PhD Candidate	Remote Sensing
Ms Jaidyn Eastaughffe	Honours student	Bioavailable N
Ms Sara Waak	Honours student	Seed germination
Mr Henry Baskerville	Honours student	Pastures and biocrusts

19 References

Armstrong, R.D., McCosker, K., Johnson, S.B., Millar, G., Walsh, K.B., Kuskopf, B., Probert, M.E., Standley, J., 1999. Legume and opportunity cropping systems in central Queensland. 1. Legume growth, nitrogen fixation, and water use. Aust. J. Agric. Res. 50, 909–924. https://doi.org/10.1071/ar98100

Asner, G.P., Elmore, A.J., Olander, L.P., Martin, R.E., Harris, A.T., 2004. GRAZING SYSTEMS, ECOSYSTEM RESPONSES, AND GLOBAL CHANGE. Annu. Rev. Environ. Resour. 29, 261–299. https://doi.org/10.1146/annurev.energy.29.062403.102142

Aspandiar, M.F., Taylor, G., Eggleton, R.A., 2003. Charters Towers region, Queensland. Regolith Landsc. Evol. Coop. Res. Cent. Landsc. Environ. Miner. Explor. B Httpleme Anu Edu AuRegLandEvolChartersTowers Pdf N.

Barger, N.N., Weber, B., Garcia-Pichel, F., Zaady, E., Belnap, J., 2016. Patterns and Controls on Nitrogen Cycling of Biological Soil Crusts, in: Biological Soil Crusts: An Organizing Principle in Drylands, Ecological Studies. Springer, Cham, pp. 257–285. https://doi.org/10.1007/978-3-319-30214-0_14

Bastin, G., Cowley, R., Friedel, M., Materne, C., 2024. Applying two remotely-sensed methods for monitoring grazing impacts in the Australian arid zone. Rangel. J. 45, 141–159. https://doi.org/10.1071/RJ23030

Belnap, J., 2003. Factors Influencing Nitrogen Fixation and Nitrogen Release in Biological Soil Crusts, in: Biological Soil Crusts: Structure, Function, and Management, Ecological Studies. Springer, Berlin, Heidelberg, pp. 241–261. https://doi.org/10.1007/978-3-642-56475-8_19

Billi, D., Potts, M., 2002. Life and death of dried prokaryotes. Res. Microbiol. 153, 7–12. https://doi.org/10.1016/S0923-2508(01)01279-7

Bowker, M.A., Maestre, F.T., Eldridge, D., Belnap, J., Castillo-Monroy, A., Escolar, C., Soliveres, S., 2014. Biological soil crusts (biocrusts) as a model system in community, landscape and ecosystem ecology. Biodivers. Conserv. 23, 1619–1637. https://doi.org/10.1007/s10531-014-0658-x

Bowker, M.A., Reed, S.C., Maestre, F.T., Eldridge, D.J., 2018. Biocrusts: the living skin of the earth. Plant Soil 429, 1–7. https://doi.org/10.1007/s11104-018-3735-1

Büdel, B., Williams, W.J., Reichenberger, H., 2018. Annual net primary productivity of a cyanobacteria-dominated biological soil crust in the Gulf Savannah, Queensland, Australia. Biogeosciences 15, 491.

Carberry, P.S., Bruce, S.E., Walcott, J.J., Keating, B.A., 2011. Innovation and productivity in dryland agriculture: a return-risk analysis for Australia. J. Agric. Sci. 149, 77–89. https://doi.org/10.1017/S0021859610000973

Cowley, R., Dyer, R., Hearnden, M., Joyce, K., 2021. Do we really need to burn the bushes? 29 years of the Kidman Springs Fire Experiment, NT.

Delgado-Baquerizo, M., Morillas, L., Maestre, F.T., Gallardo, A., 2013. Biocrusts control the nitrogen dynamics and microbial functional diversity of semi-arid soils in response to nutrient additions. Plant Soil 372, 643–654. https://doi.org/10.1007/s11104-013-1779-9

Design, R., n.d. Avenza Maps [WWW Document]. Avenza Syst. Inc. URL https://www.avenza.com/avenza-maps/ (accessed 9.4.23).

Duniway, M.C., Geiger, E.L., Minnick, T.J., Phillips, S.L., Belnap, J., 2018. Insights from Long-Term Ungrazed and Grazed Watersheds in a Salt Desert Colorado Plateau Ecosystem. Rangel. Ecol. Manag. 71, 492–505. https://doi.org/10.1016/j.rama.2018.02.007

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

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

Eldridge, D.J., Delgado-Baquerizo, M., 2017. Continental-scale Impacts of Livestock Grazing on Ecosystem Supporting and Regulating Services. Land Degrad. Dev. 28, 1473–1481. https://doi.org/10.1002/ldr.2668

Eldridge, D.J., Greene, R.S.B., 1994. Microbiotic soil crusts - a review of their roles in soil and ecological processes in the rangelands of Australia. Soil Res. 32, 389–415. https://doi.org/10.1071/sr9940389

Hartley, A., Barger, N., Belnap, J., Okin, G.S., 2007. Dryland Ecosystems, in: Marschner, P., Rengel, Z. (Eds.), Nutrient Cycling in Terrestrial Ecosystems, Soil Biology. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 271–307. https://doi.org/10.1007/978-3-540-68027-7_10

Jahodářová, E., Dvořák, P., Hašler, P., Holušová, K., Poulíčková, A., 2018. Elainella gen. nov.: a new tropical cyanobacterium characterized using a complex genomic approach. Eur. J. Phycol. 53, 39–51. https://doi.org/10.1080/09670262.2017.1362591

Karnieli, A., 1997. Development and implementation of spectral crust index over dune sands. Int. J. Remote Sens. 18, 1207–1220. https://doi.org/10.1080/014311697218368

Lenth, R.V., Bolker, B., Buerkner, P., Giné-Vázquez, I., Herve, M., Jung, M., Love, J., Miguez, F., Riebl, H., Singmann, H., 2024. emmeans: Estimated Marginal Means, aka Least-Squares Means.

Maestre, F.T., Eldridge, D.J., Soliveres, S., Kéfi, S., Delgado-Baquerizo, M., Bowker, M.A., García-Palacios, P., Gaitán, J., Gallardo, A., Lázaro, R., Berdugo, M., 2016. Structure and Functioning of Dryland Ecosystems in a Changing World. Annu. Rev. Ecol. Evol. Syst. 47, 215–237. https://doi.org/10.1146/annurev-ecolsys-121415-032311

Metternicht, G., Smith, M.S., 2020. Commentary: on the under-valuing of Australia's expertise in drylands research and practice globally. Rangel. J. 42, 253–260. https://doi.org/10.1071/RJ20055

Moreira C Fernandes, V., Giraldo-Silva, A., Roush, D., Garcia-Pichel, F., 2021. Coleofasciculaceae, a Monophyletic Home for the Microcoleus steenstrupii Complex and Other Desiccation-tolerant Filamentous Cyanobacteria. J. Phycol. 57, 1563–1579. https://doi.org/10.1111/jpy.13199

O'Reagain, P.J., Scanlan, J.C., 2013. Sustainable management for rangelands in a variable climate: evidence and insights from northern Australia. animal 7, 68–78.

Piñeiro, G., Paruelo, J.M., Oesterheld, M., Jobbágy, E.G., 2010. Pathways of Grazing Effects on Soil Organic Carbon and Nitrogen. Rangel. Ecol. Manag. 63, 109–119. https://doi.org/10.2111/08-255.1

Potts, M., 1999. Mechanisms of desiccation tolerance in cyanobacteria. Eur. J. Phycol. 34, 319–328.

Pringle, M.J., Allen, D.E., Phelps, D.G., Bray, S.G., Orton, T.G., Dalal, R.C., 2014. The effect of pasture utilization rate on stocks of soil organic carbon and total nitrogen in a semi-arid tropical grassland. Agric. Ecosyst. Environ. 195, 83–90. https://doi.org/10.1016/j.agee.2014.05.013

Radford, B.J., Thornton, C.M., Cowie, B.A., Stephens, M.L., 2007. The Brigalow Catchment Study: III.* Productivity changes on brigalow land cleared for long-term cropping and for grazing. Soil Res. 45, 512–523. https://doi.org/10.1071/SR07062

Reed, S.C., Delgado-Baquerizo, M., Ferrenberg, S., 2019. Biocrust science and global change. New Phytol. 223, 1047–1051.

Rodríguez-Caballero, E., Knerr, T., Weber, B., 2015. Importance of biocrusts in dryland monitoring using spectral indices. Remote Sens. Environ. 170, 32–39. https://doi.org/10.1016/j.rse.2015.08.034

Román, J.R., Chamizo, S., Roncero-Ramos, B., Adessi, A., De Philippis, R., Cantón, Y., 2021. Overcoming field barriers to restore dryland soils by cyanobacteria inoculation. Soil Tillage Res. 207, 104799. https://doi.org/10.1016/j.still.2020.104799

Rondeaux, G., Steven, M., Baret, F., 1996. Optimization of soil-adjusted vegetation indices. Remote Sens. Environ. 55, 95–107.

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

Shah, S.H.H., Li, Y., Wang, J., Collins, A.L., 2020. Optimizing farmyard manure and cattle slurry applications for intensively managed grasslands based on UK-DNDC model simulations. Sci. Total Environ. 714, 136672. https://doi.org/10.1016/j.scitotenv.2020.136672

Swe, T.M., Williams, W.J., Schmidt, S., Potgieter, A., Cowley, R., Mellor, V., Driscoll, C., Zhao, Y., 2023. Using digital photography to monitor changes in biocrusts and ground cover in a savanna rangeland. Rangel. J. https://doi.org/10.1071/RJ22019

Thomas, A.D., Dougill, A.J., 2007. Spatial and temporal distribution of cyanobacterial soil crusts in the Kalahari: Implications for soil surface properties. Geomorphology, Drylands: Linking landscape processes to sedimentary environments 85, 17–29. https://doi.org/10.1016/j.geomorph.2006.03.029

Torres-Cruz, T.J., Howell, A.J., Reibold, R.H., McHugh, T.A., Eickhoff, M.A., Reed, S.C., 2018. Species-specific nitrogenase activity in lichen-dominated biological soil crusts from the Colorado Plateau, USA. Plant Soil 429, 113–125. https://doi.org/10.1007/s11104-018-3580-2

Tucker, C.J., 1979. Red and photographic infrared linear combinations for monitoring vegetation. Remote Sens. Environ. 8, 127–150.

Vega-Cofre, M.V., Williams, W., Song, Y., Schmidt, S., Dennis, P.G., 2023. Effects of grazing and fire management on rangeland soil and biocrust microbiomes. Ecol. Indic. 148, 110094. https://doi.org/10.1016/j.ecolind.2023.110094

Waters, C.M., Melville, G.J., Orgill, S.E., Alemseged, Y., 2015. The relationship between soil organic carbon and soil surface characteristics in the semi-arid rangelands of southern Australia. Rangel. J. 37, 297–307. https://doi.org/10.1071/RJ14119

Weber, B., Belnap, J., Büdel, B., Antoninka, A.J., Barger, N.N., Chaudhary, V.B., Darrouzet-Nardi, A., Eldridge, D.J., Faist, A.M., Ferrenberg, S., Havrilla, C.A., Huber-Sannwald, E., Malam Issa, O., Maestre, F.T., Reed, S.C., Rodriguez-Caballero, E., Tucker, C., Young, K.E., Zhang, Y., Zhao, Y., Zhou, X., Bowker, M.A., 2022. What is a biocrust? A refined, contemporary definition for a broadening research community. Biol. Rev. 97, 1768–1785. https://doi.org/10.1111/brv.12862

Weber, B., Bowker, M., Zhang, Y., Belnap, J., 2016. Natural Recovery of Biological Soil Crusts After Disturbance, in: Biological Soil Crusts: An Organizing Principle in Drylands, Ecological Studies. Springer, Cham, pp. 479–498. https://doi.org/10.1007/978-3-319-30214-0_23

Wickham, H., 2016. Getting Started with ggplot2, in: Wickham, H. (Ed.), Ggplot2: Elegant Graphics for Data Analysis. Springer International Publishing, Cham, pp. 11–31. https://doi.org/10.1007/978-3-319-24277-4_2

Williams, W., Büdel, B., Williams, S., 2018. Wet season cyanobacterial N enrichment highly correlated with species richness and *Nostoc* in the northern Australian savannah. Biogeosciences 15, 2149–2159. https://doi.org/10.5194/bg-15-2149-2018

Williams, W., Büdel, B., Williams, S., 2017. Cyanobacterial species richness and Nostoc highly correlated to seasonal N enrichment in the northern Australian savannah. Biogeosciences Discuss 2017, 1–24. https://doi.org/10.5194/bg-2017-377

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

Williams, W.J., Eldridge, D.J., Alchin, B.M., 2008. Grazing and drought reduce cyanobacterial soil crusts in an Australian Acacia woodland. J. Arid Environ. 72, 1064–1075. https://doi.org/10.1016/j.jaridenv.2007.11.017

Williams, W.J., Schmidt, S., Zaady, E., Alchin, B., Myint Swe, T., Williams, S., Dooley, M., Penfold, G., O'reagain, P., Bushell, J., 2021. Resting Subtropical Grasslands from Grazing in the Wet Season Boosts Biocrust Hotspots to Improve Soil Health. Agronomy 12, 62.

Young, K.E., Ferrenberg, S., Reibold, R., Reed, S.C., Swenson, T., Northen, T., Darrouzet-Nardi, A., 2022. Vertical movement of soluble carbon and nutrients from biocrusts to subsurface mineral soils. Geoderma 405, 115495.

20 Appendices

Appendix 1: Biocrusts – the living skin of rangelands soils

Video production for land manager and producer education

Communication with industry has been a focus of our project attending relevant conferences, workshops and participating in field days, articles and an explanatory video; “Biocrusts - the living skin of rangelands”, with producer case study developed for producer and advisor training. The video is available on Future Beef Website and has been integrated into the rangelands management course run by DITT (NT). Education and training is underway in Northern Territory (also see Appendix 2).

In 2023, Dr Wendy Williams and PhD candidate Maria Vega travelled to Alice Springs (NT) to shoot a video about biocrusts. The OGA film crew were employed by MLA to do the shoot and met us there. We travelled to Mt Denison Station in the west where the Rain Ready Rangelands project (NT govt.) had been established. We initiated interviews with the Terry and Robert Martin (Fig. x), owners of Mt Denison, to describe how they saw biocrusts and why they were important. We also filmed at NT Livestock Industries grazing property at Old Man Plains. A range of interviews were conducted, and the resulting video provides a snapshot of how important biocrusts are in the rangelands and how they influence productivity. The end products are two video versions (long and short), first released for a preliminary viewing and feedback at the Australian Rangelands Conference (Broome, 2023) and officially in March 2024 via FutureBeef (Fig. A1.1). Link to video: https://futurebeef.com.au/biocrusts-the-living-skin-of-rangeland-soils/

Figure A1.1 (a) Mt Denison owners/managers Terry and Robert Martin, **(b)** Example of fire affected pastures and healthy biocrusts with bare degraded ground remnant from previous drought at Mt Denison Station (NT).

(a)

(b)

Appendix 2: Biocrust extension and education in the Northern Territory and Queensland

Next Gen Rangeland Management Courses

The Next Generation Land Managers Project involves Rangeland Management Courses (RMC's) that are presented by DITT staff and supported by the Northern Hub, with aims to provide further education to station hands that may not have previous experience in the industry (Fig. A2.1(a). In 2023, the course was updated to include the topic of biocrusts, it has been presented at Northern Territory stations spanning from the Barkly, to the NT/WA border of the Eastern Kimberley, and participants include Cave Creek, Rocklands, Bullo River and Helen Springs. A combined total of around 35 Station hands and managers have participated in the course thus far. The course involves a series of PowerPoints in which the biocrust slides are presented and then discussed. A paddock walk occurs at each station where participants are asked to look for biocrusts, where further discussion is encouraged, and questions are asked. Lots of positive feedback has been provided and several of the station staff have commented on the feedback form showing a keen interest in the biocrust section and curiosity to know more.

Katherine Show 2023: Biocrust display

The Department of Industry, Tourism and Trade (NT) had an Agricultural Pavilion at the Katherine Show (21st and 22nd July) where a series of educational posters were displayed (Fig. A2.1(b). A Biocrust poster was presented with Jaidyn Eastaughffe presenting the poster and answering several questions and generating discussion over the two days. The Livestock Industry team at Katherine Research Station created a "Find the Answers" competition in which participants had to answer, "How many kg of Nitrogen do biocrusts fix per hectare per year". Many students, producers and members of the general public participated.

Agriculture NT Facebook Post

Following the Soil Science Australia Conference where Jaidyn Eastaughffe presented 'Fire impacts on biocrusts in a grazed savannah' a post was made to the AgricultureNT Facebook page. With over 840 members it is a forum used for other researchers, extension officers, producers and those interested in agriculture across the Northern Territory (Fig. A2.1(c)).

Figure A2.1 (a) Northern Territory Livestock Industries extension team at Anthony Lagoon, Eva Downs Station NT trained to measure biocrust health as a part of the Rain Ready Rangelands program. Left to right: Ben Wirth, Stacey Holzapfel, Mary Williams, Caroline Pettit, Jaidyn Eastaughffe, Elle Fordyce (photo with permission Jaidyn Eastaughffe), **(b)** poster at Katherine Show, **(c)** material used for education extension (d) information sheet for Southern Queensland Landscapes and Department of Agriculture and Fisheries QLD.

(a)

What are Biocrusts?

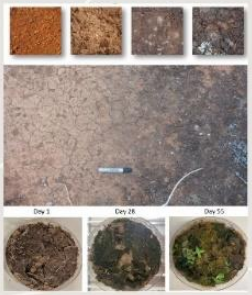
- Throughout the wet season you may have noticed a green, slimy coating on the soil surface – this is known as biocrust or biological soil crusts.
- Biocrusts are a diverse community of microorganisms including cyanobacteria, fungi, lichens, liverworts and mosses that occupy the top 1-2cm of the soil surface.
- They are a widespread phenomenon, covering roughly 12% of the terrestrial surface.
- Throughout the dry season the biocrusts dry out and become inactive, and re-activate and grow in the wet.

Biocrusts in the dry

Biocrusts in the wet (right)

What do they do?

- Biocrusts intertwine with soil particles, stabilising the soil surface helping to prevent erosion.
- They enhance moisture retention.
- Increase ecological biodiversity.
- Cyanobacteria, liverworts, algae, lichens and mosses within the biocrust photosynthesis and fix carbon like plants do.
- They fix nitrogen! They have been found to fix 5kg of nitrogen per hectare per year, making atmospheric nitrogen available to surrounding pastures.
- During the dry season the nutrient rich biocrusts breakdown and incorporate additional nutrients into the soil, helping to improve soil fertility and productivity.



Changes in biocrust cover and diversity over time in response to rain and spelling

Fire, Grazing and Biocrusts

- We are researching the impacts of fire and grazing management on biocrusts in the VRD, Barkly and in central Australia.
- Like Australia's native vegetation, biocrusts have evolved with fire and are therefore well adapted.
- Fire can enhance biocrust cover and functionality by removing litter, shrubs and trees that otherwise compete as ground cover.
- Biocrusts benefit from wet season spelling whilst biocrusts are actively growing.
- Heavy grazing and trampling can have negative effects on biocrust cover and composition and the associated benefits such as nitrogen fixation.

Biocrusts intertwining with soil particles

Biocrusts holding together the soil surface on the edge of an eroded site

Join the Agriculture Facebook Group @AgricultureNT

For more information, please contact:
Jaidyn Eastaughffe, Livestock Industries
Email: Jaidyn.Eastaughffe@nt.gov.au

For more information, go to industry.nt.gov.au

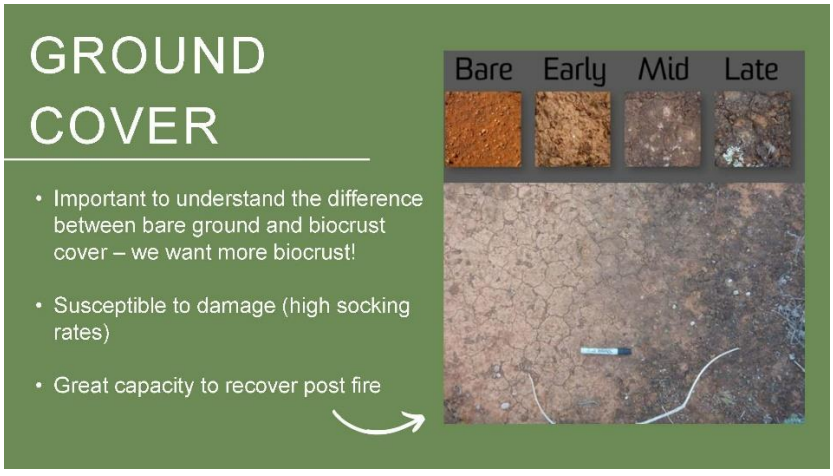
Department of Industry, Tourism and Trade
T: 08 8999 2006

NORTHERN TERRITORY GOVERNMENT | THE TERRITORY

(b)


Biocrusts
WHAT ARE THEY?


WHAT DO THEY DO?
• Increase soil stability
• Enhance moisture retention and regulate water infiltration
• Carbon fixation and sequestration
• Fix and accumulate nitrogen – incorporating N back into the soil as they break down
• Increase functional diversity and ecological biodiversity of the soil


GROUND COVER
• Important to understand the difference between bare ground and biocrust cover – we want more biocrust!
• Susceptible to damage (high socking rates)
• Great capacity to recover post fire
Bare
Early
Mid
Late

(c)



WHAT ARE BIOCRUSTS?

- In the rangelands there is an important microbiome that occupies the soil surfaces between grass plants.
- Biological soil crusts (biocrusts) form a visible skin or dark 'crust' packed with microorganisms that include cyanobacteria, bacteria, fungi, lichens, liverworts and mosses,
- Biocrusts that grow in the top 1–2 cm of the soil surface.
- They are widespread, covering roughly 12% of the Earth's ground surfaces.
- In the dry season the biocrusts dry out and become dormant and re-activate and grow in the wet season.
- Biocrusts are resilient to drought and fire.
- Biocrusts stabilise the soil surface and help to prevent erosion.
- They enhance moisture retention.
- Biocrusts improve pasture quality.

https://futurebeef.com.au/biocrusts-the-living-skin-of-rangeland-soils/

https://futurebeef.com.au/resources/biocrust-project/

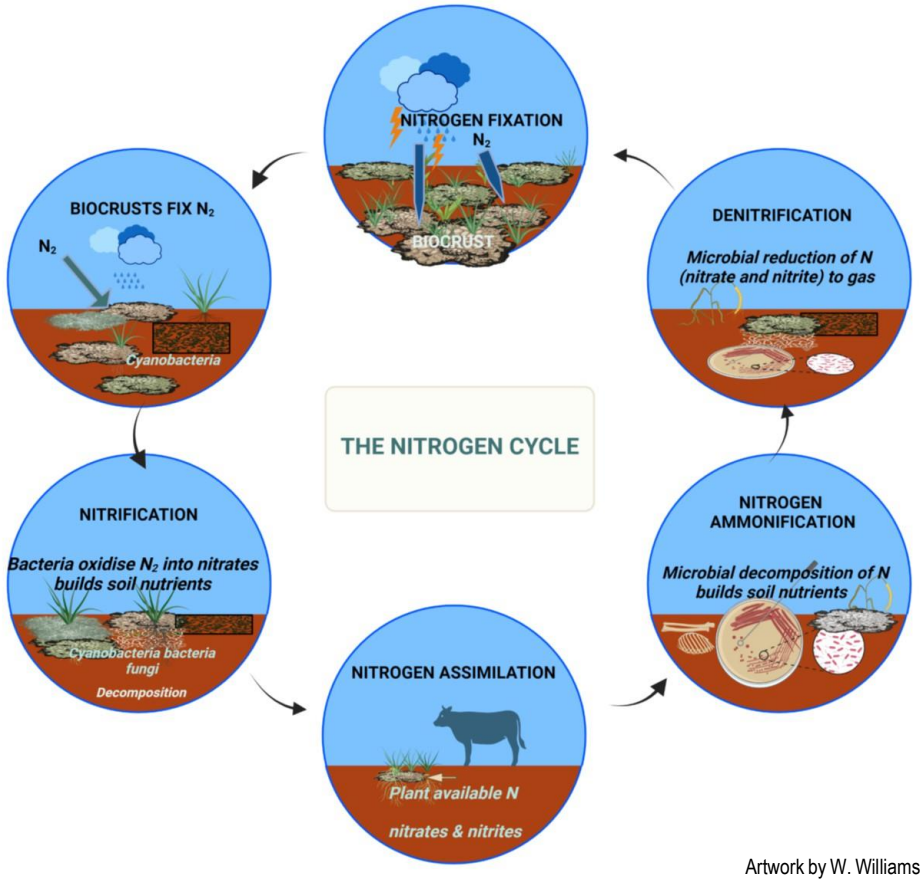
Contact: Wendy.Williams@uq.edu.au

WHAT DO BIOCRUSTS DO?

- Many biocrust microbes photosynthesise like plants, sequestering carbon and fixing atmospheric nitrogen.
- Biocrusts can **provide 5kg of nitrogen per hectare per year.**
- Heavy grazing and trampling can have negative effects on biocrusts.
- Each rainy season nutrient rich biocrusts breakdown and regrow.

BIOCRUSTS AND THE NITROGEN CYCLE

- Biocrusts release nutrients into the soil, improve soil fertility and productivity.
- Biocrusts benefit from wet season spelling when they are actively growing.
- Biocrusts are indicators of good soil health.



