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A century of livestock exclusion reveals soil microbiome impacts in arid Australia.

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Abstract

Soil microbiome responses to disturbance in arid ecosystems remains a critical knowledge gap. This study examines the soil microbiome in the north-east pastoral zone of South Australia, comparing the TGB Osborn Vegetation Reserve, established in 1925 with livestock and rabbit-proof fencing, to the adjacent grazed Koonamore Station, a merino sheep-grazing lease. Soil bacterial and fungal community compositions were analysed through amplicon sequencing, revealing that livestock grazing is associated with specific microbial abundances and community structure, with distinct spatial patterns between bare soil areas and soil under perennial vegetation. Soils from open spaces in livestock grazing areas showed more disparity through reduced microbial abundances compared to soils ungrazed for 98 years. However, soils underneath shrub canopies in grazed areas showed increased abundances and taxonomic differences in comparison to ungrazed. Further research is needed to understand the functional consequences of these soil microbiome shifts on ecosystem function and services.

Introduction

Our understanding of soil microbiome responses to disturbance in arid ecosystems remains limited, as well as the timescales required for functional rehabilitation following disturbance (Toledo et al. 2021; Zhang et al. 2023). Soil microbes form complex, highly diverse communities with significant roles in ecosystem function, yet are largely undescribed and understudied, especially in arid systems (Liu et al. 2023). Within a landscape, different microsites are conducive of different microbial communities, and as such contribute to important processes such as nutrient cycling and soil surface stabilisation. Arid lands are water and nutrient limited, so perennial vegetation which can alter the distribution of resources play vital roles in the functioning of the system (Ludwig and Tongway 1995; Morton et al. 2011). Individual perennial plants create small-scale areas of high fertility beneath the canopy surrounded by lower fertility soil, called “fertile islands” (Garcia-Moya and McKell 1970). Microbial abundance, diversity, and activity have been found to be higher in fertile islands (Goberna et al. 2007). The ameliorated conditions combined with higher substrate levels in fertile islands directly contribute to enhanced nutrient cycling by microbes when compared to open spaces (Macdonald et al. 2015). Soil microbial communities that form biological soil crusts also play major roles in ecosystem services provisioned in the open spaces as well by stabilising the soil surface and fixing atmospheric nitrogen (Belnap and Lange 2003; Castillo-Monroy et al. 2011). Identifying the differences in soil microbiomes where livestock are present and excluded in both open and within fertile islands is crucial for assessing the system wide consequences of livestock grazing. Alongside limitations in identifying microbial species and function, another significant barrier in our understanding of soil restoration and impacts of livestock grazing on soil microbial communities is that there is only a small number of long-term grazing exclusion study sites for comparison (Allington and Valone 2014). This study conducts a cross-fence comparison between a unique 98 year ungrazed reserve and adjacent livestock grazed arid rangelands to address these critical knowledge gaps. The main hypotheses to be tested are: 1) perennial vegetation fertile islands will foster unique microbial communities in comparison to open spaces, and 2) 98 years of grazing exclusion will result in substantive differences in microbial communities compared to livestock grazed areas, and the impacts will be more substantive in open spaces compared to those under plant canopies.

Methods

Sampling was conducted in chenopod shrublands of the arid north-east pastoral zone of South Australia within the TGB Osborn Vegetation Reserve (32°07′S, 139°20′E), established in 1925 with a livestock and rabbit-proof fence, and the surrounding merino sheep-grazing lease on Koonamore Station.

Soil sampling and microbiome extraction and sequencing

Three soil cores (10cm diameter to 5cm depth) were taken beneath the ‘Canopy’ of selected *Eremophila sturtii* shrubs (that are unpalatable to livestock) within 10m of the TGB Osborn Vegetation Reserve (hereafter “reserve”) exclusion fence and combined to make a single sample. The same approach was used to provide a composite soil sample from a randomly chosen paired site on ‘Open’ ground approximately 5 metres away. Composite samples were homogenised, sub-sampled into 5g tubes and stored in a -18°C freezer. Sampling was replicated for both ungrazed sites inside the ‘Ungrazed’ reserve and at ‘Grazed’ sites outside the reserve in May 2023 (Autumn; *n*=24) an in September 2023 (Spring; *n*=41).

PCR amplification and sequencing of soil samples was conducted by the Australian Genomic Research Facility (AGRF, Adelaide, Australia) on the Illumina NextSeq2000 platform (San Diego, CA, USA). DNA was extracted using the PowerSoil Soil DNA Isolation Kit (MoBio Laboratories, Solana Beach, CA, USA). Two regions of ribosomal DNA, 16S and ITS, were amplified to assay the bacterial and fungal communities using the forward and reverse primers, 341F - 806R for bacteria, and ITS1F - ITS2R for fungi. Fungi and bacterial communities were sequenced from Autumn samples (*n*=24) and only fungi was sequenced from Spring samples (*n*=41) for logistical reasons.

Bioinformatic pipeline and statistical analysis

The bioinformatics analysis involved demultiplexing, quality control, Amplicon Sequence Variant (ASV) calling, and taxonomic classification. The demultiplexed raw reads are primer trimmed and quality filtered using the cutadapt plugin followed by denoising with DADA2 (Callahan *et al.* 2016) (via q2-dada2). Taxonomy was assigned to ASVs using the q2-feature-classifier classify-sklearn naïve Bayes taxonomy classifier (Bokulich *et al.* 2018). Diversity profiling analysis was performed using QIIME 2 2019.7 (Bolyen *et al.* 2019). To assess the multivariate response of microbes to grazing and canopy cover, multivariate permutational analysis of variance was used to model the ASV inter-sample Bray-Curtis dissimilarities as a function of Canopy/Open and Grazed/Ungrazed treatments while accounting for spatial clusters (i.e., blocks) and random spatial site-pairs (shrub Canopy-Open ground pairs). To determine microbial community compositon patterns between grazing and canopy treatments and sampling date, the assigned taxonomy of fungal (at Class level) and bacterial (at Phylum level) ASVs were aggregated to visualise the broad patterns of the read counts, representing the relative abundances, of these taxa at easily distinguishable taxonomic levels. To determine if ASV's are indicative of any multivariate treatment differences, Indicator Species Analysis (De Cáceres and Legendre 2009) was conducted on the read counts, representing the relative abundances, of the fungal and on the bacterial data and the Phylum and Class compositions as a proportion of the indicator species were plotted for visualisation.

Results

Fungal community

There were 4002 fungal amplicon sequence variants (ASVs) identified across 24 Autumn-sampled sites; the most abundant Phyla were Ascomycota and Basidiomycota, by majority, and Chytridiomycota, Mortierellomycota and Rozellomycota. Fungal abundance was higher under Canopy in the Grazed compared to Ungrazed Canopy, whereas in Open sites there was considerably higher fungal abundance in the Ungrazed compared to Grazed Open soils. This result was consistent across the two seasons (**Figure 1**). The multivariate response differed between Canopy and Open sites (*p-value* = 0.001) as well as between the Ungrazed and Grazed sites (*p-value* = 0.03). The same Phyla were rank-ordered most abundant in 3911 fungal ASVs identified across 41 sites sampled in Spring, with the exception that Glomeromycota replaced Rozellomycota as the fifth-ranked taxon. The multivariate ASV abundances differed between Canopy and Open (*p-value* < 0.0001) in Spring, though these fungal community differences were not the same across all surveyed blocks (microsite*block *p-value* = 0.01). Similarly, average differences among the blocks varied between the Ungrazed and Grazed sites (block