Artwork by W. Williams 2024
created with BioRender.com

(d)

Appendix 3: Resting from grazing in the wet season boosts biocrust hotspots

agronomy

MDPI

Article

Resting Subtropical Grasslands from Grazing in the Wet Season Boosts Biocrust Hotspots to Improve Soil Health

Wendy J. Williams [1,*], Susanne Schmidt [1], Eli Zaady [2], Bruce Alchin [1], Than Myint Swe [1], Stephen Williams [1], Madeline Dooley [1], Grace Penfold [1], Peter O'Reagain [3], John Bushell [3], Robyn Cowley [4], Colin Driscoll [1] and Nicole Robinson [1]

[1] NUE Lab, School of Agriculture and Food Sciences, The University of Queensland, St Lucia, QLD 4074, Australia; susanne.schmidt@uq.edu.au (S.S.); b.alchin@uq.edu.au (B.A.); t.myintswe@uq.net.au (T.M.S.); u1077155@umail.usq.edu.au (S.W.); madeline.dooley@uq.net.au (M.D.); g.penfold@uq.net.au (G.P.); c.driscoll@uq.edu.au (C.D.); nicole.robinson@uq.edu.au (N.R.)
[2] Department of Natural Resources, Institute of Plant Sciences, Agricultural Research Organization Gilat Research Center, Negev 8531100, Israel; zaadye@volcani.agri.gov.il
[3] Queensland Department of Agriculture and Fisheries, Charters Towers, QLD 4820, Australia; peter.oreagain@daf.qld.gov.au (P.O.); john.bushell@daf.qld.gov.au (J.B.)
[4] NT Department of Industry, Tourism and Trade, Darwin, NT 0801, Australia; Robyn.Cowley@nt.gov.au
* Correspondence: wendy.williams@uq.edu.au

Abstract: Effective grazing management in Australia's semi-arid rangelands requires monitoring landscape conditions and identifying sustainable and productive practice through understanding the interactions of environmental factors and management of soil health. Challenges include extreme rainfall variability, intensifying drought, and inherently nutrient-poor soils. We investigated the impacts of grazing strategies on landscape function—specifically soil health—as the foundation for productive pastures, integrating the heterogenous nature of grass tussocks and the interspaces that naturally exist in between them. At Wambiana—a long-term research site in north-eastern Australia—we studied two soil types, two stocking rates (high, moderate), and resting land from grazing during wet seasons (rotational spelling). Rotational spelling had the highest biocrust (living soil cover), in interspaces and under grass tussocks. Biocrusts were dominated by cyanobacteria that binds soil particles, reduces erosion, sequesters carbon, fixes nitrogen, and improves soil fertility. Rotational spelling with a moderate stocking rate emerged as best practice at these sites, with adjustment of stocking rates in line with rainfall and soil type recommended. In drought-prone environments, monitoring the presence and integrity of biocrusts connects landscape function and soil health. Biocrusts that protect and enrich the soil will support long-term ecosystem integrity and economic profitability of cattle production in rangelands.

Keywords: landscape function; drylands; tropical rangelands; grazing; soil health; biocrusts; drought

check for updates

Citation: Williams, W.J.; Schmidt, S.; Zaady, E.; Alchin, B.; Myint Swe, T.; Williams, S.; Dooley, M.; Penfold, G.; O'Reagain, P.; Bushell, J.; et al. Resting Subtropical Grasslands from Grazing in the Wet Season Boosts Biocrust Hotspots to Improve Soil Health. *Agronomy* **2022**, *12*, 62. https://doi.org/10.3390/agronomy12010062

Academic Editor: Shan Lin

Received: 11 November 2021
Accepted: 21 December 2021
Published: 28 December 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

1. Introduction

Beef cattle grazing is the dominant industry in Australia's subtropical and tropical savannas and grasslands that cover much of the continent. Vast grazing properties of 10- to 100-thousand hectares require land managers to maintain pasture composition and production [1]. Inherently nutrient-poor soils and highly variable rainfall mainly driven by ENSO (El Niño/La Niña Southern Oscillation) cycles constrain the quantity and quality of forage. Significant economic loss [2] and declines in ecosystem function [3] result from a failure to manage for seasonal rainfall variability and landscape heterogeneity at large spatial scales. Northern Australia's rangelands, the focus of this study, have a distinct dry season over mild winter months followed by a hot summer wet season (2–6 months) when most pasture growth occurs. Resting the landscape (i.e., temporary cattle removal) during the dry winter months when grasses are dormant is deemed ineffective, while

resting during the summer growing season can improve composition and production of perennial grasses [2]. However, the benefits of resting are modulated by stocking rates, as resting combined with excessive stocking does not improve pasture [1,4], while conservative stocking rates with year-round grazing, or some wet season resting, facilitated recovery and productive pastures [4]. These findings demand refinement; Australia is the hottest and driest continent and profoundly impacted by climate change, it is therefore a matter of urgency to identify sustainable practices [5]. Much of the continent has experienced rainfall declines accompanied by more frequent and intensive droughts and rising temperatures [6]. Managing sustainability and profitably is a challenge for northern Australian beef producers, as cattle carrying capacity and pasture productivity is heavily influenced by month-to-month and year-to-year rainfall variability [7]. The principles of good grazing management require sound methods of landscape monitoring and understanding how land management and ecosystems interact. Our focus is soil health as a critical factor for grazing extensive rangelands. Soil and biological nitrogen fixation provide the essential nutrient elements for plant growth and productivity. In environments where nutrient cycling is limited by soil moisture, most nutrients occur within the upper few centimeters of the soil [8]. While most nutrients become available via decomposition of organic matter and soil weathering, nitrogen input occurs via bacterial (biological) fixation of atmospheric nitrogen, so that nitrogen removed through grazing and export of the herd can be replenished. Nitrogen as a renewable source is important. It is the essential building block of proteins, and accounts for over 60% of the essential nutrients. Additionally, low nitrogen often limits pasture productivity, particularly in high rainfall years [9,10]. Insufficient nitrogen availability limits both productivity and pasture quality, and low forage quality is a major constraint to cattle production that leads to poor weight gains—or even weight loss—in northern Australian rangelands [11]. Landscape function analysis (LFA) [12] is a widely used monitoring tool for quantifying soil health, soil fertility and effects of land management in context of the spatial organization of the landscape. A range of parameters link to the flow of resources across a patchy landscape, facilitate the quantification of landscape heterogeneity, and define resilience to disturbance. Here, we applied LFA's soil function indices to understand the role of microorganism communities that cover the soil surface, so-called biocrusts (also termed biological, microbiotic or cryptogamic crusts). By quantifying the presence of biocrusts with different grazing management, we examined their contribution to the nutrient content of grazed rangelands. Biocrusts form at the critical zone between the soil and atmosphere, and are a key component of soil function [13], including nutrient cycling, water infiltration, and soil stability [14]. In northern Australia's rangelands, biocrusts grow between perennial grasses and contain diverse bacterial communities and non-vascular plants such as liverworts [15]. These biocrusts are dominated by photosynthesizing cyanobacteria that exude sticky polysaccharides to bind soil particles and protect from erosion. Cyanobacteria and other diazotrophic bacteria improve soil fertility with nitrogen fixation generating bioavailable nitrogen for pasture plants [16,17]. We investigated the drivers of soil function that influence the key principles for grazing management in northern Australia [18] including: (1) manage stocking rates to meet goals for livestock production and land condition, (2) periodically rest pastures to maintain a good condition, and (3) restore pastures from poor condition to increase productivity. It follows that cattle stocking rates influence soil condition through the removal of the understory vegetation with grazing and the trampling of the soil's surface. Our study used a long-term research site that has tested cattle stocking strategies over 24 years [1] and that is representative of typical cattle properties in the region, albeit at a smaller scale. The objectives of this study were to examine the long-term impacts of heavy and moderate grazing pressure (stocking) and a combination of moderate stocking with wet season resting (rotational spelling) on several response variables of ecosystem function in two contrasting soil types. There is evidence that biocrusts growing in interspaces (open areas) between grass patches perform vital ecosystem functions. A previous study showed that interspaces with nitrogen-fixing biocrust communities had similar nutrient cycling as

the soil under grasses [8]. We therefore hypothesized that biocrust-covered interspaces are important drivers of the soil–plant continuum, providing soil stability, water infiltration, and plant-available nutrients. We expect the preferential use of interspaces by cattle as easy passageway to access pasture to be impacted by stocking density, exacerbated by rainfall deficiencies and drought [19]. We also evaluated whether periodic wet season resting from cattle provides the opportunity for biocrust recovery.

2. Materials and Methods

2.1. Site Background

The grazing trial is located on Wambiana, a working cattle station near Charters Towers, Queensland, Australia (wambianastation.com.au (accessed on 3 November 2021)). Average annual rainfall in the region is 630 mm ranging from 200 to 1400 mm with most (70%) rainfall received in the warmer summer months. The vegetation is a relatively open Eucalypt-Acacia woodland underlain by native tropical C4 tussock grasses. The native shrub *Carissa ovata* is also widespread on some soil types. Stocking strategies are set in response to rainfall and pasture availability. The trial is testing five stocking strategies replicated twice (see [20] for more detail). Paddocks are approximately 100 hectares in size and contain three main soil types (Figure 1). We studied the two main soil types: duplex soils associated with *Eucalyptus brownii* (Reid River Box) and red-yellow earths associated with *E. melanophloia* (Mugga Ironbark) [21]. The three main management strategies investigated here were: (1) moderate stocking (MSR) at the recommended 8 to 10 hectares per Adult Equivalent (8 ha/AE, 1 AE = 450 kg), (2) heavy stocking (HSR) at 4 to 5 ha/AE and, (3) moderate stocking with rotational wet season resting (R/Spell) (Figure 2). In addition, we sampled exclosures (XCL) that were small, fenced areas within the paddocks (~25 × 25 m and 5 ha in R/Spell), protected from grazing. In drought years, stock numbers in the HSR were reduced and fed supplements to ensure animal welfare (Figure 2).

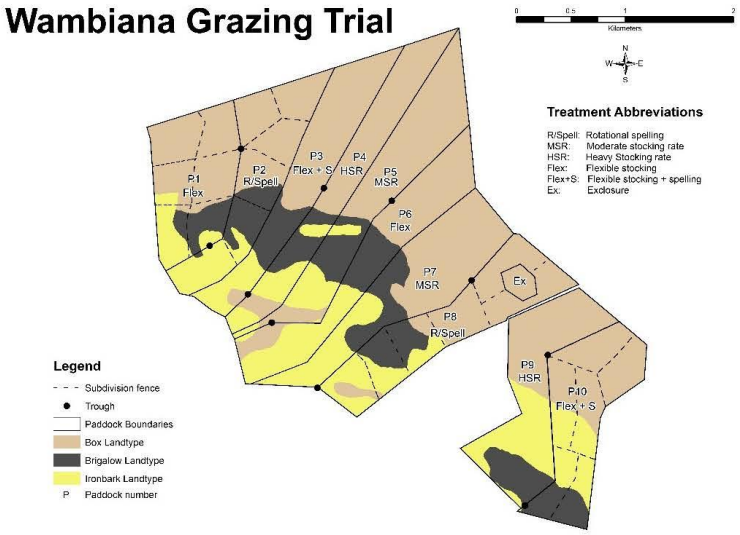


Figure 1. Wambiana Paddock plan of the landscape types and stocking strategies: heavy stocking (HSR), moderate stocking (MSR), and rotational spelling (R/Spell).

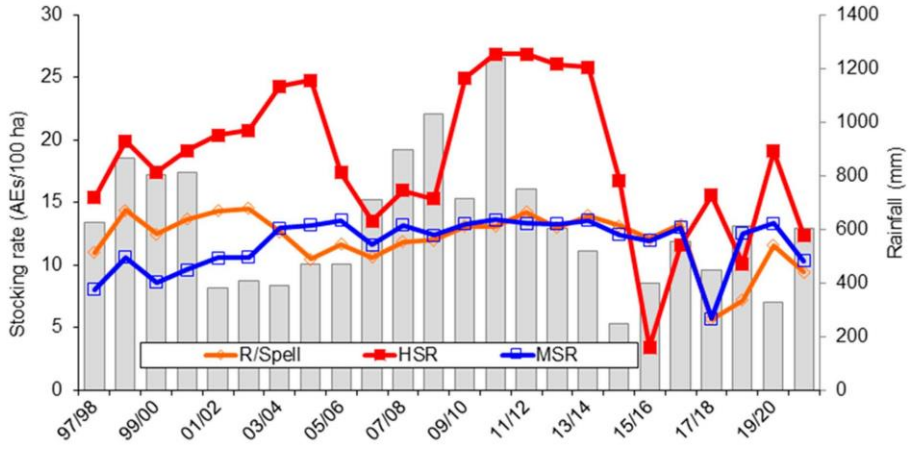


Figure 2. Stocking rates expressed as adult equivalents (AE) per 100 ha for rotational/spell (R/Spell), heavy stocking (HSR) and moderate stocking (MSR) against rainfall records at the Wambiana.

2.2. *Field Methods*

The paddocks consisted of two main soil types and were treated at two levels. Firstly, the whole paddock was treated as a management unit as there was a gradient of soils throughout. This reflects typical grazing properties that comprise large paddocks (hundreds to thousands of hectares) with changing soil types across them. Secondly, ecosystem responses across the paddock were measured within the soil types at the soil cover-biocrust level. Each long-term grazing trial paddock has several permanent one-hectare monitoring sites consisting of five 100-m transects set 20 m apart. Sites are stratified by soil type (Figure 1). Sampling was conducted in November 2020, following a season of well below average rainfall (384 mm) and a succession of five drought years, with 2014/15 the fourth driest year on record. We used the two replicate paddocks for each of the three treatments. On each of the two soil types we selected one monitoring site. Here, we selected two transects (50 m apart), then laid out a 30 m tape in the same direction as the 100 m transect. Alongside these 30 m lines, a 1 m^2 quadrat was placed at 6 m intervals (Figure 3). There were two soil types of duplex soils (DC), and red-yellow earths (RY). Two paddocks were selected for each treatment (HSR, MSR, R/Spell, and XCL), two transects per paddock and, six quadrats per transect. Exclosure (XCL) treatments were fenced areas within these paddocks with no access for stock. In total, 24 quadrats per treatment per soil type were assessed.

2.2.1. Landscape Function

Landscape function analysis (LFA) [12] has been developed to establish soil surface indicators for measuring and analyzing the nature and severity of problems in a dysfunctional or degraded ecosystem [3,22]. The conceptual framework is based on the spatial organization of clumps of grasses and shrubs that capture, accumulate, and retain resources (called patches). The interspaces (or inter-patches) are the open areas between the grass patches and can be natural 'hotspots' for biocrusts due to less competition for light, moisture, and litter. In this study, we focused on the role of these biocrust hotspots in determining the three LFA indices: stability, infiltration, and nutrient cycling. These three indices are assessed by 11 soil surface indicators (Figure 4) that are individually scored and provide the percentage level of each index. The indices are a relative measure and are independent of each other. In this study, we assumed the exclosures with no cattle grazing would be a benchmark for the best condition. The higher the index the better

the condition. The LFA complete soil surface assessment (SSA) data spreadsheet and detailed methodology is located in the LFA manual [23] and SSA details provided in the Supplementary Material (Figure S1). Our aim was to compare the different management strategies for each of the three indices that were representative of ecosystem function with a focus on the interspaces. For each quadrat, the LFA attributes were recorded and ranked (Figure S1). Later, they were separated into their dominant category: patches or interspaces. Only the interspaces were used in the data analysis and separately analyzed as either biocrust dominant (cover > 10% based on LFA category assessments) or bare soil dominant (where biocrust cover < 10%). For each treatment and soil type there were at least five quadrat replicates used in the analysis. Quadrats that matched the criteria were analyzed on separate soil surface assessment (SSA) worksheets in the LFA program.

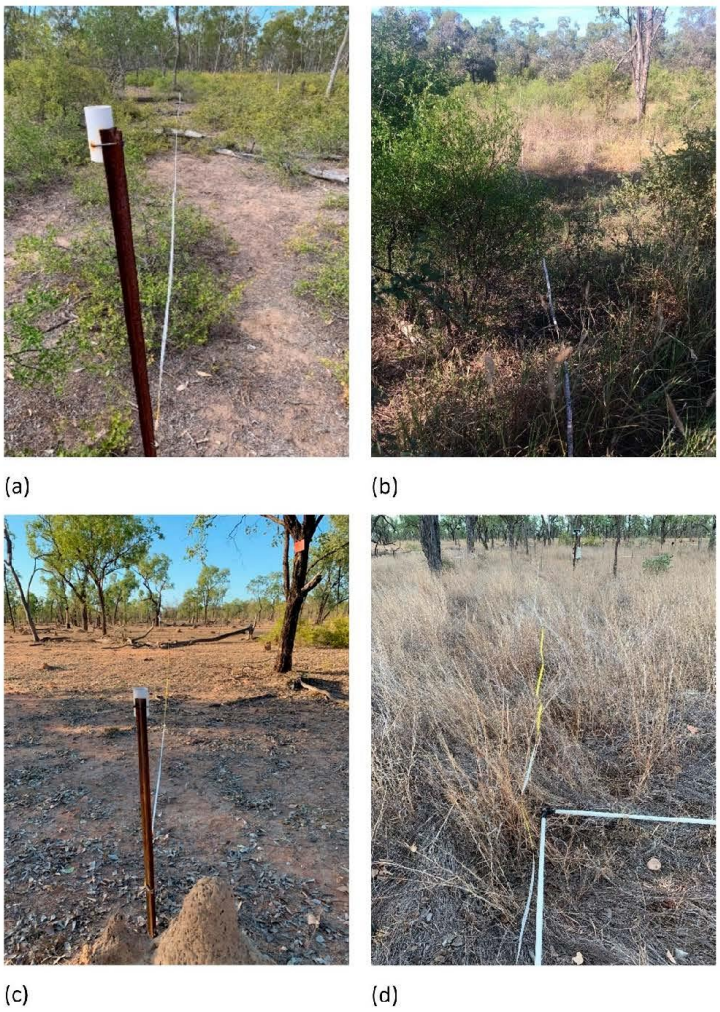


Figure 3. Box woodland transect on duplex soils (DC) with (**a**) heavy stocking (HSR) and (**b**) exclosure (XCL) no stock; Ironbark woodland on red-yellow earths (RY) with (**c**) HSR and (**d**) XCL.

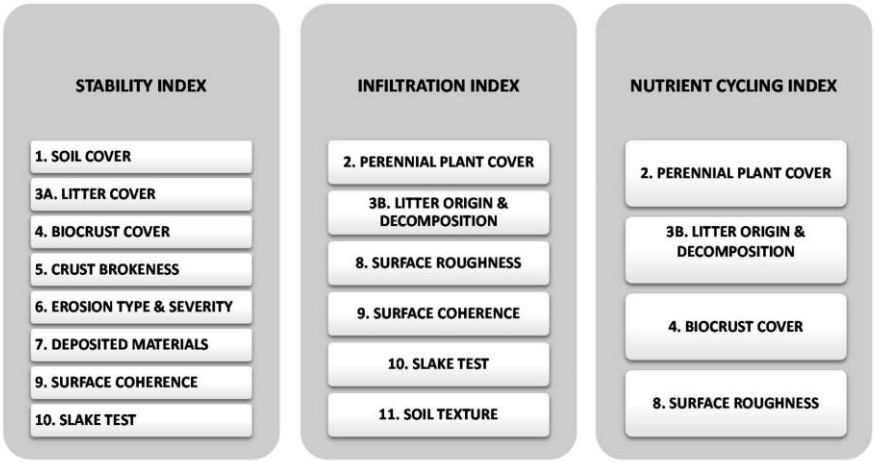


Figure 4. LFA indices (stability, infiltration, and nutrient cycling) and the contribution of the measured attributes to each.

2.2.2. Ground Cover

Ground cover was measured in each 1 m^2 quadrat (Figure 5) in two ways. Firstly the overall grass cover was recorded; *C. ovata* patches were identified separately. This was followed by estimating the ground-level cover for each component as a total percentage of the cover categories within each quadrat. These categories comprised biocrust, bare soil, basal area of grass plants, and litter cover and equaled 100%.

2.3. Biocrust Structure

Scanning Electron Microscope (SEM) Imaging

Representative biocrusts for imaging were selected from samples collected on 11–13 November 2020. The images were processed at the University of Queensland's Centre for Microscopy and Microanalysis. Double-sided carbon stickers were attached to round aluminum specimen stubs. Silver conducting paint was added to the stickers for enhanced stability of biocrust samples. Sections were made to appropriate sizes to fit on to stubs and placed using tweezers. After samples were prepared on stubs, they were coated with platinum, using the Safematic CCU-010 Compact Coating Unit. Ensuring the appropriate settings were in use, the chamber was pressurized before samples were coated for 10 s. Following platinum coating, samples were positioned on the viewing stage of the Hitachi TM4000Plus II Tabletop Scanning Electron Microscope© (Hitachi High-Technologies Corporation Tokyo, Japan). Three samples were added to the stage at one time, and individually imaged.

2.4. Statistical Analysis

We examined the differences in biocrusts, bare soil, basal grass area, and litter cover across all treatments using ANOVAs (Minitab V20, [24]) and applied Tukey's method to identify significant differences between treatments. To establish the three LFA indices for all quadrats, we processed the attributes in the LFA spreadsheet , available online accompanying the manual [22] and detailed in Section 2.2.1. Once the indices had been calculated, we examined the differences in a General Linear Model with fixed factors to look at the effect on the three stocking levels for each variable. We then used Tukey's pairwise comparison tests to determine where significant differences occurred between treatments.

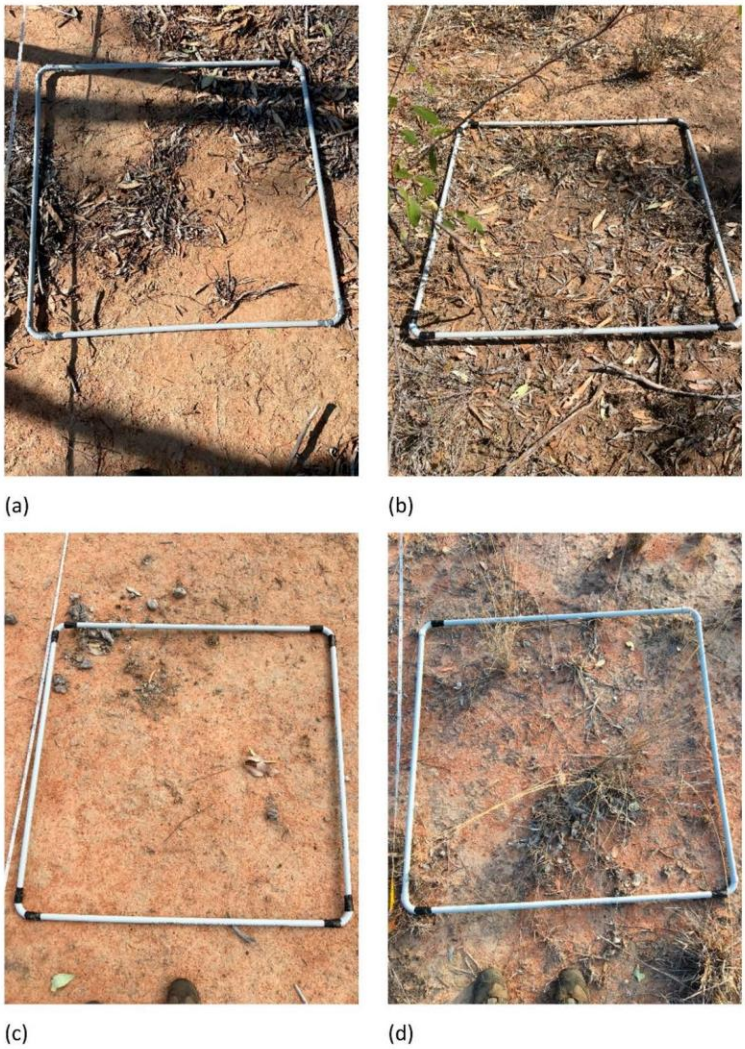


Figure 5. Examples of 1 m^2 quadrats across different soils and contrasting treatments. (**a**) DC soil, HSR treatment; (**b**) DC soil, MSR treatment; (**c**) RY soil, HSR treatment; (**d**) RY soil, MSR treatment.

3. Results

3.1. Biocrust Hotspots in Duplex Soils

The biocrust cover was significantly higher in the exclosures (XCL), and the rotational spelled paddocks (R/Spell) compared to the heavily (HSR) and moderately (MSR) stocked paddocks ($p < 0.001$). Biocrust cover across the XCL and R/Spell averaged ~34%, about double that of both MSR (18.7%) and HSR (14.6%) (Figure 6).

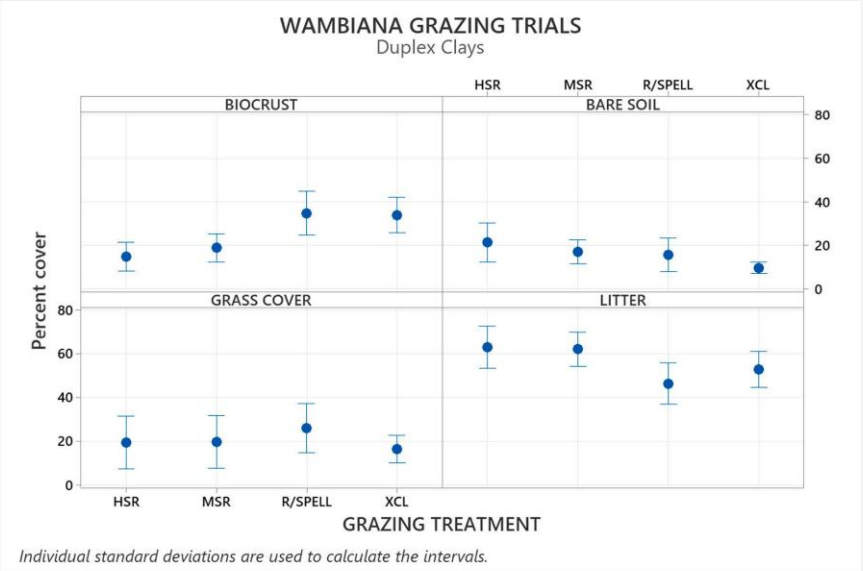


Figure 6. Duplex soils with comparisons of mean values ± SD for grass and shrub (*Carissa* sp.) and ground cover: biocrusts, bare soil (no visible biocrust), and litter cover at different stocking levels: high stocking rates (HSR), moderate stocking rates (MSR), rotational spelling at moderate stocking rates (R/Spell) and, no stock, exclosures (XCL).

In-paddock observations, followed by SEM, demonstrated well-developed and cyanobacterial dominated biocrusts in the XCL's and R/Spell treatments compared to HSR treatments that were almost completely devoid of biocrust (Figure 7). Bare ground cover was significantly lower in the exclosures (9.6%) compared with HSR ($p = 0.03$, 21.3%) but were similar between XCL, MSR, and R/Spell (9.6–16.9%). There were no significant differences between treatments for grass/shrub or litter cover (Figure 6).

3.2. Biocrusts in Red-Yellow Earths

The red-yellow earths (RY) did not significantly differ in their biocrust cover across grazing treatments; however, the bare ground in the heavily grazed paddocks was up to 2.5 times higher than the XCL, R/Spell and MSR ($p < 0.001$). Overall, the various treatments were significantly different from each other where the bare ground cover (mean % ± SD) in the XCL was the lowest (14.8 ± 11.75) and R/Spell (29.9 ± 20.7) compared to the HSR (79 ± 11.5) and MSR (51.4 ± 30.9), (Figure 8). Observations in the paddock showed that the biocrusts on the RY soils were often thin and fragile and easily broken. We followed up with SEM that confirmed cyanobacterial dominated biocrusts in the XCL, and HSR were almost devoid of biocrust (Figure 9). Grass and litter cover were both significantly different across the grazing treatments ($p < 0.001$). Although grass cover (mean % ± SD) in the XCL was by far the highest (32.4 ± 34.3), this was also highly variable. However, HSR and MSR were similar (1.6 ± 5.1% and 1.6 ± 2.4% respectively) while R/Spell grass cover was 9.9 ± 12.3%, highly variable and statistically similar to HSR and MSR. Litter cover ranged from 57.7% (XCL) to 12.7% (HSR), with a significant difference between the XCL and R/Spell ($p < 0.001$); however, these were significantly different from MSR and HSR (Figure 8).

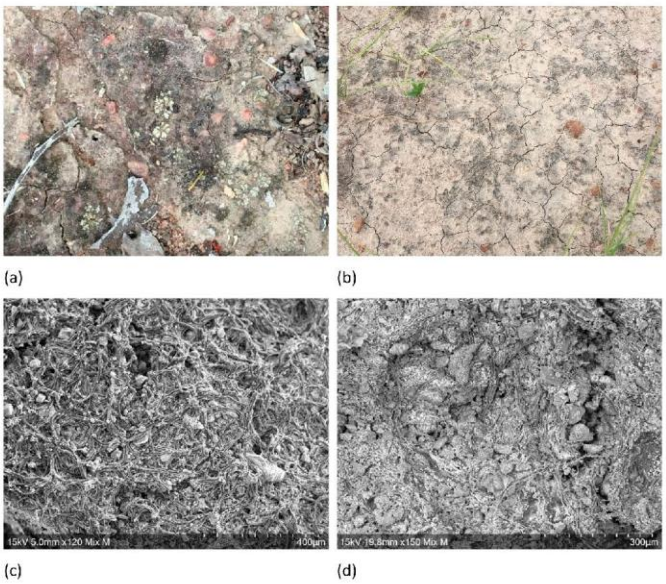


Figure 7. Duplex soils, photos and SEM images: (**a**) Biocrust in good condition (XCL) with cyanobacteria, lichens, liverworts, and mosses; (**b**) darkened patches represent poor quality cyanobacterial biocrust; (**c**) R/Spell cyanobacterial filaments with fine soil particles SEM, 120× mag; (**d**) HSR biocrust in poor condition, cyanobacterial filaments with soil, 150× mag.

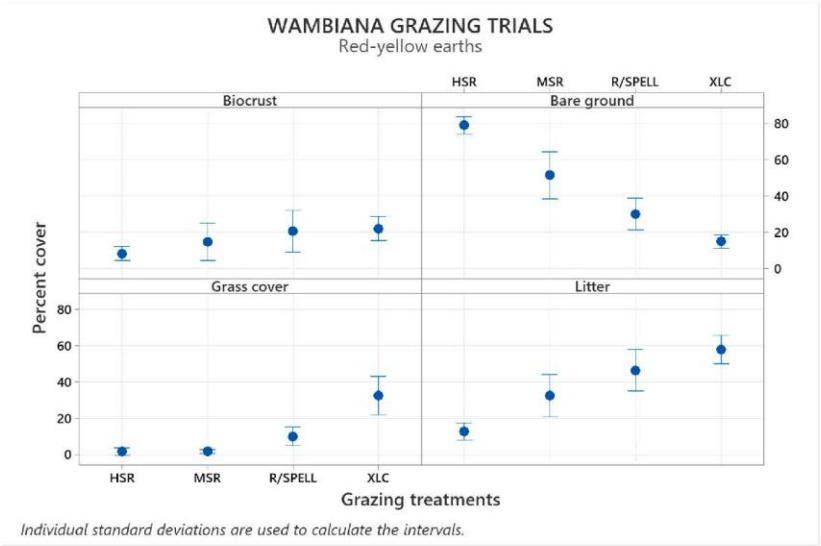


Figure 8. Red-yellow earths with comparisons of mean values ± SD for grass cover and ground cover: biocrusts, bare soil (no visible biocrust), litter, at different stocking levels: high stocking rates (HSR), moderate stocking rates (MSR), rotational spelling at moderate stocking rates (R/Spell) and, no stock, exclosures (XCL).

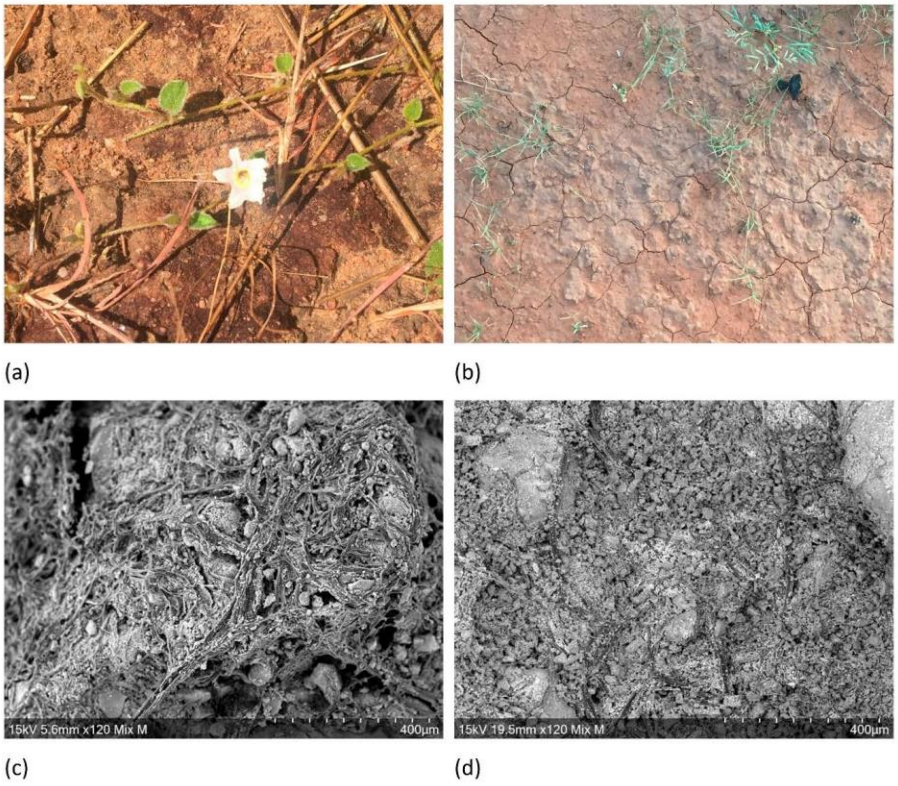


Figure 9. Red-yellow earths, photo and scanning electron microscope (SEM) images: (**a**) Red coloured biocrust in good condition (XCL) dominated by cyanobacteria; (**b**) degraded cyanobacterial biocrust seen with faint discolouration on surface; (**c**) R/Spell cyanobacterial filaments with soil particles SEM, 120× mag; (**d**) HSR biocrust in poor condition, cyanobacterial filaments with soil, 120× mag.

3.3. Landscape Function across Interspaces

Across the interspaces, all LFA indices were negatively affected by HSR management strategies, which had the lowest percentage indices for stability, infiltration, and nutrient cycling (Figure 10). However, there were varied differences between the LFA indices across all treatments, particularly in the RY soil types (Figures 8 and 11), especially in HSR that was dominated by >80% bare soil, and very low levels of biocrusts (Figure 8). Although the rotational spelling (R/Spell) had the highest average levels of landscape function of all the grazed treatments, due to the high variance, especially in the RY soil type, there were no significant differences, and it was not included in the overall analysis (Table 1).

3.3.1. Stability

The duplex soils (DC) and red-yellow earths (RY) that dominated the Box and Ironbark woodlands differed in their structure [21], and the stability of the interspaces was significantly different ($p = 0.04$) (Figure S1). In the DC soils, the exclosures (XCL) had significantly higher stability compared to the HSR paddocks ($p = 0.003$), and although not significant, XCL was somewhat different to MSR ($p = 0.06$). RY soil stability indices (mean %) had the widest ranges between 53.8% (RY, HSR) and 68.5% (XCL) (Table 1, Figure 10).

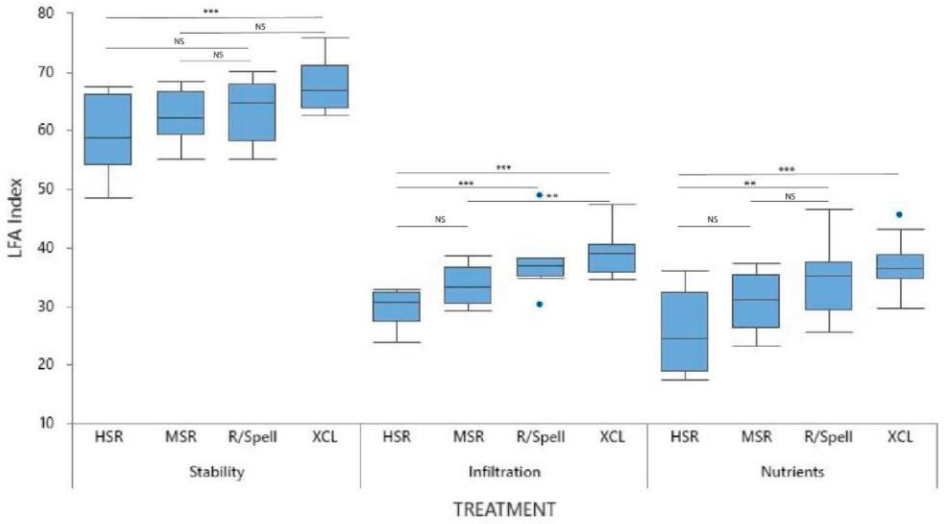


Figure 10. Overall results for LFA indices for heavy (HSR), moderate (MSR), rotational spelling (R/Spell) and exclosure (XCL) stock treatments at the paddock scale. Significant differences marked with ** ($p < 0.05$), and *** ($p < 0.001$), or NS for not significant.

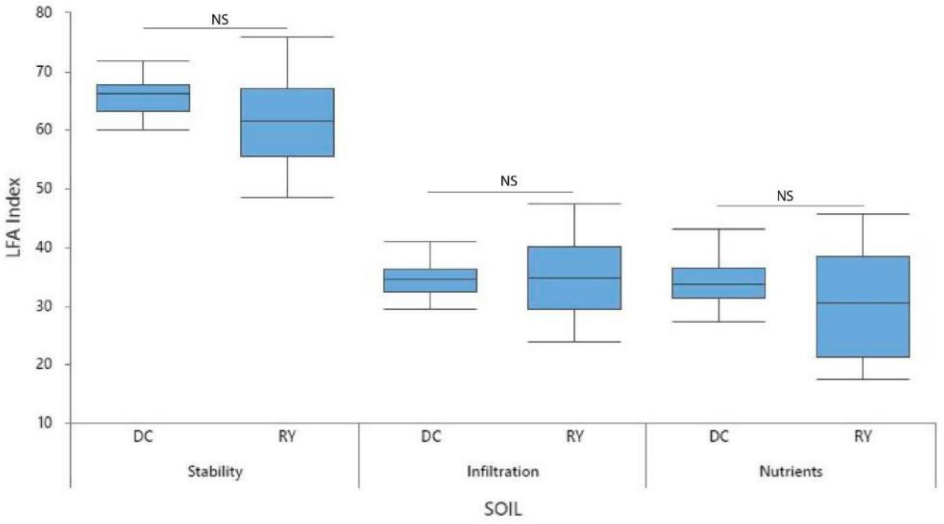


Figure 11. At a paddock scale there was high variability with no significant differences (NS) found between soil types for LFA indices for heavy (HSR), moderate (MSR), and exclosure (XCL) treatments.

Table 1. LFA Indices across all treatments (mean % ± SE) DC—Duplex soil; RY—red-yellow soil; HSR—heavy stocking rate; MSR—moderate stocking rate; R/Spell—rotational spelling (paddock resting during wet season); MSR—moderate stocking rate; XCL—exclosure.

Variable	Soil	HSR	MSR	R/SPELL	XCL
Stability	DC	65.0 ± 3.39	65.1 ± 1.64	65.1 ± 1.21	66.9 ± 1.24
	RY	53.8 ± 3.88	59.8 ± 1.94	62.1 ± 3.66	68.5 ± 2.24
Infiltration	DC	31.6 ± 0.76	34.8 ± 0.99	36.8 ± 0.82	37.1 ± 1.17
	RY	28.2 ± 1.93	32.3 ± 4.25	38.3 ± 3.89	40.6 ± 1.49
Nutrients	DC	31.8 ± 1.8	33.9 ± 3.38	35.6 ± 0.97	35.5 ± 1.86
	RY	19.4 ± 0.94	27.5 ± 4.11	33.8 ± 4.67	36.7 ± 1.55

3.3.2. Infiltration

DC and RY soils did not significantly differ in their infiltration indices ($p = 0.89$) (Figure 11), however XCL had significantly higher infiltration compared to HSR ($p < 0.001$), and MSR ($p = 0.009$) (Figure 10). RY infiltration indices (mean %) also showed the widest range between 28.2% for the HSR and 40.6% for the XCL (Table 1).

3.3.3. Nutrient Cycling

At the paddock scale nutrient cycling across the interspaces was significantly different ($p = 0.05$) however there were no significant differences between the DC and RY soil types (Figure 11). The XCL had significantly higher nutrient cycling levels than both the HSR paddocks ($p < 0.001$), and for the MSR paddocks ($p = 0.03$) over both soil types. Yet, due to the high variability in DC soils (Table 1), HSR and MSR were not significantly different ($p = 0.13$). High litter levels in DC likely contributed to this (Figure 6). Nutrient cycling indices in the RY soils also widely differed (mean %) from 19.4% (HSR) to 36.7% (XCL) (Table 1).

4. Discussion

4.1. Sustaining Landscape Function during Drought

The understory of northern Australian savannas and grasslands is dominated by perennial tussock grasses providing pasture and protecting the soil surface from erosion. Loss of these grasses due to drought or excessive grazing pressure by cattle results in a loss of pasture condition, and an increase in bare ground, soil loss, and unpalatable weed invasion [1]. In landscapes that are intact and managed sustainably, the soil surface of the interspaces between grass tussocks is covered with biocrusts, which protect soil from erosion, ensure water infiltration, and add organic carbon and nitrogen to the soil [25,26]. Our landscape function and soil health study occurred after five years of drought at a long-term grazing trial (Figure 2 [1]). Irrespective of the grazing strategy applied, landscape function was compromised, compared to ungrazed exclosures, likely due to the prolonged deficiency from well below-average rainfall. Despite this, we found strong evidence that rotational spelling during the wet season, combined with a moderate stocking rate, improved both biocrust and pasture cover. Resting paddocks from livestock grazing in the wet season to allow pasture plant recruitment and growth is recommended as an important management strategy. Leaving pasture areas to rest can deliver rapid improvements, provided stocking rates had not been excessive [1]. Prior to the point when drought starts to affect the landscape, understanding the role of the interspaces between grass tussocks is critically important [19]. We showed that, by examining landscape function during a drought year, following five years of below-average rainfall, these interspaces significantly contributed to the three key areas of maintaining a functional landscape: nutrient cycling, infiltration, and stability.

4.2. Biocrusts Protect the Soil during Drought

Biocrust cover across the interspaces on both soil types provided protection and contributed to soil nutrient cycling. Biocrust cover in the wet season spelled paddocks was almost as extensive as cattle exclosures on duplex soils. Furthermore, the biocrust cover was about twice that of the moderately stocked paddocks and more than double that of the heavily stocked paddocks. Significantly, neither of these latter strategies had any form of spelling. The biocrust cover was highly visible across the paddocks. The grasses had almost disappeared, leaving large tracts of bare ground with large patches of non-desirable and prickly unpalatable *C. ovata* shrub dominating the understory (Figure 3a). As cattle avoid *C. ovata* patches and preferentially used interspaces there is a consequential increase in grazing pressure in the interspaces between the *C. ovata* [4]. We predict that, should the drought conditions continue, increased grazing pressure on the remaining perennial grasses and trampling will lead to a more rapid decline in land condition. Once a threshold of biocrust removal is reached, the cover loss becomes exponential, and the topsoil is vulnerable to erosion [27]. In sandy and loam soils of the Australian Mallee regions, mechanically disturbed biocrusts had soil losses increase 1.6 times. Post-disturbance, the soil loss was 6.7 times the erosion target [27]. Removal of the biocrust increased the risk of erosion from less than five percent to greater than twenty percent [27]. We found a similar occurrence in the red-yellow earths (sandy loams), where the loss of biocrust in all the stocked paddocks has resulted in a significant loss of landscape function across all three soil health indices. Although the exclosure had on average >20% biocrust cover, sandy loams require >31% cover to protect them and maintain soil transport below erosion limits of 5 g m^2 [27]. It should be noted that the grass cover in the exclosure and rotational spelled red-yellow soil averaged 10%, thus in combination with the biocrusts, it would provide adequate protection from soil loss. In red-yellow soils with rotational spelling, the biocrust cover was around the 20% threshold, although the impact of the drought meant that treatment differences were non-significant. At Wambiana, unprotected soil would be washed away by the overland flow of water from heavy rains, and following the loss of biocrust cover [28]. The most pronounced degradation was observed in the heavy stocking rate with over 80% bare ground with little to no protection from biocrusts, which only covered 10%, and were poorly developed (Figures 5, 7 and 9). On the more stable duplex clay-richer soils, in the exclosure and rotational spelled sites, the biocrust cover was well over the threshold (~34%). By comparison, moderate and heavy stocking rates without spelling had less than 15% cover and consequently were highly vulnerable to soil loss. Due to natural aggregation promoting biocrust cover, the duplex (clay richer) soils are inherently more stable than the red-yellow (sandy loam) soils. In contrast, sandier soils are dependent on the biocrust for their stability [23]. The stability between soil types and treatments is a critical factor for water infiltration and nutrient cycling. When the landscape loses stability, soil loss is inevitable, compounding the factors influencing infiltration (surface cover and cohesion) and nutrient loss increase. While landscape function is a continuum along a gradient of gains or losses, after a certain point, the losses occur exponentially [9,25].

4.3. Biocrust Hotspots—The Engine Room of the Landscape

In these landscapes, biocrust cover occupying the interspaces provides an important source of nutrients, and when degraded or removed, results in the loss of the three functional roles it provides. In northern Australia, biocrusts are a considerable component of the rangelands that contribute significantly to the carbon and nitrogen content of the soils [13,26]. At the study site, wet season resting from grazing boosted biocrust hotspots in the interspaces across the duplex soils. This proved advantageous in also increasing nutrient cycling in rotational spelled paddocks to similar levels as the exclosures. LFA suggests the interspaces were biocrust hotspots that influenced nutrient cycling and infiltration. On a small scale, spatial heterogeneity of biocrusts may not appear to influence nutrient cycles [29] however as demonstrated in XLCs and R/Spell, (DC) had more biocrust and better functional indices. In the DC soils nutrient cycling was significantly higher in the

XCLs (~35%) compared to HSR (about 4% difference, Table 1), similarly for R/Spell, with higher variability. In contrast, for the HSR in the RY soils the nutrient cycling index was considerably lower in the DC soils at around 19% and almost half that of XCL and R/Spell (34–37%), and still significantly lower than MSR (27%) (Table 1). Landscape function represents a sequence of processes operating to support and maintain the biogeochemical engine room of the landscape [9]. It is important to fully understand the components of the landscape that contribute to these processes. As a consequence of the natural gradation between soil types across the landscape there were no significant differences between landscape function indices (Figure 11). Yet, there were significant differences between stability, infiltration, and nutrient cycling due to the other variables assessed. We have focused on the role of the biocrusts in the interspaces between the plants. This is underpinned by the primary role of cyanobacteria (Figures 7 and 9) that dominate these biocrusts to act as ecosystem engineers [27]. Cyanobacterial soil crusts are known to modulate the landscape, redistribute water resources, create habitats that allow for the introduction of other species, and increase biodiversity [28]. Other studies have shown the net positive effect of biocrusts on infiltration [29]. Cyanobacteria also contribute significant amounts of carbon and nitrogen to the soil [13,26,30], and are thus instrumental in building soil nutrients [31]. Through the cyanobacterial extra-cellular-polymeric matrix (ECM) that binds cyanobacteria together, biocrusts are integrated into the soil surface particles [32], with the ECM stickiness also trapping dust particles (Figures 7 and 9). Cyanobacteria and its ECM influence the physicochemical and hydrological properties of the soil [29,33] and in northern Australia they play an important role in regulating its seasonal productivity [16,30].

4.4. Managing and Monitoring the Interspaces

The interspaces are the areas first impacted by drought where excessive trampling can occur as cattle seek out grasses. As the interspaces increase in size and lose biocrust cover, exposure to the elements (particularly from the overland flow of water) will result in soil loss. In this study, there was a strong link between these interspaces and the presence of biocrusts that influenced all three landscape function indices, nutrient cycling, stability, and infiltration. In these landscapes, biocrusts can provide resilience to the impacts of drought, but heavy stocking severely limits the contribution of biocrusts across all land types. Drought and grazing are known to reduce biocrust presence and diversity [19]. It follows that understanding the role of the interspaces and the common and widespread occurrence of biocrusts that landscape function can also be determined by monitoring the presence/absence and extent of biocrusts in these interspaces. During drought, the risks of landscape function declining by overstocking escalate. Resting during part or all of a wet season provides a period with limited soil surface disturbances when biocrusts are at the height of productivity [30], allowing them to rapidly recolonize the soil surfaces. In the interstitial spaces, biocrusts will in turn provide a nutrient-rich micro-climate conducive to native grass establishment and often inhibitive to weeds [31]. Monitoring interspaces can therefore be a key tool for understanding the level of landscape health or decline early in the drought cycle.

5. Concluding Remarks

Management at the paddock scale needs to incorporate ecosystem services provided by perennial plants, biocrusts, and leaf litter to better understand the influence they have on productive pastures, soil stability, infiltration, and nutrient cycling. Our results support the recommended practice of wet season (rotational) spelling in Northern Australia. Maintaining and monitoring biocrusts that occur in the interspaces can be an important management strategy combined with understanding the carrying capacity of paddocks. Other data from the present trial shows, that while pasture condition has declined the most in the HSR, it has also declined significantly in the other more 'sustainable' treatments, most likely due to the effects of drought (pers. comm. P. O'Reagain). Is the inevitable result of grazing a decline in land condition? We do not necessarily think this is the case;

however, continuing to monitor the extent of recovery post-drought would be beneficial. As demonstrated with the DC soils, under good management, grazing can achieve similar outcomes to exclosures. Furthermore, recent research has highlighted the benefits of using biocrusts and cyanobacteria to facilitate landscape recovery post-disturbance [32–35]. Future studies could incorporate the application of biocrust inoculum to degraded areas to promote functional recovery.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agronomy12010062/s1, Figure S1: LFA Manual.

Author Contributions: Conceptualization, W.J.W., S.S., B.A., P.O., and R.C.; Data curation, W.J.W., T.M.S., and C.D.; Formal analysis, W.J.W., and C.D.; Funding acquisition, W.J.W., S.S., and R.C.; Investigation, W.J.W., E.Z., B.A., T.M.S., S.W., M.D., G.P., P.O., and J.B.; Methodology, W.J.W., S.S., P.O., J.B., R.C., and C.D.; Project administration, N.R.; Supervision, S.S., E.Z., B.A., and N.R.; Writing—original draft, W.J.W. and C.D.; Writing—review and editing, S.S., E.Z., B.A., S.W., M.D., G.P., P.O., R.C., C.D., and N.R. All authors have read and agreed to the published version of the manuscript.

Funding: This project is funded by Meat & Livestock Australia as part of "Boosting natural regeneration of the nitrogen capital in grazing lands" (B.PAS. 0502) in collaboration with The University of Queensland, Queensland Department of Agriculture & Fisheries, Northern Territory Department of Primary Industries.

Acknowledgments: We would like to acknowledge the traditional guardians of the Gudjal Lands where Wambiana is located. We sincerely appreciate and thank Michael and Michelle Lyons for their cooperation, assistance, and hospitality as the owners of Wambiana Station, whose help and advice has been invaluable. We especially thank Laiza Sherar who volunteered her time and assisted in driving, field work and data collation.

Conflicts of Interest: There is no conflict of interest or ethical consideration for this research.

References

1. O'Reagain, P.J.; Scanlan, J.C. Sustainable Management for Rangelands in a Variable Climate: Evidence and Insights from Northern Australia. *Animal* **2013**, *7*, 68–78. [CrossRef] [PubMed]
2. Scanlan, J.C.; McIvor, J.G.; Bray, S.G.; Cowley, R.A.; Hunt, L.P.; Pahl, L.I.; MacLeod, N.D.; Whish, G.L. Resting Pastures to Improve Land Condition in Northern Australia: Guidelines Based on the Literature and Simulation Modelling. *Rangel. J.* **2014**, *36*, 429–443. [CrossRef]
3. Tongway, D.J. Soil and Landscape Processes in the Restoration of Rangelands. *Rangel. J.* **1990**, *12*, 54–57. [CrossRef]
4. Ash, A.J.; Corfield, J.P.; McIvor, J.G.; Ksiksi, T.S. Grazing Management in Tropical Savannas: Utilization and Rest Strategies to Manipulate Rangeland Condition. *Rangel. Ecol. Manag.* **2011**, *64*, 223–239. [CrossRef]
5. Ash, A.; Thornton, P.; Stokes, C.r.s.; Togtohyn, C. Is Proactive Adaptation to Climate Change Necessary in Grazed Rangelands? *Rangel. Ecol. Manag.* **2012**, *65*, 563–568. [CrossRef]
6. Godde, C.M.; Garnett, T.; Thornton, P.K.; Ash, A.J.; Herrero, M. Grazing Systems Expansion and Intensification: Drivers, Dynamics, and Trade-Offs. *Glob. Food Secur.* **2018**, *16*, 93–105. [CrossRef]
7. Holt, J.A.; Coventry, R.J. Nutrient Cycling in Australian Savannas. *J. Biogeogr.* **1990**, *17*, 427–432. [CrossRef]
8. Housman, D.C.; Yeager, C.M.; Darby, B.J.; Sanford, R.L.; Kuske, C.R.; Neher, D.A.; Belnap, J. Heterogeneity of Soil Nutrients and Subsurface Biota in a Dryland Ecosystem. *Soil Biol. Biochem.* **2007**, *39*, 2138–2149. [CrossRef]
9. Mott, J.J.; Williams, J.; Andrew, M.H.; Gillison, A.N. Australian Savanna Ecosystems. In *Ecology and Management of the World's Savannas*; Australian Academy of Sciences: Canberra, Australia, 1985; pp. 56–82.
10. McKeon, G.M.; Day, K.A.; Howden, S.M.; Mott, J.J.; Orr, D.M.; Scattini, W.; Weston, E.J. Northern Australian Savannas: Management for Pastoral Production. *J. Biogeogr.* **1990**, 355–372. [CrossRef]
11. Hill, J.O.; Coates, D.B.; Whitbread, A.M.; Clem, R.L.; Robertson, M.J.; Pengelly, B.C.; Hill, J.O.; Coates, D.B.; Whitbread, A.M.; Clem, R.L.; et al. Seasonal Changes in Pasture Quality and Diet Selection and Their Relationship with Liveweight Gain of Steers Grazing Tropical Grass and Grass–Legume Pastures in Northern Australia. *Anim. Prod. Sci.* **2009**, *49*, 983–993. [CrossRef]
12. Tongway, D.; Hindley, N. Landscape Function Analysis: A System for Monitoring Rangeland Function. *Afr. J. Range Forage Sci.* **2004**, *21*, 109–113. [CrossRef]
13. Pointing, S.B.; Belnap, J. Microbial Colonization and Controls in Dryland Systems. *Nat. Rev. Microbiol.* **2012**, *10*, 551–562. [CrossRef]
14. Eldridge, D.J.; Poore, A.G.B.; Ruiz-Colmenero, M.; Letnic, M.; Soliveres, S. Ecosystem Structure, Function, and Composition in Rangelands Are Negatively Affected by Livestock Grazing. *Ecol. Appl.* **2016**, *26*, 1273–1283. [CrossRef]

15. Williams, W.J.; Büdel, B.; Reichenberger, H.; Rose, N. Cyanobacteria in the Australian Northern Savannah Detect the Difference between Intermittent Dry Season and Wet Season Rain. *Biodivers. Conserv.* **2014**, *23*, 1827–1844. [CrossRef]
16. Williams, W.; Büdel, B.; Williams, S. Wet Season Cyanobacterial N Enrichment Highly Correlated with Species Richness and *Nostoc* in the Northern Australian Savannah. *Biogeosciences* **2018**, *15*, 2149–2159. [CrossRef]
17. Barger, N.N.; Weber, B.; Garcia-Pichel, F.; Zaady, E.; Belnap, J. Patterns and Controls on Nitrogen Cycling of Biological Soil Crusts. In *Biological Soil Crusts: An Organizing Principle in Drylands*; Ecological Studies; Springer: Cham, Switzerland, 2016; pp. 257–285; ISBN 978-3-319-30212-6.
18. Hunt, L.P.; McIvor, J.G.; Grice, A.C.; Bray, S.G. Principles and Guidelines for Managing Cattle Grazing in the Grazing Lands of Northern Australia: Stocking Rates, Pasture Resting, Prescribed Fire, Paddock Size and Water Points—A Review. *Rangel. J.* **2014**, *36*, 105–119. [CrossRef]
19. Williams, W.J.; Eldridge, D.J.; Alchin, B.M. Grazing and Drought Reduce Cyanobacterial Soil Crusts in an Australian Acacia Woodland. *J. Arid Environ.* **2008**, *72*, 1064–1075. [CrossRef]
20. O'Reagain, P.; Bushell, J.; Holloway, C.; Reid, A.; O'Reagain, P.; Bushell, J.; Holloway, C.; Reid, A. Managing for Rainfall Variability: Effect of Grazing Strategy on Cattle Production in a Dry Tropical Savanna. *Anim. Prod. Sci.* **2009**, *49*, 85–99. [CrossRef]
21. Aspandiar, M.F.; Taylor, G.; Eggleton, R.A. Charters Towers Region, Queensland. In *Regolith Landscape Evolution Across Australia: A Compilation of Regolith Landscape Case Studies with Regolith Landscape Evolution Models*; Cooperative Research Centre for Landscape Environments and Mineral Exploration: Perth, Australia, 2003.
22. Tongway, D.J.; Ludwig, J.A. *Restoring Disturbed Landscapes: Putting Principles into Practice*; Island Press: Washington, DC, USA, 2011; ISBN 1-59726-580-2.
23. Tongway, D.; Hindley, N. *Landscape Function Analysis: Procedures for Monitoring and Assessing Landscapes—with Special Reference to Minesites and Rangelands*; CSIRO Sustainable Ecosystems: Canberra, Australia, 2004.
24. Data Analysis Software | Statistical Software Package. Available online: https://www.minitab.com/content/www/websites/en-us/products/minitab.html (accessed on 3 November 2021).
25. Williams, W.J.; Büdel, B. Species Diversity, Biomass and Long-Term Patterns of Biological Soil Crusts with Special Focus on Cyanobacteria of the Acacia Aneura Mulga Lands of Queensland, Australia. *Algol. Stud.* **2012**, *140*, 23–50. [CrossRef]
26. Eldridge, D.J.; Zaady, E.; Shachak, M. Microphytic Crusts, Shrub Patches and Water Harvesting in the Negev Desert: The Shikim System. *Landsc. Ecol.* **2002**, *17*, 587–597. [CrossRef]
27. Eldridge, D.J.; Leys, J.F. Exploring Some Relationships between Biological Soil Crusts, Soil Aggregation and Wind Erosion. *J. Arid Environ.* **2003**, *53*, 457–466. [CrossRef]
28. Bowker, M.A.; Belnap, J.; Bala Chaudhary, V.; Johnson, N.C. Revisiting Classic Water Erosion Models in Drylands: The Strong Impact of Biological Soil Crusts. *Soil Biol. Biochem.* **2008**, *40*, 2309–2316. [CrossRef]
29. Bowker, M.A.; Maestre, F.T.; Eldridge, D.; Belnap, J.; Castillo-Monroy, A.; Escolar, C.; Soliveres, S. Biological Soil Crusts (Biocrusts) as a Model System in Community, Landscape and Ecosystem Ecology. *Biodivers. Conserv.* **2014**, *23*, 1619–1637. [CrossRef]
30. Büdel, B.; Williams, W.J.; Reichenberger, H. Annual Net Primary Productivity of a Cyanobacteria-Dominated Biological Soil Crust in the Gulf Savannah, Queensland, Australia. *Biogeosciences* **2018**, *15*, 491. [CrossRef]
31. Slate, M.L.; Callaway, R.M.; Pearson, D.E. Life in Interstitial Space: Biocrusts Inhibit Exotic but Not Native Plant Establishment in Semi-Arid Grasslands. *J. Ecol.* **2019**, *107*, 1317–1327. [CrossRef]
32. Chamizo, S.; Mugnai, G.; Rossi, F.; Certini, G.; De Philippis, R. Cyanobacteria Inoculation Improves Soil Stability and Fertility on Different Textured Soils: Gaining Insights for Applicability in Soil Restoration. *Front. Environ. Sci.* **2018**, *6*, 49. [CrossRef]
33. Williams, W.; Chilton, A.M.; Schneemilch, M.; Williams, S.; Neilan, B.A.; Driscoll, C. Microbial Biobanking–Cyanobacteria-Richtopsoil Facilitates Mine Rehabilitation. *Biogeosciences* **2019**, *16*, 1–16. [CrossRef]
34. Bowker, M.A. Biological Soil Crust Rehabilitation in Theory and Practice: An Underexploited Opportunity. *Restor. Ecol.* **2007**, *15*, 13–23. [CrossRef]
35. Román, J.R.; Roncero-Ramos, B.; Chamizo, S.; Rodríguez-Caballero, E.; Cantón, Y. Restoring Soil Functions by Means of Cyanobacteria Inoculation: Importance of Soil Conditions and Species Selection. *Land Degrad. Dev.* **2018**, *29*, 3184–3193. [CrossRef]

Appendix 4: Effects of grazing and fire management on rangeland soil and biocrust microbiomes.

Ecological Indicators 148 (2023) 110094

Contents lists available at ScienceDirect

Ecological Indicators

journal homepage: www.elsevier.com/locate/ecolind

Effects of grazing and fire management on rangeland soil and biocrust microbiomes

Maria V. Vega-Cofre [a], Wendy Williams [b], Young Song [a], Susanne Schmidt [b,*], Paul G. Dennis [a,*]

[a] School of Earth and Environmental Sciences, The University of Queensland, Brisbane, QLD 4072, Australia
[b] School of Agriculture and Food Sciences, The University of Queensland, Brisbane, QLD 4072, Australia

ARTICLE INFO

Keywords:
Drylands
Grazing
Rangelands
Fire management
Ranching
Biocrusts
Microbiome

ABSTRACT

Biocrusts play important roles in rangeland ecosystems by protecting soil surfaces and fixing carbon and nitrogen. Their responses to rangeland management practices, however, are poorly understood. Here, we characterised the impacts of cattle grazing and fire management (controlled 2- or 4-yearly burning *versus* no fire) on the diversity and composition of biocrust and associated soil bacterial communities (0–1 cm depth) in a long-term (30–60 years) field experiment in the Northern Territory, Australia. Both experiments were replicated on two soil types (vertosol, calcarosol). For the grazing experiment, we also characterised samples from 0 to 10 cm depth. Significant effects of grazing on bacterial community composition were only detected in the vertosol, where it was generally associated with enrichments of cyanobacterial taxa in the 0–1 cm samples, and more varied responses in 0–10 cm samples. In contrast, despite some minor reductions in the relative abundances of *Bacillus* populations in more frequently burned sites (late season 2-yearly burning), we did not observe any significant impacts of fire management on the overall composition of bacterial communities. Our findings indicate that the presence of livestock in rangelands increases the proportional representation of cyanobacteria within biocrust and associated soil microbiomes, and that these communities, at least from a taxonomic perspective, are not strongly impacted by fire management.

1. Introduction

Australia is the world's driest inhabited continent with approximately 70% of land area considered arid or semi-arid (DCCEEW, 2022; Peel et al., 2007). These regions are predominantly used as rangelands for extensive cattle grazing (ABARES, 2022). Due to the vast managed land areas involved (200,000 ha and larger), it is not feasible to use mineral fertilisers to maintain soil fertility and compensate for nutrient losses. Hence, the fertility of rangeland soils is largely dependant on the activities of soil microorganisms that mobilise nutrients from soil, replenish nitrogen (N) via N fixation, and produce extracellular matrices that stabilise soil structure and thereby reduce erosion (Büdel et al., 2016; Eldridge et al., 2020a). Within rangelands, many of these organisms are associated with biologically diverse biocrusts, which grow on, and in, the upper soil and cover surfaces between tussock grasslands and stands of herbaceous and woody vegetation (Belnap and Lange, 2001; Williams et al., 2014).

Previous studies indicate that the coverage, biomass, and composition of biocrusts is influenced by rangeland management strategies including: 1) grazing intensity, which is being adapted in response to increases in the frequency and severity of drought and flooding in some Australian rangelands (O'Reagain and Scanlan, 2013; Bastin et al., 1993; Smith et al., 2007); and 2) fire, which is used to control woody vegetation and promote pasture growth (Cowley et al., 2014). For example, excessive trampling has been shown to reduce biocrust coverage (Williams et al., 2022; Williams et al., 2008) and biomass by up to 80% (Belnap and Eldridge, 2001). Furthermore, depending on its frequency and intensity, fire can shift biocrust community composition in favor of algae or cyanobacteria (Eldridge and Bradstock, 1994), result in bare soil (Aanderud et al., 2019), halve biocrust cover (Palmer et al., 2020), or increase biocrust cover (O'Bryan et al., 2009). Hence, rangeland management is likely to influence the microbial communities that help to protect soil surfaces and compensate for the impacts of grazing and nutrient loss. Most of these studies, however, were based on visual observations and/or a microscope – methods known to be ineffective for detecting the vast majority of microorganisms, but which can be characterised using modern DNA sequencing-based approaches (Chilton et al., 2018; Maier et al., 2018; Miralles et al., 2020a; Miralles et al.,

* Corresponding authors.
E-mail addresses: susanne.schmidt@uq.edu.au (S. Schmidt), p.dennis@uq.edu.au (P.G. Dennis).

https://doi.org/10.1016/j.ecolind.2023.110094
Received 7 December 2022; Received in revised form 20 February 2023; Accepted 28 February 2023
Available online 8 March 2023

2020b; Moreira-Grez et al., 2019; Pombubpa et al., 2020). Consequently, there is an opportunity to identify the bacterial taxa present in biocrusts and soil, and evaluate their responses to environmental factors.

In this study, we compared the impacts of grazing (sites with or without cattle) and fire management (sites with controlled 2- or 4-yearly burning *versus* those with no fire) on the diversity and composition of biocrust and associated soil bacterial communities. Our research was performed at a long-term rangeland fire experiment in the Northern Territory, Australia. Prior to sampling, the grazing and fire treatments had been continuously applied for approximately 60 and 30 years, respectively. Both experiments were replicated on two soil types (vertosol, calcarosol). For the grazing experiment, we also considered two soil depths (0–1 and 0–10 cm). Bacterial communities were characterised using high-throughput 16S rRNA gene amplicon sequencing, and we tested the hypotheses that biocrust bacterial communities differ between: 1) soil types, 2) grazed and non-grazed sites, and that these effects vary between depths, 3) fire treatments, with larger effects anticipated in plots with more frequent fires.

2. Materials and methods

2.1. Site descriptions

Field sampling was performed at two adjacent sites at the Victoria River Research Station (Kidman Springs, Northern Territory, Australia) (16°07′04.2″ S, 130°57′28.7″ E), located on two distinct soil types, a vertosol and a calcarosol. The vertosol had a clay texture (40% sand: 11% silt: 49% clay), while that of the calcarosol was sandy clay (53% sand: 11% silt: 36% clay). The dominant vegetation associated with the vertosol included: Rosewood (*Terminalia volucris* R.Br.), Bauhinia (*Bauhinia cunninghamii* Benth), Feathertop wire grass (*Aristida latifolia* Domin), Curly blue grass (*Dichanthium fecundum* S.T.Blake), Golden beard grass (*Chrysopogon fallax* S.T.Blake), Flinders grass (*Iseilema* spp.), *Flemingia pauciflora* Benth and Native millet (*Panicum decompositum* R. Br.). The dominant vegetation associated with the calcarosol included: Bloodwood (*Corymbia terminalis* (F.Muell.) K.D.Hill & L.A.S.Johnson), Silver box (*Eucalyptus pruinose* Schauer), Conkerberry (*Carissa lanceolata* R.Br.), Common hakea (*Hakea arborescens* R.Br.), Black speargrass (*Heteropogan contortus* (L.) P. Beauv. ex Roem. & Schult.), Native couch (*Brachyachne convergens* (F.Muell.) Stapf), Bottlewasher (*Enneapogon polyphyllus* (Domin) N.T.Burb.), Batchelors buttons (*Gomphrena canescens* R.Br.) and Blue heads (*Spermacoce stenophylla* F.Muell) (Lebbink et al., 2018). The sites are at 100 m elevation, characterised by hot wet summers and mild dry winters. During the wet season (Nov-Apr), temperatures fluctuate between 33.9 °C – 39.1 °C (min–max) (1996–2012) with 119.4 mm mean precipitation (1996–2022), and the dry season (May-Oct) 30.7 °C – 35.2 °C (min–max) (1996–2012) with 6.5 mm mean precipitation (1996–2022) (https://www.bom.gov.au, rainfall station No. 14847, accessed 12th August 2022). At calcarosol and vertosol sites, stocking rates of *Bos indicus* cattle averaged 10 and 12 adult equivalents per km^2, respectively (Lebbink et al., 2018).

2.2. Effects of grazing on bacterial communities of biocrust and underlying soils

Two long-term non-burned and non-grazed exclosures have been in place for 60 years on both soil types. Each of these exclosures cover approximately 20 ha. We sampled inside and within one meter of the outside of the exclosures to determine the effects of grazing without managed fires on biocrust and the underlying soil (Fig. S1).

2.3. Effects of fire on bacterial communities of biocrust

Long-term fire experiments were established in 1993 and comprise 16 experimental plots (160 m × 160 m) arranged as a 4 × 4 grid with each plot separated by a fire break (Cowley et al., 2014). We focussed on three of the six treatments implemented at each site (i.e. the vertosol and calcarosol): 1) non-burned controls, 2) controlled burning every two years in the late dry season, and 3) controlled burning every four years in the late dry season (Fig. S1). There are two plots for each treatment, and four plots for the non-burned controls (Cowley et al., 2014). Each site, is fenced, but has a gate in each corner that remains open to permit grazing except during the wet season (c. 6–7 months) following a burn. This is a recommended grazing management strategy to provide a recovery period post-fire (Cowley et al., 2014).

2.4. Sample collection

Biocrust samples were collected over a 25 × 25 m homogenous area from each plot according to the protocol of the Australian Microbiome Initiative (Bissett et al., 2016). Using a 5 cm diameter corer, biocrust samples were collected at 0–1 cm and 0–10 cm depth using sterile containers and maintained in a coolbox until being transferred to −20 °C storage within approximately 4 h. In total, six samples from each, the unburnt, non-grazed 60 year exclosure and adjacent grazed paddock were collected. There were three samples per depth from the two paddocks, calcarosol and vertosol.

We also collected three replicates of contrasting fire treatments from the top 5 cm soil, from the intensive fire treatment (late dry season every 2 years), and the recommended fire management at the time (late dry season every 4 years), compared with the non-burned plots (control). These samples were collected from Victoria River Research Station (Northern Territory) in June 2017, two years post fire, in the dry season.

2.5. DNA extraction, 16S rRNA gene amplification, and sequencing

DNA extraction, 16S rRNA gene amplification, and sequencing DNA were performed according to protocols used by the Australian Microbiome Initiative (https://www.earthmicrobiome.org/protocols-and-standards/dna-extraction-protocol/), at the Australian Genome Reserarch Facility (AGRF). Briefly, DNA was extracted from 250 mg soil in triplicate using the Qiagen DNeasy® PowerSoil Kit according to manufacturer's instructions.

Bacterial 16S rRNA genes were amplified by polymerase chain reaction (PCR) using the primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 519R (5′-GWATTACCGCGGCKGCTG-3′) (Lane, 1991), each modified on the 5′ end to contain the Illumina overhang adapter for compatibility with the P5 and i7 Nextera XT indices, respectively. PCR reactions contained: 1 μl DNA samples in 1X ImmoBuffer (Bioline), 200 nM of dNTPs (Invitrogen), 2.5 mM $MgCl_2$, 1 Unit Immolase DNA Polymerase (Bioline), and 500 nM of each primer. This reaction was made up to a total volume of 25 μl with molecular biology grade water. Thermocycling conditions were as follows: 95 °C for 10 min; then 35 cycles of 94 °C for 30 sec, 55 °C for 10 sec, 72 °C for 45 sec; followed by 72 °C for 10 min. Blank extraction controls and negative amplification controls were verified by gel electrophoresis.

Amplicons were purified and normalised in concentration using SequelPrep Normalisation Plate Kits according to manufacturer instructions (Invitrogen). Equal volumes of the normalised amplicon samples were then pooled and sequenced on an Illumina MiSeq and a MiSeq Reagent Kit v3 (600 cycles; Illumina) according to the manufacturer's instructions.

2.6. Processing of sequence data

Raw sequence data were processed according (Forstner et al., 2020). Briefly, USEARCH (v10.0.240) (Edgar, 2010) was used for primer removal and trimming to 250 bp using fastx_truncate. High-quality forward reads were then identified using fastq_filter (-fastq_maxee = 1) and duplicate sequences were removed using fastx_uniques. Operational taxonomic units (OTU) were generated by clustering sequences at

97% similarity, and potential chimeras were identified and removed (cluster_otus). An OTU table was the generated using otutab. SILVA SSU (v138) (Quast et al., 2012) taxonomy was assigned using BLASTN (v2.3.0 +) (Zhang et al., 2000) within the feature classifier of QIIME2 (v2017.9) (Bolyen et al., 2018), and OTUs classified as chloroplasts, mitochondria, archaea or eukaryotes were then removed using the BIOM tool suite (McDonald et al., 2012). De-novo alignments of the representative OTU sequences were generated using MAFFT (v7.221) (Katoh and Standley, 2013), masked (QIIME2), and then used to generate a midpoint-rooted phylogenetic tree using FastTree (v2.1.9) (Price et al., 2010) in QIIME2. The OTU table was then rarefied to 37,350 sequences per sample. Weighted UniFrac (Lozupone and Knight, 2005), the mean numbers of observed OTUs (Sobs), Shannon's Diversity Index, and Faith's Phylogenetic diversity (Faith's PD) were calculated using QIIME2.

2.7. Statistical analyses

The effects of treatments on univariate (i.e. numbers of observed OTUs (Sobs), Shannon's Diversity Index, and Faith's Phylogenetic Diversity Index (PD)) and multivariate (i.e. Hellinger transformed OTU relative abundances, and weighted UniFrac) response variables were evaluated using ANOVA with Tukey's HSD posthoc comparisons, and PERMANOVA (Anderson, 2001) respectively. PERMANOVA was implemented using the function adonis in the R vegan package (Oksanen et al., 2017). OTUs associated with significant treatments were identified using indicator species analyses (Dufrêne and Legendre, 1997) implemented using the indval function in the R labdsv package (Roberts, 2016). The effects of treatments on significant indicator OTUs were also evaluated using ANOVA. All analyses were performed using R (R Core Team, 2021).

3. Results

3.1. The effects of grazing on bacterial diversity

Bacterial communities in the grazed sites were dominated by members of the *Acidobacteriota*, *Actinobacteriota*, *Chloroflexota*, *Cyanobacteria*, *Firmicutes*, and *Proteobacteria* (Fig. 1). At the OTU level, bacterial community composition was significantly influenced by soil, depth, and their interaction (Table 1). A significant compositional effect of grazing was also observed, although this was dependent on soil type (Table 1). The vertosol had larger relative abundances of *Chloroflexota* (Anaerolineaceae), and several *Actinobacteriota* (OTUs 1 and 70), *Cyanobacteria* (OTUs 25, 4275 and 20519), and *Proteobacteria* (OTUs 8 and 46) populations (Fig. 1). The calcarosol was positively associated with multiple representatives of the *Actinobacteriota* (OTUs 10, 14, 17, 35, 50, and 56), and some *Acidobacteriota* (OTU 142), *Cyanobacteria* (OTUs 4, 13, 19 and 58), and Firmicutes (OTUs 20 and 21).

Given the strong effect of soil type, we focussed on the effects of depth and grazing within each soil separately (Table 1). Depth had a stronger effect on bacterial community composition than grazing and was significant in both soils. We observed significant effects of grazing in the vertosol, but not in the calcarosol. Indicator analysis identified 12 OTUs that were significantly associated with depth and/or grazing in vertosol (Fig. 2; Table S1). Of these, just two (OTUs 22 and 25) were associated with depth only (ANOVA); and one (OTU 4) was associated with grazing only (Table S1). The remaining nine OTUs were

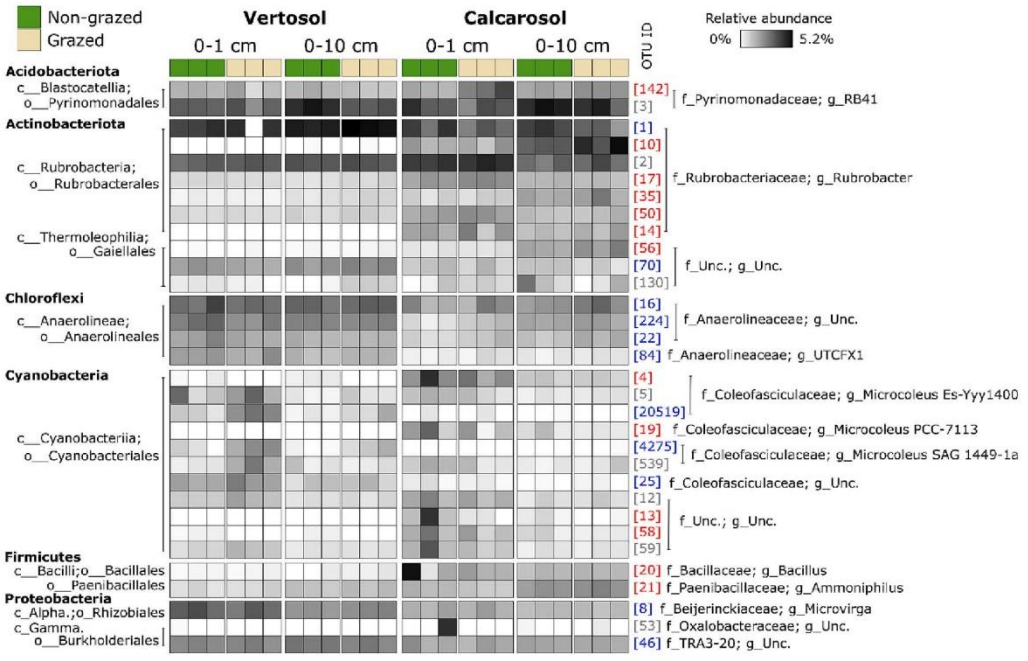


Fig. 1. Heatmap summarising the frequencies of bacterial OTUs present at ≥ 1% mean relative abundance within any treatment group. Relative abundances are Hellinger transformed. The numbers in brackets are OTU IDs and are consistent between figures. OTUs identified as being significant soil indicators ($P < 0.05$, indicator analysis) are shown as Blue (vertosol) or Red (calcarosol) OTU IDs. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Results from PERMANOVA models summarising the main and interactive effects of soil (vertosol and calcarosol), grazing (with and without) and depth (0–1 cm and 0–10 cm) on the composition of bacterial communities, as well as the effect of grazing and depth within each soil separately.

Predictor	OTU relative abundances				Weighted UniFrac distances			
	F value	R^2 value	*P* value		*F* value	R^2 value	*P* value	
Both sites								
Soil	14.48	35.0	<0.001	***	31.61	41.5	<0.001	***
Depth	4.32	9.8	0.003	**	16.81	22.1	<0.001	***
Grazing	1.76	4.0	0.058	.	2.09	2.7	0.091	.
Soil: Depth	2.67	6.0	0.026	*	3.05	4.0	0.028	*
Soil: Grazing	1.83	4.1	0.048	*	3.43	4.5	0.019	*
Depth: Grazing	1.06	2.4	0.287		1.42	1.9	0.216	
Soil: Depth: Grazing	1.15	2.6	0.265		1.69	2.2	0.151	
Within vertosol								
Depth	2.95	20.9	<0.001	***	4.80	27.3	<0.001	***
Grazing	1.98	14.0	0.003	**	2.77	15.8	0.002	**
Depth: Grazing	1.19	8.4	0.146		2.00	11.4	0.029	*
Within calcarosol								
Depth	3.99	27.2	<0.001	***	4.46	30.4	<0.001	***
Grazing	1.63	11.1	0.067	.	1.21	8.2	0.202	
Depth: Grazing	1.03	7.0	0.342		1.03	7.0	0.319	

significantly influenced by both treatments (Table S1), including an *Acidobacteriota* (OTU 3), three *Actinobacteriota* (OTUs 1, 35, and 130), and five *Cyanobacteria* (OTUs 4275, 539, 4, 20519, 59, and 12) (Fig. 2).

In the top 0–1 cm of soil, which has the largest proportion of biocrust organisms, grazing significantly increased the relative abundances of two *Microcoleus SAG1449-1a* (OTUs 4275 and 539; Fig. 2) populations, a representative of the *Microcoleus Es-Yyy1400* (OTU 20519), and two unidentified members of the *Cyanobacteriales* (OTUs 59 and 12) (Fig. 2). Yet, grazing effects on these populations were not detected within the deeper (0–10) cm soil (Fig. 2). Significant grazing-associated changes in relative abundances within 0–10 cm depth included increases in an actinobacterial *Rubrobacter* (OTU 35) and a *Gaiellales* (OTU 130) population, and decreases in an acidobacterial *RB41* (OTU 3), and another cyanobacterial population, *Microcoleus Es-Yyy1400* (OTU 4) (Fig. 2).

The alpha diversity of bacterial communities in the grazed sites differed significantly between soils, with all metrics indicating that communities in the vertosol were slightly more diverse than those in the calcarosol (Tables 2 and S2). No significant depth or grazing effects were observed in the vertosol (Table 2). Within the calcarosol, however, there were a small number of significant effects. These comprised: 1) small but significant grazing-assocated increases in Shannon's Diversity and Faith's Phylogenetic Diversity Indices in the upper 0–1 cm depth; and 2) a small but significant grazing-assocated decrease in the number of observed OTUs in the deeper (0–10 cm) soil (Table 2).

3.2. The effects of fire on bacterial diversity

In the fire experiment, bacterial communities were dominated by representatives of the *Acidobacteriota*, *Actinobacteriota*, *Chloroflexota*, *Cyanobacteria*, *Firmicutes*, and *Proteobacteria* (Fig. 3). At the OTU level, the composition of these communities differed significantly between soils, but not between fire treatments, irrespective of the site (PERMANOVA, Table 3). The main differences between biocrusts formed on different soils were that those on the vertosols had larger relative abundances of several *Chloroflexota* (*Anaerolineaceae*) and a couple of *Proteobacteria* (*Rhizobiales* and *Burkholderiales*) populations, while those on the calcarosol were positively associated with multiple representatives of the *Cyanobacteria* (*Cyanobacteriales*) and *Firmicutes* (*Bacillus*) (Fig. 3).

For OTUs present at ≥ 1% mean relative abundance within any treatment group (i.e. those in Fig. 3), we also applied ANOVA. While more prone to type 1 errors, this approach indicated that two *Bacillus* populations (OTUs 6 and 20; Fig. 3) were not only significantly influenced by soil type, but also by fire, irrespective of site (Table S3). In both cases, this effect was associated with a slight decrease in relative abundance in the Late 2Y relative to other treatments (Fig. 3).

The alpha diversity of biocrust bacterial communities differed significantly between soils, but not between fire treatments, irrespective of the site (Tables 3 and S4). Biocrust microbiomes associated with the vertosols were slightly more diverse than those associated with the calcarosols (Table 4).

4. Discussion

In this study, we used high-throughput 16S rRNA gene amplicon sequencing to characterise the composition of biocrust and associated soil bacterial communities within a long-term rangeland management experiment focussing on the impacts of grazing and fire on two soil types. As hypothesised, bacterial diversity differed significantly between soil types and depths; however, significant impacts of grazing were only detected in the vertosol, and effects of fire were relatively minor, despite some evidence that *Bacillus* populations were enriched under more frequent fire regimes.

4.1. Bacterial communities and the influence of different soils and depths

Our results indicate that biocrust and associated soil bacterial communities were dominated by members of the *Acidobacteriota*, *Actinobacteriota*, *Chloroflexota*, *Cyanobacteria*, *Firmicutes*, and *Proteobacteria*, which is in agreemeent with previous studies of biocrusts and arid soils (Chilton et al., 2022, 2018; Makhalanyane et al., 2015; Moreira-Grez et al., 2019; Condon et al., 2020). Similarly, as observed in many studies, bacterial diversity differed between soils and depths (Bowker et al., 2016; Bu et al., 2016; Pombubpa et al., 2020; Zhang et al., 2022). These differences may reflect variation in edaphic, climatic and ecological properties between locations, soils and depths (Eldridge et al., 2020b; Moreira-Grez et al., 2019; Pombubpa et al., 2020). The availability of light is a likely explanation for the observed reductions in cyanobacterial relative abundances at depth, as these taxa are known to be capable of photosynthesis (Lange, 2001). In our study, members of the *Rubrobacteraceae* were positively associated with the calcarolsol – a red and relatively coarse textured soil when compared with the vertosol. These taxa have been previously reported to dominate biocrusts and arid/semi-arid sandy soils in other parts of Australia and beyond (Holmes et al., 2000; Makhalanyane et al., 2015; Moreira-Grez et al., 2019). In contrast, the vertosol was positively associated with members of the *Chloroflexi*, which have been shown to be more frequent in agricultural soils and correlated with net primary production in arid regions (Trivedi et al., 2016).

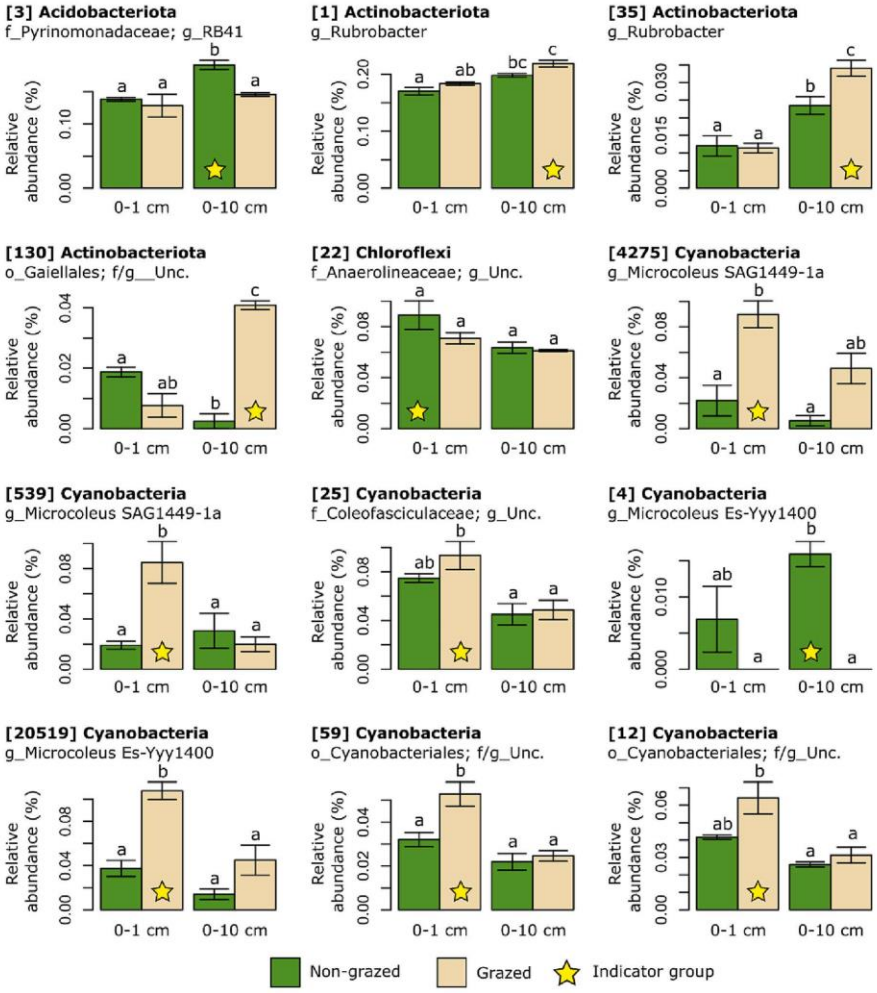


Fig. 2. Bacterial OTUs that were identified as significant indicators of depth or grazing in the vertosol using indicator analysis. The specific group for which each OTU is indicative is highlighted with a star. Error bars represent standard errors of the means and the letter above the bars indicate significant differences ($P < 0.05$) between treatments according to ANOVA with *post hoc* Tukey tests.

4.2. Influence of grazing on biocrust and associated soil bacterial communities

As previously observed in Australian rangelands (Eldridge et al., 2020b), we found significant effects of grazing on the composition of biocrust and associated soil bacterial communities. This effect, however, was weaker than that of depth and was only detected in the vertosol – indicating that the impacts of grazing on biocrust microbiomes need to be investigated across a wide range of soils to provide relevant advise to rangeland managers. The differences between soils in our study may reflect their physicochemical properties (Steven et al., 2013), vegetation composition, or stocking rates, which were based on pasture availability, with 10 and 12 adult equivalents per km^2 for the calcarosol and vertosol, respectively (Lebbink et al., 2018). Vertosols are known to swell and shrink with changing soil moisture due to their heavy clay content (Temga et al., 2019), and the trampling of grazing animals can exacerbate cracking due to the loss of vegetation, which helps to bind soil (Taddese et al., 2002). Hence, the effects of grazing on biocrust microbiomes may have been more pronounced on the vertosol due to its more dynamic surface conditions.

On the surface of the vertosol, grazing was associated with increases in the relative abundances of *Microcoleus* populations (OTUs 539, 20519, and 4275), and other members of the *Cyanobacteriales* (OTUs 59 and 12). *Microcoleus* spp. are non-heterocystous cyanobacteria that lack the ability to fix atmospheric nitrogen, but are known to be pioneer species in biocrust formation that help to stabilise soil (Büdel et al., 2016). Their cyanosphere has also been shown to support the growth of nitrogen-fixing bacteria (Couradeau et al., 2019), although from the

Table 2
Means and standard deviations for each alpha diversity metric in the grazing experiment. The letters represent significant differences ($P < 0.05$) between treatments according to ANOVA with post hoc Tukey tests. Metrics include the observed numbers of OTUs (Sobs), Shannon's Diversity Index (Shannon), and Faith's Phylogenetic Diversity Index (PD).

Soil	Depth	Grazing	Sobs	Shannon	PD
Vertosol	0–1 cm	Non-Grazed	6186 ± 220^{a}	10.59 ± 0.1^{a}	680 ± 13^{a}
		Grazed	5954 ± 356^{a}	10.36 ± 0.1^{ab}	655 ± 25^{a}
	0–10 cm	Non-Grazed	6041 ± 363^{a}	10.46 ± 0.1^{ab}	661 ± 25^{a}
		Grazed	5749 ± 61^{ab}	10.46 ± 0^{ab}	645 ± 13^{ab}
Calcarosol	0–1 cm	Non-Grazed	5001 ± 314^{c}	9.8 ± 0.2^{c}	524 ± 32^{c}
		Grazed	5746 ± 326^{abc}	10.29 ± 0.1^{b}	592 ± 27^{bd}
	0–10 cm	Non-Grazed	5936 ± 148^{a}	10.44 ± 0.1^{ab}	596 ± 14^{bd}
		Grazed	5044 ± 150^{bc}	10.27 ± 0.1^{b}	542 ± 3^{cd}

assigned taxonomy alone, is not possible to confidently associate this trait with any of the taxa we observed to respond to grazing.

At depth, grazing was associated with a decrease in the relative abundance of an *Acidobacteriota* (f_ *Pyrinomonadaceae*) population (OTU 3), and increases in the relative abundances of *Actinobacteriota* (g_*Rubrobacter* and o_*Gaiellales*) populations (OTUs 1, 35, and 130). The drivers of these changes and their consequences are not evident but may be related to inputs of urine and faeces, and/or physical disturbances including mixing of biocrust organisms with the underlying soil. For example, *Rubrobacter* spp. are recognised to be frequent members of bacterial biocrusts (Chilton et al., 2018; Li et al., 2020), which tend to dominate early stages of biocrust development (Weber et al., 2016).

4.3. Fire effects on the composition of the bacterial communities

Overall, we detected no significant impacts of fire on the diversity and composition of biocrust and associated soil bacterial communities. The only exceptions to this were reductions in the relative abundances of two *Bacillus* populations, although these were detected by performing separate analyses on individual OTUs, which is more prone to type 1 errors. Our main finding that fire did not influence bacterial diversity is in agreement with the studies of Peterson et al. (xxxx) and Palmer et al. (2022), but in contrast with that of O'Bryan et al. (2009), which indicated that fire enhanced biocrust bacterial diversity. In principle, the intense heat associated with a fire may be expected to kill microbes, which is supported by the study of Aanderud et al., (2019), who observed reductions in the biomass and richness of biocrust microbial communities immediately after fire. One year later, however, these parameters were indistinguishable from those associated with non-burned biocrusts (Aanderud et al., 2019). Hence, the fact that we

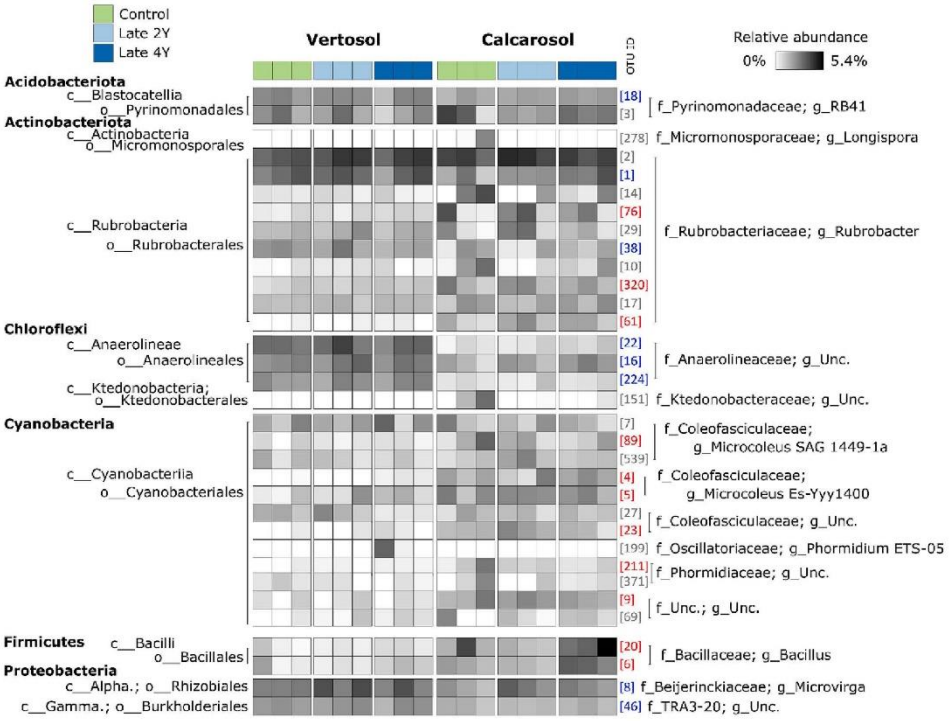


Fig. 3. Heatmap summarising the frequencies of bacterial OTUs present at ≥ 1% mean relative abundance within any treatment group. Relative abundances are Hellinger transformed. The numbers in brackets are OTU IDs and are consistent between figures. OTUs identified as being significant soil indicators ($P < 0.05$, indicator analysis) are shown as Blue (vertosol) or Red (calcarosol) OTU IDs. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3
Results from PERMANOVA models summarising the main and interactive effects of soil (vertosol and calcarosol) and fire management (control, Late 2Y, Late 4Y) on the composition of biocrust bacterial communities.

Predictor	OTU relative abundances				Weighted UniFrac distances			
	F value	R^2 value	*P* value		*F* value	R^2 value	*P* value	
Soil	6.04	27.0	<0.001	***	15.62	46.6	<0.001	***
Fire	1.16	10.4	0.277		1.94	11.6	0.081	.
Soil: Fire	0.99	8.9	0.435		1.01	6.0	0.395	

Table 4
Means and standard deviations for each alpha diversity metric in the fire experiment. The letters represent significant differences ($P < 0.05$) between treatments according to ANOVA with post hoc Tukey tests. Metrics include the observed numbers of OTUs (Sobs), Shannon's Diversity Index (Shannon), and Faith's Phylogenetic Diversity Index (PD).

Soil	Fire	Sobs	Shannon	PD
Vertosol	Control	5899 ± 129^{ab}	10.69 ± 0.1^{ab}	648 ± 9^{ab}
	Late.2Y	5450 ± 715^{ab}	10.5 ± 0.2^{abc}	605 ± 56^{abc}
	Late.4Y	6443 ± 364^{b}	10.76 ± 0.2^{b}	573 ± 20^{b}
Calcarosol	Control	4845 ± 852^{ab}	9.97 ± 0.3^{c}	506 ± 67^{c}
	Late.2Y	5412 ± 272^{ab}	10.26 ± 0.2^{abc}	550 ± 19^{ac}
	Late.4Y	5544 ± 53^{ab}	10.16 ± 0.1^{ac}	550 ± 8^{ac}

observed no differences in bacterial communities between fire treatments may reflect the fact that they had at least two years to recover after burning. Additionally, as outlined by Palmer et al. (2022), some cyanobacteria produce extracellular polysaccharide matrices that contribute to heat resistance (Kimura et al., 2015) and can withstand temperatures of 100 °C (Mager, 2010), at least temporarily. Biocrusts have also been shown to rehydrate from less impacted soil layers deeper in the profile, which may help the organisms within them to survive (DeBano, 2000; Xu et al., 2021). Lastly, landscape mosaics and vegetation cover play a role in fire intensity, with vegetation interspaces being associated with cooler fires (Bowker et al., 2004). This may also explain why we observed no differences in bacterial diversity, as our samples were collected from such interspaces.

4.4. Conclusions

Biocrust and associated soil microorganisms are thought to play important roles in replenishing the nutrients lost from rangelands upon the export of grazing livestock. By understanding how rangeland management influences these communities, practices can be optimised to help maintain ecosystem services that they mediate. Our study contributes to understanding the diversity and composition of biocrust and associated bacterial communities in semi-arid tropical savannas in northern Australian rangelands. Our results indicate that grazing can alter the composition of biocrust bacterial communities, and that these communities exhibit negligible changes in response to fire, especially with increasing years of recovery. Importantly, we observed different responses to grazing on the two soils studies. A logical next step is to understand how these structural changes influence the functioning of biocrust microbiomes, with consequences for the provision of ecosystem services, such that more sustainable rangeland management practices can be identified. Furthermore, the reasons why the effects of rangeland management on biocrust communities differs between soils needs proper investigation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

We would like to acknowledge funding from Meat & Livestock Australia (MLA) and the Australian Microbiome Initiative (AMI) through Bioplatforms Australia via the Australian Commonwealth Government through the National Collaborative Research Infrastructure Strategy. We gratefully acknowledge AMI and its contributors for providing the sequence and edaphic data used in this work.

Author contributions

SS and PGD secured funding. WW and SS collected samples. PGD analysed data. MVC and PGD wrote the paper with input from all authors.

Data accessibility

The 16S rRNA gene amplicon sequences associated with this study are available through the Bioplatforms Australia Data Portal for The Australian Microbiome Initiative using the BASE 16S Amplicon sample IDs: 42344-42385 (https://data.bioplatforms.com/organization/australian-microbiome, accessed 29 July 2022).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2023.110094.

References

Aanderud, Z.T., Bahr, J., Robinson, D.M., Belnap, J., Campbell, T.P., Gill, R.A., McMillian, B., St Clair, S., 2019. The burning of biocrusts facilitates the emergence of a bare soil community of poorly-connected chemoheterotrophic bacteria with depressed ecosystem services. Front. Ecol. Evol. 7, 467.

Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecol. 26, 32–46.

Bastin, G.N., Pickup, G., Chewings, V.H., Pearce, G., 1993. Land degradation assessment in central Australia using a grazing gradient method. Rangel. J. 15, 190–216.

Belnap, J., Eldridge, D., 2001. Disturbance and recovery of biological soil crusts, in: Biological Soil Crusts: Structure, Function, and Management. Springer, pp. 363–383.

Belnap, J., Lange, O.L., 2001. Biological soil crusts: structure, function, and management. Springer.

Bissett, A., Fitzgerald, A., Meintjes, T., Mele, P.M., Reith, F., Dennis, P.G., Breed, M.F., Brown, B., Brown, M.V., Brugger, J., 2016. Introducing BASE: the Biomes of Australian Soil Environments soil microbial diversity database. Gigascience 5, s13742-016-0126–5.

Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C., Al-Ghalith, G.A., Alexander, H., Alm, E.J., Arumugam, M., Asnicar, F., 2018. QIIME 2: Reproducible, interactive, scalable, and extensible microbiome data science. PeerJ Preprints.

Bowker, M.A., Belnap, J., Rosentreter, R., Graham, B., 2004. Wildfire-resistant biological soil crusts and fire-induced loss of soil stability in Palouse prairies, USA. Appl. Soil Ecol. 26, 41–52.

Bowker, M.A., Belnap, J., Büdel, B., Sannier, C., Pietrasiak, N., Eldridge, D.J., Rivera-Aguilar, V., 2016. Controls on distribution patterns of biological soil crusts at micro- to global scales, in: Biological Soil Crusts: An Organizing Principle in Drylands. Springer, pp. 173–197.

Bu, C., Zhang, P., Wang, C., Yang, Y., Shao, H., Wu, S., 2016. Spatial distribution of biological soil crusts on the slope of the Chinese Loess Plateau based on canonical correspondence analysis. Catena 137, 373–381.

Büdel, B., Dulić, T., Darienko, T., Rybalka, N., Friedl, T., 2016. Cyanobacteria and algae of biological soil crusts, in: Biological Soil Crusts: An Organizing Principle in Drylands. Springer, pp. 55–80.

Chilton, A.M., Nguyen, S.T., Nelson, T.M., Pearson, L.A., Neilan, B.A., 2022. Climate dictates microbial community composition and diversity in Australian biological soil crusts (biocrusts). Environ. Microbiol.

Chilton, A.M., Neilan, B.A., Eldridge, D.J., 2018. Biocrust morphology is linked to marked differences in microbial community composition. Plant Soil 429, 65–75.

Condon, L.A., Pietrasiak, N., Rosentreter, R., Pyke, D.A., 2020. Passive restoration of vegetation and biological soil crusts following 80 years of exclusion from grazing across the Great Basin. Restor. Ecol. 28, S75–S85.

Couradeau, E., Giraldo-Silva, A., De Martini, F., Garcia-Pichel, F., 2019. Spatial segregation of the biological soil crust microbiome around its foundational cyanobacterium, Microcoleus vaginatus, and the formation of a nitrogen-fixing cyanosphere. Microbiome 7, 1–12.

Cowley, R.A., Hearnden, M.N., Joyce, K.E., Tovar-Valencia, M., Cowley, T.M., Pettit, C. L., Dyer, R.M., 2014. How hot? How often? Getting the fire frequency and timing right for optimal management of woody cover and pasture composition in northern Australian grazed tropical savannas. Kidman Springs Fire Experiment 1993–2013. Rangel. J. 36, 323–345.

DeBano, L.F., 2000. The role of fire and soil heating on water repellency in wildland environments: a review. J. Hydrol. 231, 195–206.

Dufrêne, M., Legendre, P., 1997. Species assemblages and indicator species: the need for a flexible asymmetrical approach. Ecol. Monogr. 67, 345–366.

Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26, 2460–2461.

Eldridge, D.J., Bradstock, R.A., 1994. The effect of time since fire on the cover and composition of cryptogamic soil crusts on a eucalypt shrubland soil. Cunninghamia 3, 521–527.

Eldridge, D.J., Reed, S., Travers, S.K., Bowker, M.A., Maestre, F.T., Ding, J., Havrilla, C., Rodriguez-Caballero, E., Barger, N., Weber, B., 2020a. The pervasive and multifaceted influence of biocrusts on water in the world's drylands. Glob. Change Biol. 26, 6003–6014.

Eldridge, D.J., Travers, S.K., Val, J., Wang, J.-T., Liu, H., Singh, B.K., Delgado-Baquerizo, M., 2020b. Grazing regulates the spatial heterogeneity of soil microbial communities within ecological networks. Ecosystems 23, 932–942.

Forstner, C., Orton, T.G., Wang, P., Kopittke, P.M., Dennis, P.G., 2020. Wastewater treatment processing of silver nanoparticles strongly influences their effects on soil microbial diversity. Environ. Sci. Technol. 54, 13538–13547.

Holmes, A.J., Bowyer, J., Holley, M.P., O'Donoghue, M., Montgomery, M., Gillings, M.R., 2000. Diverse, yet-to-be-cultured members of the Rubrobacter subdivision of the Actinobacteria are widespread in Australian arid soils. FEMS Microbiol. Ecol. 33, 111–120.

Katoh, K., Standley, D.M., 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol. Biol. Evol. 30, 772–780.

Kimura, S., Tomita-Yokotani, K., Igarashi, Y., Sato, S., Katoh, H., Abe, T., Sonoike, K., Ohmori, M., 2015. The heat tolerance of dry colonies of a terrestrial cyanobacterium, Nostoc sp. HK-01. Biol. Sci. Space 29, 12–18.

Lane, D. J, 1991. 16S/23S rRNA sequencing. Nucleic Acid Tech. Bact. Syst. 115–175.

Lange, O.L., 2001. Photosynthesis of soil-crust biota as dependent on environmental factors, in: Biological Soil Crusts: Structure, Function, and Management. Springer, pp. 217–240.

Lebbink, G., Fensham, R., Cowley, R., 2018. Vegetation responses to fire history and soil properties in grazed semi-arid tropical savanna. Rangel. J. 40, 271–285.

Li, J.-Y., Jin, X.-Y., Zhang, X.-C., Chen, L., Liu, J.-L., Zhang, H.-M., Zhang, X., Zhang, Y.-F., Zhao, J.-H., Ma, Z.-S., 2020. Comparative metagenomics of two distinct biological soil crusts in the Tengger Desert. China. Soil Biol. Biochem. 140, 107637.

Lozupone, C., Knight, R., 2005. UniFrac: a new phylogenetic method for comparing microbial communities. Appl. Environ. Microbiol. 71, 8228–8235.

Mager, D.M., 2010. Carbohydrates in cyanobacterial soil crusts as a source of carbon in the southwest Kalahari. Botswana. Soil Biol. Biochem. 42, 313–318.

Maier, S., Tamm, A., Wu, D., Caesar, J., Grube, M., Weber, B., 2018. Photoautotrophic organisms control microbial abundance, diversity, and physiology in different types of biological soil crusts. ISME J. 12, 1032–1046.

Makhalanyane, T.P., Valverde, A., Gunnigle, E., Frossard, A., Ramond, J.-B., Cowan, D. A., 2015. Microbial ecology of hot desert edaphic systems. FEMS Microbiol. Rev. 39, 203–221.

McDonald, D., Clemente, J.C., Kuczynski, J., Rideout, J.R., Stombaugh, J., Wendel, D., Wilke, A., Huse, S., Hufnagle, J., Meyer, F., 2012. The Biological Observation Matrix (BIOM) format or: how I learned to stop worrying and love the ome-ome. Gigascience 1, 2047-217X-1–7.

Miralles, I., Lázaro, R., Sánchez-Marañón, M., Soriano, M., Ortega, R., 2020a. Biocrust cover and successional stages influence soil bacterial composition and diversity in semiarid ecosystems. Sci. Total Environ. 709, 134654.

Miralles, I., Soria, R., Lucas-Borja, M.E., Soriano, M., Ortega, R., 2020b. Effect of biocrusts on bacterial community composition at different soil depths in Mediterranean semi-arid ecosystems. Sci. Total Environ. 733, 138613.

Moreira-Grez, B., Tam, K., Cross, A.T., Yong, J.W., Kumaresan, D., Nevill, P., Farrell, M., Whiteley, A.S., 2019. The bacterial microbiome associated with arid biocrusts and the biogeochemical influence of biocrusts upon the underlying soil. Front. Microbiol. 2143.

O'Bryan, K.E., Prober, S.M., Lunt, I.D., Eldridge, D.J., 2009. Frequent fire promotes diversity and cover of biological soil crusts in a derived temperate grassland. Oecologia 159, 827–838.

O'Reagain, P.J., Scanlan, J.C., 2013. Sustainable management for rangelands in a variable climate: evidence and insights from northern Australia. Animal 7, 68–78.

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., 2017. vegan: community ecology package [Internet]. Version.

Palmer, B., Hernandez, R., Lipson, D., 2020. The fate of biological soil crusts after fire: a meta-analysis. Glob. Ecol. Conserv. 24, e01380.

Palmer, B., Lawson, D., Lipson, D.A., 2022. Years After a Fire, Biocrust Microbial Communities are Similar to Unburned Communities in a Coastal Grassland. Microb. Ecol. 1–17.

Peel, M.C., Finlayson, B.L., McMahon, T.A., 2007. Updated world map of the Köppen-Geiger climate classification. Hydrol. Earth Syst. Sci. 11, 1633–1644.

Peterson, M.J., Pressler, Y., Knight, C.A., Hannusch, H.J., Lodge, A.G., Starns, H.D., Tolleson, D.R., Blazier, J.C., Rogers, W.E., Smith, A.P., n.d. The Interactive Effects of Drought and Fire on Soil Microbial Communities in a Semi-Arid Savanna.

Pombubpa, N., Pietrasiak, N., De Ley, P., Stajich, J.E., 2020. Insights into dryland biocrust microbiome: geography, soil depth and crust type affect biocrust microbial communities and networks in Mojave Desert, USA. FEMS Microbiol. Ecol. 96, fiaa125.

Price, M.N., Dehal, P.S., Arkin, A.P., 2010. FastTree 2–approximately maximum-likelihood trees for large alignments. PloS One 5, e9490.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O., 2012. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 41, D590–D596.

Roberts, D.W., 2016. labdsv: ordination and multivariate analysis for ecology. 2016. R Package Version 1, 8.

Smith, D.M.S., McKeon, G.M., Watson, I.W., Henry, B.K., Stone, G.S., Hall, W.B., Howden, S.M., 2007. Learning from episodes of degradation and recovery in variable Australian rangelands. Proc. Natl. Acad. Sci. 104, 20690–20695.

Steven, B., Gallegos-Graves, L.V., Belnap, J., Kuske, C.R., 2013. Dryland soil microbial communities display spatial biogeographic patterns associated with soil depth and soil parent material. FEMS Microbiol. Ecol. 86, 101–113.

Taddese, G., Saleem, M.M., Ayalneh, W., 2002. Effect of livestock grazing on physical properties of a cracking and self-mulching Vertisol. Aust. J. Exp. Agric. 42, 129–133.

Team, R.c., 2021. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, p. 2012.

Temga, J.P., Azinwi, T.P., Basga, D.S., Gouban, H., Abossolo, M., Nguetnkam, J.P., Bitom, D.L., 2019. Characteristics, classification and genesis of vertisols under seasonally contrasted climate in the Lake Chad Basin, Central Africa. J. Afr. Earth Sci. 150, 176–193.

Trivedi, P., Delgado-Baquerizo, M., Anderson, I.C., Singh, B.K., 2016. Response of soil properties and microbial communities to agriculture: implications for primary productivity and soil health indicators. Front. Plant Sci. 7, 990.

Weber, B., Bowker, M., Zhang, Y., Belnap, J., 2016. Natural recovery of biological soil crusts after disturbance, in: Biological Soil Crusts: An Organizing Principle in Drylands. Springer, pp. 479–498.

Williams, W.J., Eldridge, D.J., Alchin, B.M., 2008. Grazing and drought reduce cyanobacterial soil crusts in an Australian Acacia woodland. J. Arid Environ. 72, 1064–1075.

Williams, W.J., Büdel, B., Reichenberger, H., Rose, N., 2014. Cyanobacteria in the Australian northern savannah detect the difference between intermittent dry season and wet season rain. Biodivers. Conserv. 23, 1827–1844.

Williams, W.J., Schmidt, S., Zaady, E., Alchin, B., Myint Swe, T., Williams, S., Dooley, M., Penfold, G., O'reagain, P., Bushell, J., 2022. Resting Subtropical Grasslands from Grazing in the Wet Season Boosts Biocrust Hotspots to Improve Soil Health. Agronomy 12, 62.

Xu, H.-F., Raanan, H., Dai, G.-Z., Oren, N., Berkowicz, S., Murik, O., Kaplan, A., Qiu, B.-S., 2021. Reading and surviving the harsh conditions in desert biological soil crust: the cyanobacterial viewpoint. FEMS Microbiol. Rev. 45, fuab036.

Zhang, Z., Schwartz, S., Wagner, L., Miller, W., 2000. A greedy algorithm for aligning DNA sequences. J. Comput. Biol. 7, 203–214.

Zhang, J., Xu, M., Xu, M.-X., 2022. Characterising the diversity and functionality of the microbial community within biocrusts associated with different vegetation communities and soil habitats. Appl. Soil Ecol. 175, 104458.

Appendix 5: Using digital photography to monitor changes in biocrusts and ground cover in a savanna rangeland.

CSIRO PUBLISHING

SPECIAL ISSUE | RESEARCH PAPER
https://doi.org/10.1071/RJ22019

THE RANGELAND JOURNAL

Using digital photography to monitor changes in biocrusts and ground cover in a savanna rangeland

Than Myint Swe[A], Wendy J. Williams[A,*], Susanne Schmidt[A], Andries Potgieter[B], Robyn Cowley[C], Vincent Mellor[D], Colin Driscoll[A] and Yan Zhao[B]

For full list of author affiliations and declarations see end of paper

***Correspondence to:**
Wendy J. Williams
NUE Lab, School of Agriculture and Food Sciences, The University of Queensland, St Lucia, Qld 4072, Australia
Email: wendy.williams@uq.edu.au

Received: 5 August 2022
Accepted: 1 February 2023
Published: 9 March 2023

Cite this:
Than Myint Swe *et al.* (2023)
The Rangeland Journal
44(5–6), 263–278. doi:10.1071/RJ22019

ABSTRACT

Biocrusts form a living soil cover in Australia's northern savannas, delivering essential ecosystem services. More accessible tools are needed to quantify and monitor ground cover, including biocrusts, as current methodologies are time-consuming, expensive, or specialised. At Victoria River Research Station (Northern Territory, Australia), long-term fire research plots were used to monitor the response of low vegetative ground and soil covers for different burning intervals and seasons. Mobile phone photographs were analysed using machine-learning software and a derived decision tree-based segmentation model (DTSM). The resulting data were compared to visual in-field assessment by trained researchers. Visual assessments and photographs were taken at two time points during the post-fire recovery period, mid-wet and dry seasons, at three burning intervals (2, 4, and 6 years) and for two different burning times, early or late dry season. DTSM-derived grass and litter cover were statistically similar to field observations in the burnt and unburnt plots. Biocrust cover derived from DTSM also matched field observations in fire treatments and unburnt control plots in the dry season, except when obscured by grass or litter. In the wet season, DTSM underestimated biocrust cover in some treatments, and DTSM did not detect biocrust obscured under dense grass cover. Nevertheless, biocrust pigment analysis confirmed a significant presence of biocrusts both on seemingly bare soil and under the grass canopy. We concluded that mobile phone photographs are suitable for monitoring dry-season ground cover. When similar colours of grass and litter cover were combined, the modelled accuracy reached 95–97%. With some refinements, DTSM analysis of photographs could accurately quantify the impact of fire disturbance on biocrusts and grass cover. However, it would be advantageous to improve the model by additional field records to determine how much biocrust occurs under the grass. This study provides land managers with an efficient method of recording ground cover over time to aid land-condition assessments.

Keywords: biocrusts, fire, ground cover, mobile phone photos, monitoring, rangelands, savanna, soil health.

Introduction

Monitoring vast areas of savanna exposed to climate variability spanning decadal timescales has to be carefully considered to implement effective management strategies. Ground-based monitoring of extensive native grasslands provides fine scale data on grass condition, weed invasion, nutrient cycling, and soil surface condition (Eyre *et al.* 2011). Such data can then be linked to remote sensing platforms using satellite or aircraft and applied to large-scale analysis of landscape function. By connecting scales, remotely sensed ecological indices of disturbance combined with high resolution ground cover monitoring can provide greater power to models to identify ecological function at a landscape scale (Ward and Kutt 2009; Tongway and Ludwig 2012). Furthermore, there is a need for spatially hierarchical and complimentary measures that use targeted surveillance and landscape-scale monitoring to provide information for rangeland management (Eyre *et al.* 2011).

The structure of woodlands and grasslands in the northern Australian dry savannas (600–800 mm average annual rainfall) is primarily driven by variations in climate, being conditioned to monsoon-driven summer rains and dry winters. In the lead-in to the wet season, storms with lightning strikes ignite dry vegetation and can cause large-scale hot fires (Kilinc and Beringer 2007; Bradstock 2010). Historically, fire has been used throughout the year by Indigenous Traditional Landowners to manage ecosystems and avoid vast wildfires (Preece 2002), and fire is used by livestock producers to enhance pasture production by managing woody vegetation cover (Cowley *et al.* 2014). Fire can negatively affect vegetation, fauna survival (Preece 2002) and the integrity of the soil surface (Barger *et al.* 2016). In addition to fire, extensive grazing by livestock and other herbivores can damage surface stability and disrupt soil nutrient cycling, which is most pronounced during drought periods when affected landscapes lack vegetation cover and soils are prone to degradation and erosion (Williams *et al.* 2021).

In this study, we addressed the need for easy-to-execute and efficient methods of monitoring dry savanna ground cover at fine spatial scales, with the view of providing indicators that can be scaled to satellite-level imaging (Barnetson *et al.* 2017). A key factor influencing soil degradation across landscapes is low or non-existent ground cover that ultimately results in the degradation of the soil surface, concomitant with a loss of nutrients and loss of ecosystem function (e.g. water cycling, carbon storage, vegetation growth). Tongway (2010) identified key components of landscapes with low vegetation cover and damaged soil surfaces that directly influence ecological function. These components include grass and canopy cover that reduce the impact of rain splash erosion, and litter fall as indicative of a functional nutrient cycle. An important element of soil and ecosystem integrity is biological soil crusts, i.e. biocrusts, which bind soil particles, regenerate carbon and nitrogen, and are integral to soil stability and nutrient cycling (Evans and Lange 2001; Bowker *et al.* 2014; Williams *et al.* 2018; Büdel *et al.* 2018).

In the rangelands biocrusts form a living soil cover, delivering essential ecosystem services. Accessible tools are needed for quantifying and monitoring ground cover, including biocrusts, as current methodologies are time-consuming, expensive, or specialised. Loss of grass, canopy cover and biocrusts in dry savanna, in combination with an overall low vegetative cover, can rapidly result in a degraded landscape. When such a landscape is affected by fire, the ground cover is burnt, with ash as a transient soil cover and biocrusts integrated into the soil surface, either burnt or inactive, until the next rain (Brianne *et al.* 2020). At this point, the landscape is at its most vulnerable state with much of the soil bare and prone to wind and water erosion (Flores *et al.* 2020).

To address the challenges of current methods for analysing biocrusts and their recovery post-disturbance at the field level (e.g. paddocks or grazing regions), we aimed to develop an easy-to-use alternative utilising the power of mobile phones and high-performance computing. Over the past decade, high-resolution photography (red, green, blue, RGB) and digital technologies with machine learning (ML) have enabled new methodologies for measuring plant canopy (Guo *et al.* 2017), plant nutrition estimations (Shi *et al.* 2021) and assessment of biomass for grasslands (Possoch *et al.* 2016).

Following on from successful close-range unmanned aerial vehicle (UAV) imagery analysis of biocrusts (Havrilla *et al.* 2020), we hypothesised that ground cover including biocrusts could be effectively quantified using ML based on RGB reflectance. To test our hypothesis, we compared field data with mobile phone images of ground cover plots. We expected no significant differences between the two methods. With a focus on rapid assessment tools for monitoring ground cover, our overarching aims were to (i) evaluate whether the proposed methodology would work as a tool for use by land managers, and (ii) examine how it performs under different conditions, such as burning regimes and across seasons.

Materials and methods

Study site

Field data were collected at the Victoria River Research Station (VRRS, Kidman Springs, Northern Territory; 16.12°S, 130.96°E; Fig. 1*a*). The climate is dominated by a summer wet season from November to April, and a drier period with little to no rain from May to October. The annual average temperature range is 20.1–34.9°C and average annual rainfall is 753.9 mm (Bureau of Meteorology 2021). Rainfall for the 12 months covering the pre-fire and wet season sample times is shown in Fig. 2.

In 1993, a long-term fire research program was established at VRRS to investigate the best timing for fire in terms of burning interval (years between fire) and season (Cowley *et al.* 2014). Within a fenced-off area, 16 experimental plots (160 m × 160 m) set on a 4 × 4 grid pattern were established (Fig. 1*b*), with two replicates for each fire treatment: early (E) or late (L) dry-season burning, with fire intervals of 2, 4 and 6 years and four unburnt control plots (Fig. 1*c*, *d*); firebreaks separated each plot.

Research data were collected only from the open eucalypt woodland (Conkerberry Paddock) trial site, with pastures dominated by grasses *Heteropogon contortus*, *Enneapogon* spp. and *Dichanthium fecundum* (Cowley *et al.* 2014). Grasses are underlain by red calcarosol soils, with soil texture from 0 down to 10 cm comprising clay (32%), silt (16%), sand (53%) and pH 7.9 (Allen *et al.* 2011). VRRS is a cattle research station, and the fire trials are open to cattle at the end of the first wet season following fire, grazing density is at industry recommended continuous stocking rates (Cowley *et al.* 2014). As we sampled during and at

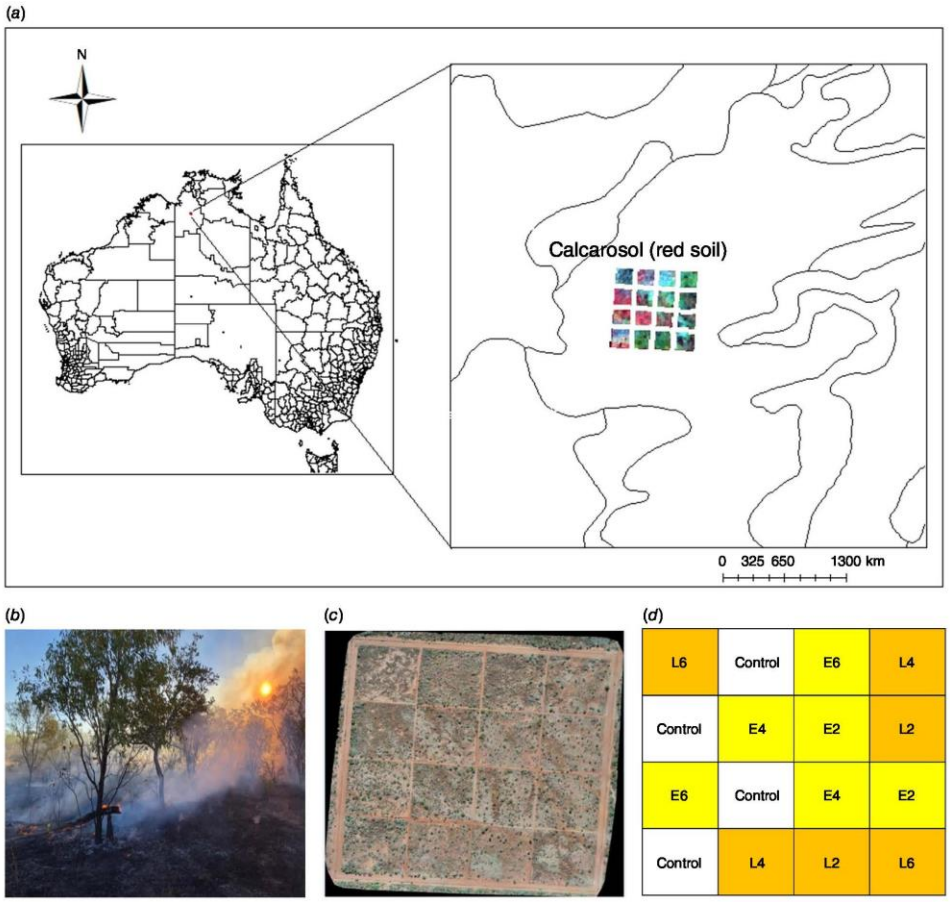


Fig. 1. (*a*) Location of Kidman Springs Research Station experimental plots where long-term fire research has been implemented since 1993. (*b*) Burning a plot in the early dry season (2019). (*c*) UAV image of calcarosol fire plots. (*d*) Location of the treatments across the experimental site with a size of each plot of 160 m × 160 m. E, early dry-season fire (cooler fire); and L, late dry-season fire (hotter fire); numbers represent the years between fire (2, 4, 6 years) and unburnt control plots.

the end of the wet season, there was no grazing during this period.

Field measurements

Biocrusts and ground cover

The assessment of ground cover included both low vegetative cover (annual and perennial grasses) and litter accumulation. Bare soil was divided into visible biocrust cover and bare soil with no apparent biocrust cover. These four parameters (herein called microsites) were selected for ground cover descriptions that could be repeated in future studies to determine key functional indices representative of post-fire recovery such as soil stability and nutrient cycling (as described by Tongway 2010).

Fire plots were burnt in the dry season in mid-June or late October 2019. The first wet-season rains were recorded in early December 2019. Total rainfall for the 2019–2020 wet season was 602.5 mm with the last rain fall in May. The rainfall distribution suggests that the grasses and biocrusts had their maximum chance of recovery post-wet season (Büdel *et al.* 2018). To measure early recovery, initial data collections for ground cover occurred mid-wet season (February 2020) after approximately 260 mm rainfall in

the preceding 2 months. Dry season measurements were made in July 2020. The sampling times were intended to document the recovery of grass and biocrusts post-fire during recovery and following a full wet season.

To assess ground cover, two 30-m transects were established centrally in each fire-treatment plot in the eucalypt woodland site. This study had seven treatments, with two plots each for the early and late fire seasons at 2-, 4-, and 6-year intervals and utilised two of the four unburnt plots as control treatments (Fig. 1*c*, *d*). Due to limited site access in the mid-wet season, measurements were carried out on one plot per treatment ($n = 7$), while two plots per treatment were sampled in the dry season ($n = 14$; Fig. 1*d*). At each treatment plot, 1-m^2 quadrats were placed at 5-m intervals, commencing at 0 m alongside the 30-m transect ($n = 6$ per transect; Table 1). Overall covers of grass and small shrubs were visually estimated for each quadrat (Fig. 3*c*); ground cover, including that under the grass canopy and small shrubs, was also estimated. Biocrust, bare soil, litter and basal area of the grass cover were visually estimated and recorded as a percentage relative to total cover. The four values made up 100% of the ground cover. The overall grass canopy cover was estimated separately. Field observers were trained over several quadrats to assess cover attributes, and data calibrations were performed by experienced biocrust researchers.

Taking RGB photographs in the field

RGB photographs of each quadrat were collected for use in EasyPCCr to determine ground-cover attributes. In each plot, photographs of the 1-m^2 quadrat were taken at about 1.5-m height with a mobile phone (iPhone 11 pro; Fig. 2*c*, *d*, Table 1). There were $n = 336$ quadrats measured and photographed in the dry season and $n = 168$ in the wet season.

Image-classification method

An image-classification software (EasyPCCr) was used to determine the different cover percentages within each photo. EasyPCCr is based on a decision tree-based segmentation model (DTSM) and is an effective method to extract

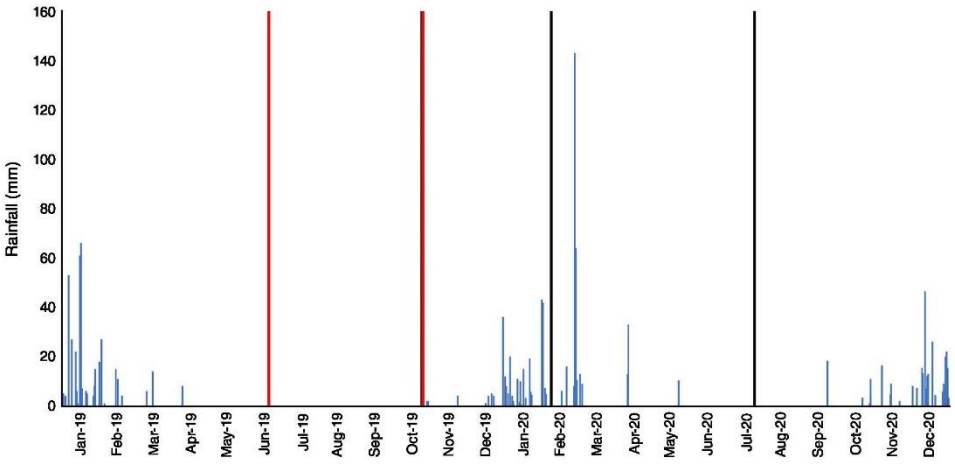


Fig. 2. VRRS (Kidman Springs) daily rainfall from January 2019 to December 2020 (source: Bureau of Meteorology 2021). Key: daily rainfall (blue bars), burning dates (23 June and 29 October 2019, red bars) and sample collection dates (18 February and 23 July 2020, black bars).

Table 1. Field sampling strategy to measure ground cover (grass, biocrust, litter and bare ground).

Sample timing	Soil type	Fire interval (years)	Plots 160 × 160 m (treatments)	Fire season	Total quadrats treatment (1 m^2)	Total quadrats analysed
Mid-wet season (Feb. 2020)	Calcarosol	2, 4, 6, unburnt (control)	7	Early (cool), Late (hot) ($n = 2$)	12	7 × 2 × 12 = 168
Dry season (July 2020)	Calcarosol	2, 4, 6, unburnt (control)	14	Early (cool), Late (hot) ($n = 2$)	12	14 × 2 × 12 = 336

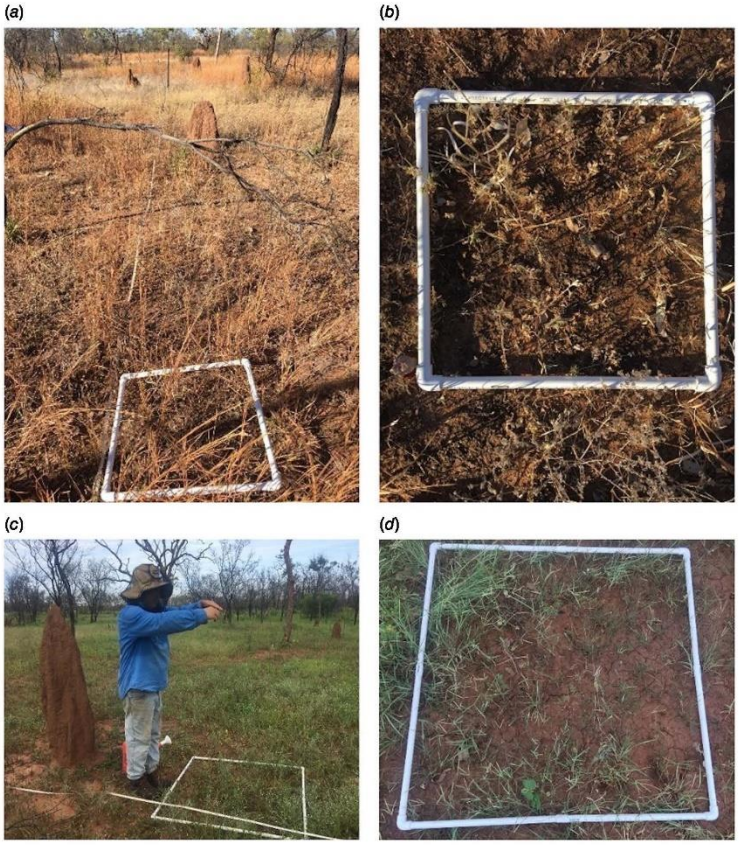


Fig. 3. Collecting ground-cover field data in wet and dry seasons. (*a*) Dry-season example of a 30-m transect, (*b*) ground cover, including grass, biocrust, litter and bare soil measured in 1 m^2 quadrat, (*c*) wet-season ground cover measured and mobile phone image taken, (*d*) example of dark biocrust cover visible beneath and adjacent to new grass growth in the wet season.

areas of plant foliage from RGB photographs that are taken in natural light conditions. This method defines individual classes for each object relating to specific plant and non-plant characteristics within an area. By this means, ML models are trained to recognise the classes (Guo *et al.* 2013). We used the EasyPCCr tool to determine the percentage of cover types from RGB photographs of sample quadrats across different fire treatments and time since fire.

Biocrust field collections and pigment analysis

To confirm the presence of biocrusts, replicate micro-core samples of soil surfaces (up to 1 cm depth) were taken from each microsite. We had previously determined that the biocrusts at this site were dominated by photosynthetic cyanobacteria, some of which are surface dwelling and others subsurface (within first few millimetres). These samples provided confirmation of biocrust presence, especially under the grass canopy and litter where they are not always clearly visible. Three micro-cores (3.56 cm^2 × 1 cm depth, average weight 5 g) of visible biocrust or apparently bare soil were collected from all treatment microsites (grass, litter, biocrust and bare ground). However, biocrust under litter was collected only during the dry season (July 2020). There was virtually no litter cover mid-wet season to provide sample points, because this had been recently burnt. The samples were dried and individually stored in sealed Falcon tubes and transported back to the laboratory. To preserve the chlorophyll (pigment), analysis was undertaken within 2 weeks of sample collection. Prior to extraction,

all samples were moistened with equal amounts of water to facilitate cell rehydration. Biocrust pigment content analysis (chlorophyll *a*, *b*, *c* concentrations) was undertaken using a 2-h dark extraction with a 1:5 ratio of di-methyl-sulfoxide (DMSO; Barnes *et al.* 1992), and the concentrations were calculated using Wellburn's (1994) equations. Results are shown as biocrust pigment and data were converted to micrograms of chlorophyll per square metre for all treatments and microsites. Heat maps were used to demonstrate the chlorophyll concentrations across all treatments.

Quantifying ground cover by using decision tree-based segmentation model (DTSM)

The photographs were processed to obtain the cover of grass, biocrusts, litter and bare soil, using DTSM. The model uses a classification and regression tree algorithm (Barnetson *et al.* 2019) to create a series of nodes to stepwise discriminate the photo elements under different light conditions. Variables of colour in this model include red (*R*), green (*G*), blue (*B*), hue (*H*), saturation (*S*), value (*V*), lightness (*L**), red/green intensity (*a**) and yellow/blue intensity (*b**). This covers any differences in range and texture, works well in situations with clearly ldefined background colour classes. The study used the EasyPCCr tool, which provides a graphical user interface (GUI) for implementing the model using sample data, model training and generating classification maps (Guo *et al.* 2017).

Preparation of photographs and defining region of interest (ROI)

For each RGB image, the entire frame was used for locating the training pixels and then creating a training dataset for each cover type (Fig. 4). The image was then cropped to the quadrat frame and loaded into the model for comparisons across photographs. This was undertaken using the ROI tool in EasyPCCr. The training dataset was generated for individual photographs because of different light conditions and the DTSM segmentation was also applied to each image.

The vegetation cover included green leaves and stems of annual and perennial plants. The identification of litter captured debris, including stones, dry branches, and dead grasses. Biocrusts were identifiable by their dark colour (pigment) compared to the natural red colour of the soil. Due to the nature of an overhead photograph, ML-based cover estimates were determined from the visible areas of biocrust in RGB image analysis. This contrasts with the field observations where biocrusts that occurred under the grass canopy were included in the ground-cover measurements. Bare soil were areas that had no distinguishable biocrust on the soil surface.

DTSM application and accuracy evaluation

The DTSM algorithm was adopted to create the decision tree, and the noise reduction filter (size 100) was applied to reduce the misclassification of the training photographs. This step was needed to enhance photo quality and thus increase accuracy of the classification and segmentation of individual classes from the model. The analysis was carried out in the EasyPCCr tool that showed the output of percentage cover (%Cov) of individual classes in each photo (Fig. 5). DTSM generates individual class data as cover portions that total 100% and are provided as surface cover for each training photo.

The verification process is an important step that determines the accuracy of an algorithm model or ML tool. In this study, verification of the model was implemented by comparing statistical analysis data between DTSM-developed ground cover and field-collected ground cover. The DTSM step in EasyPCCr produced the four ground-cover classes, i.e. grass cover, litter, visible biocrust and bare soil, which were identified in training photographs. This information provided the means to analyse the effects of fire treatments and how the landscape has recovered.

Statistical analysis

For each grass, litter, visible biocrust, and bare soil measurement, a single value was compiled for each plot by averaging values from all quadrats for wet and dry seasons derived from individual experimental plots. This provided two values (mid-wet season and dry season) per treatment. The research data were analysed using R-studio statistic software (Su *et al.* 2021).

We used DTSM to analyse the grass and litter combinations within 1 m^2 and the field-observed biocrust percentage cover (%Cov ± s.e.) for both the mid-wet season and dry season. In the case of the field observations, grass and litter cover were above 100% because litter could occur under the grass canopy. To obtain a more accurate percentage for comparisons with modelled results, we calculated the combination of both and divided it by the total percentage of all ground cover (microsites). Grass and litter combined cover was then analysed to determine the difference between DTSM and field measurements. Biocrust %Cov was analysed in R statistics software. The combination of grass and litter data were analysed using three-way analysis of variance (ANOVA) and Tukey's honestly significant difference *post hoc* test (HSD) to identify significant differences. A simple linear regression analysis was applied to explore relationships among various ground covers in both seasons. Biocrust pigment variation (Chl) for microsites was assessed using one-way ANOVA and Tukey's HSD and the heat maps.

Results

Modelled grass cover compared to field measurements

In the wet season, there were no significant differences between the modelled cover and the field measurements

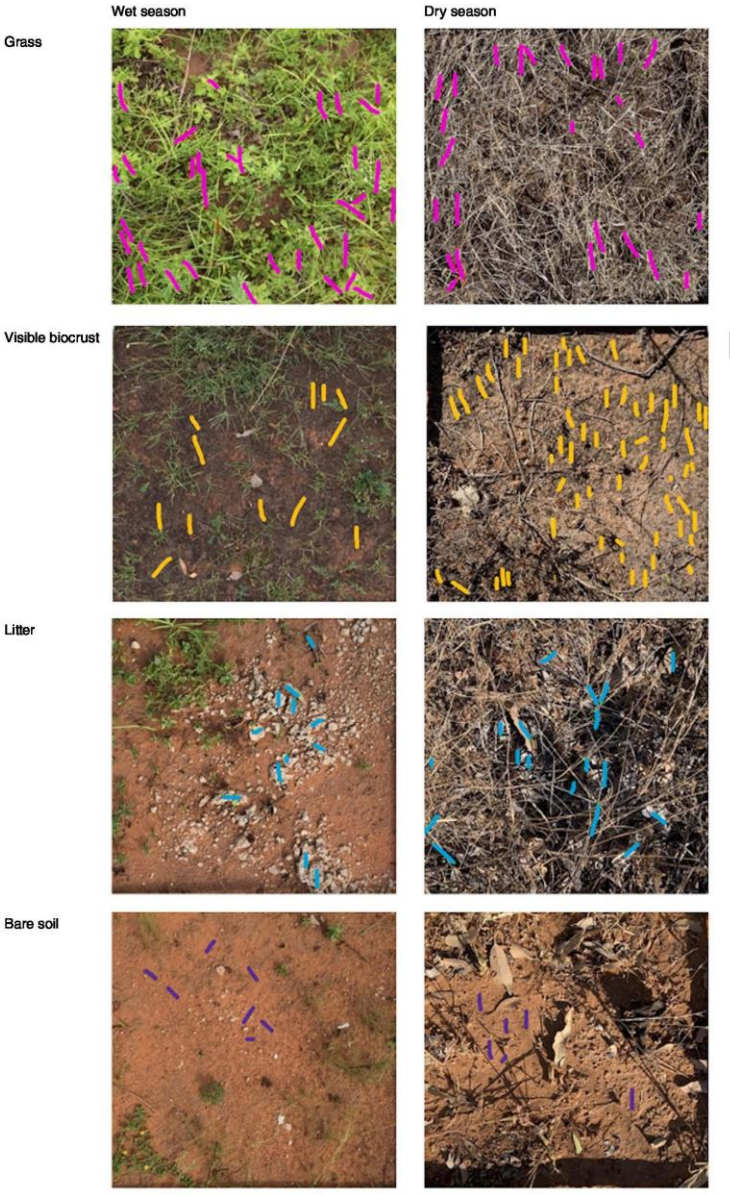


Fig. 4. Examples of 'trained' ground-cover classes for grass, visible biocrust, litter and bare soil in decision tree-based segmented model (DTSM) for machine learning. The coloured pink, yellow, blue or purple lines were drawn on the individual class to 'train' the model to recognise them as individual classes (note the black line in visible biocrust picture and bare soil picture in the dry season is the shadow cast by the quadrat-frame).

($P > 0.05$). There was, on average, a difference of 8.4%Cov between modelled (48%Cov ± 2.54) and field measurements (56%Cov ± 3.53; Fig. 6). Across the early season burning treatments, the average difference in modelled grass cover (49.4%Cov ± 8.81) was 8.2% lower than field observations (57.6%Cov ± 8.81). In the wet season, modelled grass cover in the late-season burning treatments was lower than the field observations. In the unburnt control

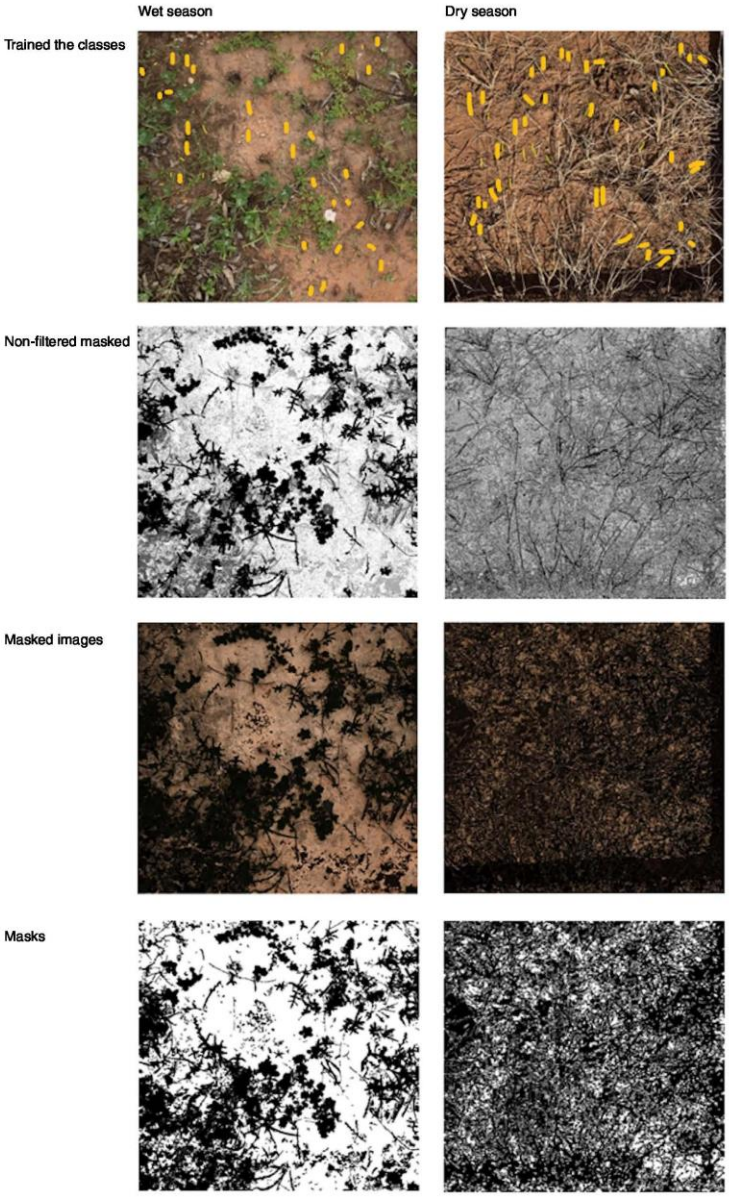


Fig. 5. DTSM output images for visible biocrust in the wet and dry season. The biocrust was identified by drawing straight lines (yellow colour) on the digital photograph on both dark and light visible biocrust.

plots, the modelled grass cover was lower than that in the field data.

In the dry season, there were significant differences between modelled and field methods in estimated grass cover. Modelled grass cover was higher than field observation across fire treatments and control plots ($P < 0.01$). For example, in the 4-year early burn plots, grass cover (modelled) was 38.8%Cov, with an average difference of 13.5%,

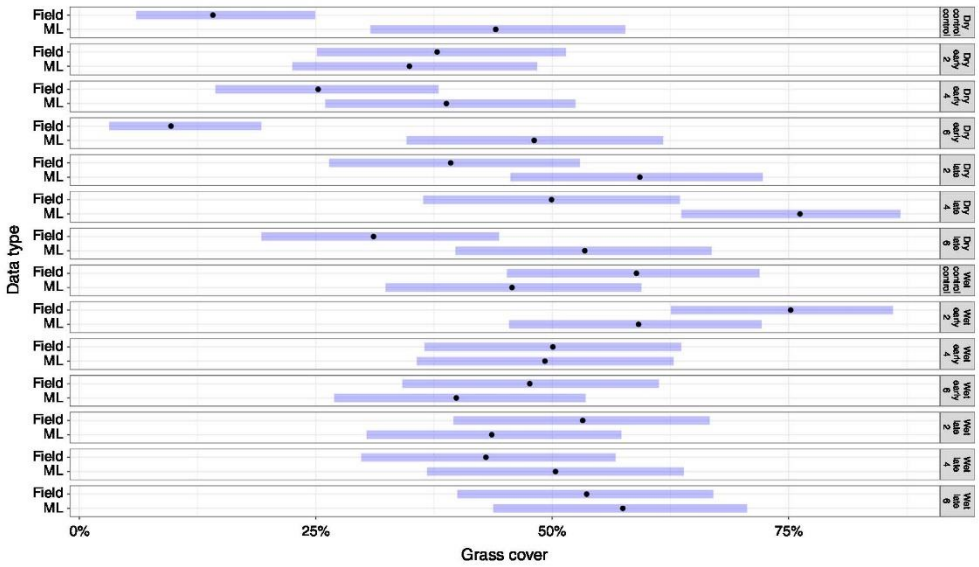


Fig. 6. Comparisons of mean grass cover between field data and machine learning in February 2020 and July 2020. Bars that do not overlap are significantly different.

compared to field data. In contrast, modelled grass cover for the 2-year early burn plots (34.9%Cov ± 2.52) was a near match to field observations (37.8%Cov ± 6.48). However, there were no significant differences ($P = 0.07$) between modelled and field data across the early burning treatments, except for the early 6-year burnt plots ($P < 0.001$). In the late-burn plots, the modelled grass cover for the late 2-year and late 4-year plots was 59.3%Cov (±1.8) and 76.2%Cov (±7.61) respectively, and significantly ($P < 0.001$) different from the field observations of late burn every 2-year plots, 39.3%Cov ± 6.6, and late burn every 4-year plots 49.9%Cov ± 7.15, (Fig. 6). Field observations of grass cover in the wet season (56% ± 3.53) were higher than those in the dry season (30% ± 5.41), whereas modelled grass cover in the wet season (48% ± 2.54) was lower than that in the dry season (51% ± 5.29).

Modelled grass and litter cover compared to field measurements

When grass and litter cover were estimated together, there was greater similarity between methodology estimates. For the wet season, grass and litter %Cov derived from photographs averaged 8.8% (±2.6) across all treatments and was comparable to field measurements, with an average of 1% difference between them (Fig. 7). We also measured grass and litter cover across fire-plot treatments in July 2020 during the dry season (rain ceased in May). Modelled grass and litter %Cov across all treatments was 20.9% (±0.6), compared to field observations of 22.21% (±0.32). Furthermore, no significant differences were observed between fire treatments and the unburnt control plots in either wet or dry seasons ($P = 0.99$; Fig. 7) where there was an average cover difference of 1%. Both field observations and modelled grass cover in the wet season were slightly lower than those in the dry season.

Relationships between microsites

In both seasons, when grass and litter cover were analysed in combination, as their cover increased, the modelled biocrust cover decreased (Fig. 8). There was a strong inverse relationship between grass and litter cover compared with visible biocrust cover in both the wet ($R^2 = 0.95$) and the dry seasons ($R^2 = 0.95$; Fig. 8). Additionally, biocrust from the field observation decreased when the combined grass and litter coverage in the plot increased, especially in the dry season ($R^2 = 0.80$). DTSM can also distinguish between biocrust and bare soil in both seasons (wet, $R^2 = 0.15$; dry, $R^2 = 0.09$). Yet, the relationship between bare and biocrust from field observations in the wet season showed that as bare cover increased, the biocrust cover decreased ($R^2 = 0.80$).

Modelled biocrust cover compared to field measurements

In the wet season, modelled biocrust cover (34%Cov ± 2.0) differed significantly from field measurements when averaged

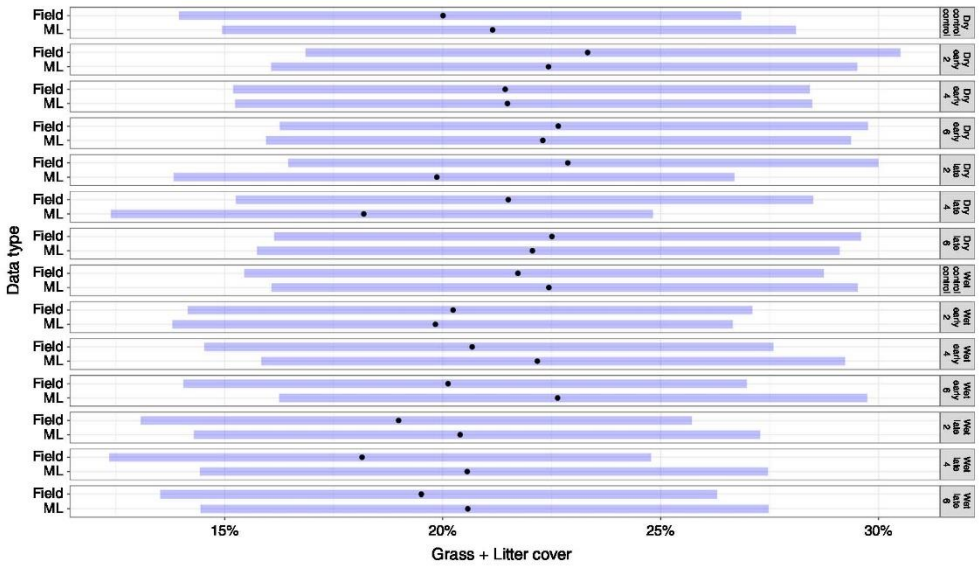


Fig. 7. Comparisons of grass and litter combined as mean cover between field data and machine learning (ML) in the mid-wet season (February 2020) and dry season (July 2020). Bars that do not overlap are significantly different.

across all treatments (39%Cov ± 3.2; $P = 0.04$; Fig. 9). These differences were more obvious in early burning treatments, such as the 6-yearly early burning treatment ($P < 0.01$) where the modelled cover was, on average, 20% lower than the observed field result (Fig. 9). Furthermore, in the unburnt control plots, detected biocrust cover (20%Cov ± 2.1) was also significantly lower than field observations (57%Cov ± 5.8) ($P < 0.01$). In contrast, modelled biocrust cover (33%Cov ± 3.3) was comparable with field observations (42%Cov ± 6.2) in late-burning treatments ($P = 0.21$; Fig. 9).

In the dry season, modelled biocrust cover across all burnt and unburnt treatments was comparable to field observations (Fig. 9). Biocrust cover in early burning plots averaged 30%, with no significant differences between DTSM-modelled cover and field observations. Similar data were generated for modelled biocrust cover on control plots (18%Cov ± 2.8) and field observations (15%Cov ± 5.6), although DTSM-modelled biocrust cover in late-burn plots (25%Cov ± 2.7) differed significantly from field observations (53%Cov ± 4.4). However, there were no overall differences between modelled biocrust cover and field observations in either fire treatments and control plots in the dry season ($P = 0.06$), with the exception of the late 4-year burning treatment (Fig. 9).

Biocrust pigment content across microsites

Biocrust pigment content based on chlorophyll concentration (Chl) was analysed across three microsites, i.e. biocrust, grass and bare soil in both wet and dry seasons (Fig. 10). Overall, bare soil microsites had the lowest and biocrust microsites the highest pigment concentrations. Biocrust pigment was recorded under litter but only in the dry season (Supplementary Fig. S1).

In the wet season, the highest average pigment concentration was detected in biocrust microsites in the control plots (162 ± 35 mg Chl/m^2; $P < 0.01$). In the burnt treatments, the early season 6-yearly (E6) burn plot was not significantly different from the unburnt controls (112 ± 12 mg Chl/m^2, $P = 0.35$) whereas L6 (14 ± 1 mg Chl/m^2) and L4 (20 ± 3 mg Chl/m^2) had significantly lower pigment concentration compared to the other treatments ($P < 0.01$), in contrast to E6 which had high concentrations in the biocrust microsites.

Furthermore, in the wet season, microsites under grass canopy also had the highest pigment concentrations in the unburnt control plots (92 ± 19 mg Chl/m^2) and E2 (75 ± 5 mg Chl/m^2), whereas L6 (29 ± 1 mg Chl/m^2) had significantly ($P < 0.01$) lower pigment concentrations in grass microsites. Microsites under grass in E6 (38 ± 4 mg Chl/m^2) and E4 (45 ± 1 mg Chl/m^2) had also significantly lower pigment concentrations than did the unburnt control ($P < 0.01$). In the wet season, although low, L6 bare microsites had significantly ($P < 0.05$) higher pigment concentrations (26 ± 5 mg Chl/m^2) than did other burnt bare microsites, including E2 (10 ± 2 mg Chl/m^2), L2 (8 ± 2 mg Chl/m^2), unburnt control (8 ± 2 mg Chl/m^2) and

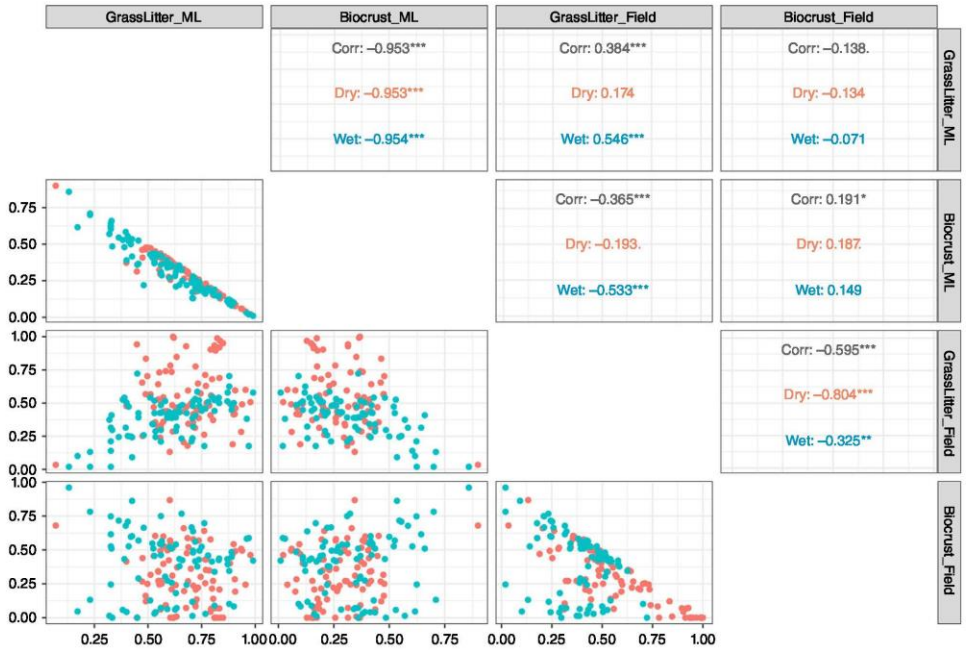


Fig. 8. Relationship between ML and field observations for grass, litter, and visible biocrust in February 2020 and July 2020, post-fire recovery. *Asterisks represent the level of significance. ML, machine learning.

E6 (2 ± 2 mg Chl/m^2; $P < 0.01$). Pigment concentration in bare microsites E4 (17 ± 4 mg Chl/m^2) and L4 (14 ± 1 mg Chl/m^2) was significantly higher than E6 ($P < 0.04$).

In the dry season, L6 had the highest pigment concentrations for all burnt treatments and in the unburnt control plots; however, they were not significantly ($P = 0.16$) different. The highest pigment in biocrust microsites ranged between (L6) and 128 ± 35 mg Chl/m^2 (L2). The lowest pigment recorded was in E4 (45 ± 11 mg Chl/m^2) and the unburnt control (61 ± 31 mg Chl/m^2). Litter microsites in the control had the highest pigment (236 ± 48 mg Chl/m^2) together with E4 (190 ± 116 mg Chl/m^2), but both showing the large variability between sample points.

L6 also had the highest pigment concentration under the grass canopy (108 ± 13 mg Chl/m^2), whereas E6 had the lowest pigment concentration (17 ± 2 mg Chl/m^2; $P < 0.01$). Similarly, the control had low pigment (34 ± 7 mg Chl/m^2), significantly different from L2 (86 ± 5 mg Chl/m^2; $P < 0.01$), L4 (104 ± 7 mg Chl/m^2) and E2 (81 ± 17 mg Chl/m^2; $P < 0.03$). E4 (42 ± 7 mg Chl/m^2) was significantly ($P < 0.01$) lower than L6 and L2.

Similarly, L6 had the highest pigment concentration in dry-season bare microsites (86 ± 38 mg Chl/m^2) and the lowest in the unburnt control plots (14 ± 3 mg Chl/m^2), although differences were not significant ($P = 0.06$). For example, E6 (37 ± 3 mg Chl/m^2), L2 (35 ± 3 mg Chl/m^2), E2 (29 ± 9 mg Chl/m^2) and E4 and L4 were both 25 ± 3 mg Chl /m^2.

Discussion

Seasonal impacts on ML accuracy

Building good 'training' data was a crucial part of the photographic analysis. If the object was correctly defined, DTSM provided accurate data. In the dry season, even though grass and litter cover were recorded under sunny conditions in the training photographs, it was difficult to obtain accurate training data when these ground-cover classes were presenting the same or similar colours. For example, the grass colour resembled litter, dry leaves and dead grass, and ML over-estimated grass cover. In effect, the grass cover measured by ML was a combination of dry litter and dry grass, whereas observers could visually separate the two. Also, dry-season grass and litter cover in the 2-yearly burning treatments and unburnt control were significantly higher than those in other treatments.

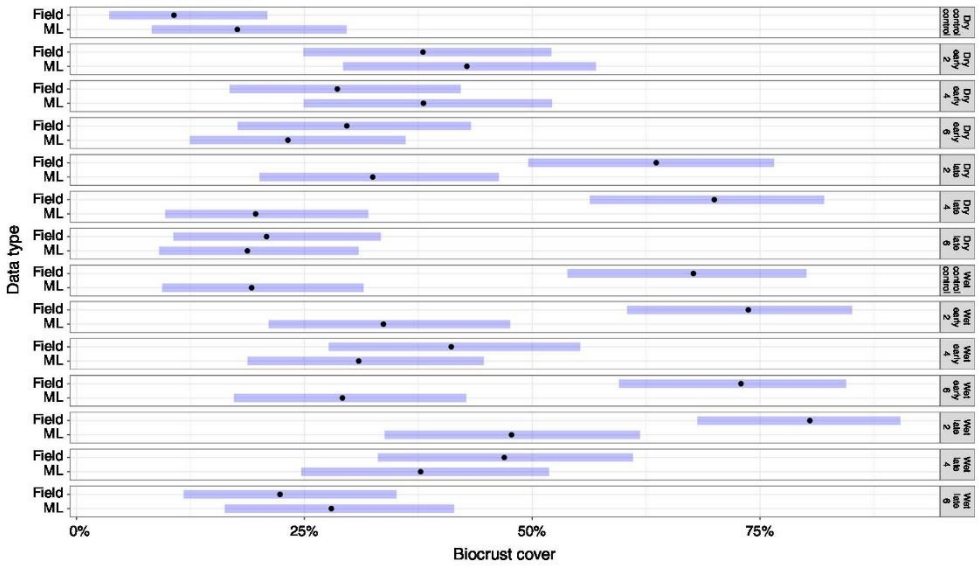


Fig. 9. Comparison of biocrust recovery cover collected with trained field observers versus machine learning derived from phone images in the mid-wet season (February 2020) and dry season (July 2020). Bars that do not overlap are significantly different.

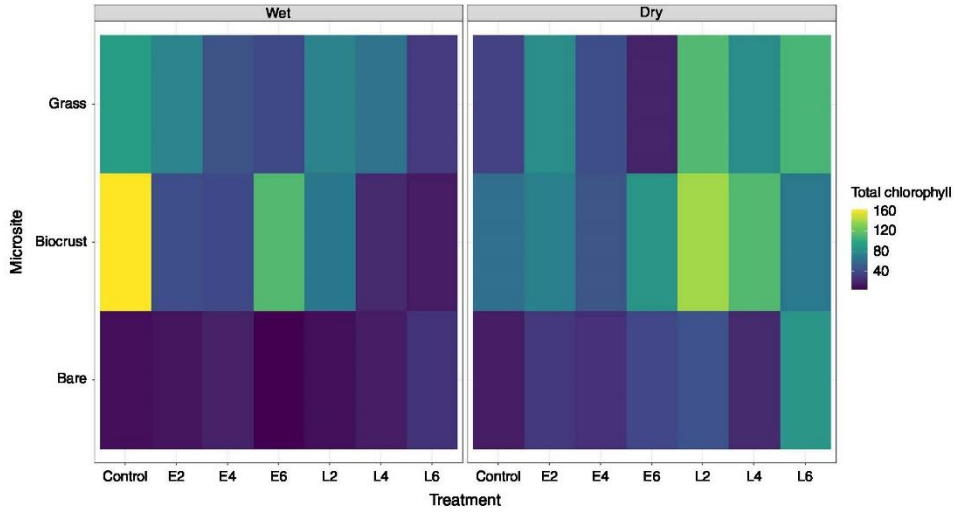


Fig. 10. Pigment concentrations (mg chlorophyll/m^2) for calcarosols at three microsites (biocrust, grass, bare soil) in the wet season (February 2020) and dry season (July 2020) for unburnt controls and early season (E) and late-season (L) fires implemented in 2-, 4-, and 6-yearly intervals.

Thus, one of the challenges of the DTSM model was to separate objects of the same colour among different trained classes. We approached this problem in two ways. First, we refined the training with attention to detail and colour differentiation. Second, we confirmed the poorer differentiation by ML, by combining litter and grass. When we performed these steps, there were no significant differences between ML and observers. Future applications could couple these measurements as a positive indicator of ground cover and soil-surface stability (Williams *et al.* 2021).

For wet-season field measurements, high soil water content and active biological processes indicated by pigment (Belnap *et al.* 2016) improved biocrust identification of biocrust presence (Blanco-Sacristán *et al.* 2019, 2021). In this case, as the biocrust was sometimes difficult to see (owing to the early stage of recovery), *in situ* pigment detection verified its presence. Furthermore, the DTSM model could not detect biocrust cover located under high density of grass, shrubs, or litter cover. This was demonstrated in the regression models (Fig. 7).

Measuring biocrusts using ML

Methods such as UAV (Havrilla *et al.* 2020; Blanco-Sacristán *et al.* 2021), airborne sensing (Weber *et al.* 2008; Rodríguez-Caballero *et al.* 2014) and satellite imagery (Panigada *et al.* 2019) can map landscapes, but have limitations because of environmental conditions obscuring biocrust quantification with dry- versus wet-season variability, sun position without clouds, and using conventional indices (e.g. normalised difference vegetation index (NDVI); Karnieli *et al.* 2001; Belnap *et al.* 2016). Such methods are also unsuitable for direct use by most land managers, although there is a need for land manager-based on-ground monitoring.

In the dry season, biocrusts were quite clearly visible because of their darkened colour contrasting against the red calcarosol soils. However, in many cases, there was biocrust under grass and shrubs (recorded by observers) that was not discernible by the ML methods we used, as a consequence of the grass canopy covering the biocrust. During the wet season, biocrust pigment concentrations derived from microsites demonstrated that biocrusts were as prevalent under the grass canopy as they were in the interspaces between the grass plants and, to a lesser extent, in apparently bare soil (Fig. 10). Biocrust pigment was not measured under litter in the wet season because of the scarcity of litter so soon after fire. Nevertheless, dry-season pigment measurements taken in 2020 reconfirmed the presence of biocrusts across all microsites including under litter (data not shown). In some instances, dry-season pigment was less concentrated than the wet-season one, whih is likely to be indicative of the inactive state of the biocrusts.

There was a high correlation between the increase in grass and litter cover and a decreased biocrust cover (Fig. 7). This demonstrated the limitation of this method in detecting biocrust cover that was not visible in the photo as it was obscured by the grass canopy. Another factor that contributed to decreased accuracy in biocrust cover detection was likely to be the colour effect. As there were two visible biocrust colours (light and dark), and light patches of biocrust mixed with bare soil and small grasses, detection was often difficult, leading potentially to underestimation of biocrust presence. In previous studies, the classification of biocrusts was identified as dark and light biocrust by using UAV images, but the identification of light-coloured cyanobacteria has presented challenges in UAV images when separating from bare soil and surface roughness, with shadowing from biocrust microtopography. These surface cover classes are difficult to separate due to the reflectance in the RGB zone and when real-life colours are similar (Havrilla *et al.* 2020). To resolve these problems, further field measurements that estimate biocrusts under the grass canopy as a separate measure to visible areas could be used to establish a suitable crust cover index for different landscapes.

ML comparisons with alternative methods

We analysed ground cover, including biocrust, grass, litter, and bare soil to determine whether digital photographs were suitable. We applied ML techniques to aid in the quantification of these microsites following different burning treatments and in wet and dry seasons. Comparing data obtained by trained biocrust field-observers with ML analysis of photographs, we demonstrated that it was feasible to monitor microsite recovery post-fire disturbance by using a mobile phone camera.

Information derived from photographs, generated by ML by using the DTSM algorithm, generally showed high accuracies in detecting cover of each class when compared with field observation. There were exceptions in some cases, such as biocrust cover in the wet season when grass tussocks obscured biocrusts. Yet, the proposed method provided a high level of accuracy (88%) for biocrust cover in contrasting conditions, including the dry season, and following late burning in the wet season.

However, it was apparent that there was a lower level of accuracy for early dry-season burning, most likely a consequence of the increase in litter, although it could also be related to observer errors. In addition, observers did not consider any differences in plant species that may influence accuracies. Further ground-truthing and adjustments to the model that are specific to seasonal conditions and biocrusts are therefore required.

Post-fire recovery monitoring tools

The Australian savanna landscapes are extensive and often subject to managed and natural fires (Cowley *et al.* 2014). Furthermore, rainfall is spatially variable, resulting in better recovery in some paddocks than others, or even within

paddocks that can be as large 2000 ha or more (O'Reagain *et al.* 2009). Even though it appears feasible that UAVs and satellite imagery encompass the large areas, resolution can range between 10 and 30 m (multi-spectral), with small-scale attributes of the ground cover mixed.

The benefit of digital photographs is the capacity to collect relatively large numbers of high-resolution images at a small scale. Photos can be acquired seasonally with minimal effort (e.g. at fixed sentinel points) while performing tasks such as checking cattle, water points and fences. Once the ML tools have been applied for specific regions with similar characteristics, their applications are time- and cost-effective. The EasyPCCr method has been proven efficient in cropping systems (Guo 2018; Tresch *et al.* 2019) and, as demonstrated, can be readily applied to the savanna grasslands such as the landscapes in this study. Decision-making regarding ground cover and local conditions, especially for post-fire or drought recovery, can then be based on biocrust presence as an indicator of soil health and land condition (Williams *et al.* 2021). We acknowledge that not all land managers would have the capacity to analyse their photographs, although field extension officers could be trained to develop the DTSM model for their local region.

Conclusions

Advantages of ML methods

The method developed here required minimal time to collect field data and used simple equipment and relatively easy data processing to quantify biocrust and other ground-cover types at a fine scale. Similarly, quantifying grass and litter cover following early and late-season burning provided results between methods with 95–97% similarity. The exception was an underestimation of grass and litter cover produced by the DTSM algorithm for the 2-year early season burning treatment in the wet season, which was likely to be a result of small grasses being shaded by the photographer's shadow and quadrat frame. However, statistically there was no difference between grass cover in this treatment plot when combined with litter cover when DTSM was compared with field observations. Thus, the DTSM model provided accurate results under various light conditions, and, like other studies, produced the best results in sunny conditions (Guo *et al.* 2017).

The methodology effectively detected ground-cover classes from photographs taken with a mobile phone in combination with 'off-the-shelf' image-analysis software package and DTSM learning algorithm. Our derived sensing metric showed moderate to high accuracies in classifying different ground cover when compared with observations by specialists in the field. We concluded that the methodology developed in this study effectively quantified ground cover, including grass and biocrust, as potential indicators of primary productivity and soil health.

A constraint for quantifying biocrusts was high grass cover in the wet season such as the unburnt control plots or early 6-yearly burn plots where biocrusts were often obscured by grass tussocks. Nevertheless, we confirmed that there was a considerable biocrust presence under grass tussocks, which was confirmed by the pigment analysis. This created one of the key differences between methods with observer estimations of microsite cover, incorporating cover underneath grass canopy and in the interspaces between grass tussocks. Thus, to accurately analyse biocrust and litter cover, the estimates for biocrusts and litter under the grass canopy need to be built into the model. In terms of landscape function, it appeared that dry-season photographs were more informative. With promising findings, next steps in this research should be expanded to analyse other soil types so that a land manager-based management tool can be developed.

Supplementary material

Supplementary material is available online.

References

Allen EB, Steers RJ, Dickens SJ (2011) Impacts of fire and invasive species on desert soil ecology. *Rangeland Ecology & Management* **64**, 450–462. doi:10.2111/REM-D-09-00159.1

Barger NN, Weber B, Garcia-Pichel F, Zaady E, Belnap J (2016) Patterns and controls on nitrogen cycling of biological soil crusts. In 'Biological soil crusts: an organizing principle in drylands'. (Eds B Weber, B Büdel, J Belnap) pp. 257-285. (Springer: Cham, Switzerland)

Barnes JD, Balaguer L, Manrique E, Elvira S, Davison AW (1992) A reappraisal of the use of DMSO for the extraction and determination of chlorophylls *a* and *b* in lichens and higher plants. *Environmental and Experimental Botany* **32**, 85–100. doi:10.1016/0098-8472(92)90034-Y

Barnetson J, Phinn S, Scarth P, Denham R (2017) Assessing Landsat fractional ground-cover time series across Australia's arid rangelands: separating grazing impacts from climate variability. *International Archives of the Photogrammetry, Remote Sensing and Spatial Information Sciences* **XLII-3/W2**, 15–26. doi:10.5194/isprs-archives-XLII-3-W2-15-2017

Barnetson J, Phinn S, Scarth P (2019) Mapping woody vegetation cover across Australia's arid rangelands: utilising a machine-learning classification and low-cost Remotely Piloted Aircraft System. *International Journal of Applied Earth Observation and Geoinformation* **83**, 101909. doi:10.1016/j.jag.2019.101909

Belnap J, Weber B, Büdel B (2016) Biological soil crusts as an organizing principle in drylands. In 'Biological soil crusts: an organizing principle in drylands'. (Eds B Weber, B Büdel, J Belnap) pp. 3–13. (Springer)

Blanco-Sacristán J, Panigada C, Tagliabue G, Gentili R, Colombo R, Ladrón de Guevara M, Maestre FT, Rossini M (2019) Spectral diversity successfully estimates the α-diversity of biocrust-forming lichens. *Remote Sensing* **11**, 2942. doi:10.3390/rs11242942

Blanco-Sacristán J, Panigada C, Gentili R, Tagliabue G, Garzonio R, Martín MP, Ladrón de Guevara M, Colombo R, Dowling TPF, Rossini M (2021) UAV RGB, thermal infrared and multispectral imagery used to investigate the control of terrain on the spatial distribution of dryland biocrust. *Earth Surface Processes and Landforms* **46**, 2466–2484. doi:10.1002/esp.5189

Bowker MA, Maestre FT, Eldridge D, Belnap J, Castillo-Monroy A, Escolar C, Soliveres S (2014) Biological soil crusts (biocrusts) as a model system in community, landscape and ecosystem ecology.

Biodiversity and Conservation **23**, 1619–1637. doi:10.1007/s10531-014-0658-x

Bradstock RA (2010) A biogeographic model of fire regimes in Australia: current and future implications. *Global Ecology and Biogeography* **19**, 145–158. doi:10.1111/j.1466-8238.2009.00512.x

Brianne P, Rebecca H, David L (2020) The fate of biological soil crusts after fire: a meta-analysis. *Global Ecology and Conservation* **24**, e01380. doi:10.1016/j.gecco.2020.e01380

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

Bureau of Meteorology (2021) Climate statistics for Australian locations. Available at http://www.bom.gov.au/climate/averages/tables/cw_014847.shtml [accessed 20 June 2021]

Cowley RA, Hearnden MN, Joyce KE, Tovar-Valencia M, Cowley TM, Pettit CL, Dyer RM (2014) How hot? How often? Getting the fire frequency and timing right for optimal management of woody cover and pasture composition in northern Australian grazed tropical savannas. Kidman Springs Fire Experiment 1993–2013. *The Rangeland Journal* **36**, 323–345. doi:10.1071/RJ14030

Evans RD, Lange OL (2001) Biological soil crusts and ecosystem nitrogen and carbon dynamics. In 'Biological soil crusts: structure, function, and management'. (Eds J Belnap, OL Lange) pp. 263–279. (Springer)

Eyre TJ, Fisher A, Hunt LP, Kutt AS (2011) Measure it to better manage it: a biodiversity monitoring framework for the Australian rangelands. *The Rangeland Journal* **33**, 239–253. doi:10.1071/RJ10071

Flores BM, Staal A, Jakovac CC, Hirota M, Holmgren M, Oliveira RS (2020) Soil erosion as a resilience drain in disturbed tropical forests. *Plant and Soil* **450**, 11–25. doi:10.1007/s11104-019-04097-8

Guo W (2018) Automated characterization of plant growth and flowering dynamics using RGB images. In 'Smart plant factory'. (Ed. T Kozai) pp. 385–393. (Springer)

Guo W, Rage UK, Ninomiya S (2013) Illumination invariant segmentation of vegetation for time series wheat images based on decision tree model. *Computers and Electronics in Agriculture* **96**, 58–66. doi:10.1016/j.compag.2013.04.010

Guo W, Zheng B, Duan T, Fukatsu T, Chapman S, Ninomiya S (2017) EasyPCC: benchmark datasets and tools for high-throughput measurement of the plant canopy coverage ratio under field conditions. *Sensors* **17**, 798. doi:10.3390/s17040798

Havrilla CA, Villarreal ML, DiBiase JL, Duniway MC, Barger NN (2020) Ultra-high-resolution mapping of biocrusts with unmanned aerial systems. *Remote Sensing in Ecology and Conservation* **6**, 441–456. doi:10.1002/rse2.180

Karnieli A, Kokaly RF, West NE, Clark RN (2001) Remote sensing of biological soil crusts. In 'Biological soil crusts: structure, function, and management'. (Eds J Belnap, OL Lange) pp. 431–455. (Springer)

Kilinc M, Beringer J (2007) The spatial and temporal distribution of lightning strikes and their relationship with vegetation type, elevation, and fire scars in the Northern Territory. *Journal of Climate* **20**, 1161–1173. doi:10.1175/JCLI4039.1

O'Reagain P, Bushell J, Holloway C, Reid A (2009) Managing for rainfall variability: effect of grazing strategy on cattle production in a dry tropical savanna. *Animal Production Science* **49**, 85–99. doi:10.1071/EA07187

Panigada C, Tagliabue G, Zaady E, Rozenstein O, Garzonio R, Di Mauro B, De Amicis M, Colombo R, Cogliati S, Miglietta F, Rossini M (2019) A new approach for biocrust and vegetation monitoring in drylands using multi-temporal Sentinel-2 images. *Progress in Physical Geography: Earth and Environment* **43**, 496–520. doi:10.1177/0309133319841903

Possoch M, Bieker S, Hoffmeister D, Bolten A, Schellberg J, Bareth G (2016) Multi-temporal crop surface models combined with the RGB vegetation index from UAV-based images for forage monitoring in grassland. *The International Archives of the Photogrammetry, Remote Sensing and Spatial Information Sciences* **XLI-B1**, 991–998. doi:10.5194/isprs-archives-XLI-B1-991-2016

Preece N (2002) Aboriginal fires in monsoonal Australia from historical accounts. *Journal of Biogeography* **29**, 321–336. doi:10.1046/j.1365-2699.2002.00677.x

Rodríguez-Caballero E, Escribano P, Cantón Y (2014) Advanced image processing methods as a tool to map and quantify different types of biological soil crust. *ISPRS Journal of Photogrammetry and Remote Sensing* **90**, 59–67. doi:10.1016/j.isprsjprs.2014.02.002

Shi P, Wang Y, Xu J, Zhao Y, Yang B, Yuan Z, Sun Q (2021) Rice nitrogen nutrition estimation with RGB images and machine learning methods. *Computers and Electronics in Agriculture* **180**, 105860. doi:10.1016/j.compag.2020.105860

Su Y-g, Liu J, Zhang Y-m, Huang G (2021) More drought leads to a greater significance of biocrusts to soil multifunctionality. *Functional Ecology* **35**, 989–1000. doi:10.1111/1365-2435.13761

Tongway DJ (2010) Teaching the assessment of landscape function in the field: enabling the design and selection of appropriate restoration techniques. *Ecological Restoration* **28**, 182–187. doi:10.3368/er.28.2.182

Tongway DJ, Ludwig JA (2012) Planning and implementing successful landscape-scale restoration. In 'Restoration ecology'. (Eds J van Andel, J Aronson) pp. 30–42. (John Wiley & Sons, Ltd)

Tresch L, Mu Y, Itoh A, Kaga A, Taguchi K, Hirafuji M, Ninomiya S, Guo W (2019) Easy MPE: extraction of quality microplot images for UAV-based high-throughput field phenotyping. *Plant Phenomics* **2019**, 2591849. doi:10.34133/2019/2591849

Ward DP, Kutt AS (2009) Rangeland biodiversity assessment using fine scale on-ground survey, time series of remotely sensed ground cover and climate data: an Australian savanna case study. *Landscape Ecology* **24**, 495–507. doi:10.1007/s10980-009-9324-1

Weber B, Olehowski C, Knerr T, Hill J, Deutschewitz K, Wessels DCJ, Eitel B, Büdel B (2008) A new approach for mapping of biological soil crusts in semidesert areas with hyperspectral imagery. *Remote Sensing of Environment* **112**, 2187–2201. doi:10.1016/j.rse.2007.09.014

Wellburn AR (1994) The spectral determination of chlorophylls *a* and *b*, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. *Journal of Plant Physiology* **144**, 307–313. doi:10.1016/S0176-1617(11)81192-2

Williams W, Büdel B, Williams S (2018) Cyanobacterial species richness and Nostoc highly correlated to seasonal N enrichment in the northern Australian savannah. *Biogeosciences* **15**(7), 2149–2159. doi:10.5194/bg-15-2149-2018

Williams WJ, Schmidt S, Zaady E, Alchin B, Myint Swe T, Williams S, Dooley M, Penfold G, O'Reagain P, Bushell J, Cowley R, Driscoll C, Robinson N (2021) Resting subtropical grasslands from grazing in the wet season boosts biocrust hotspots to improve soil health. *Agronomy* **12**, 62. doi:10.3390/agronomy12010062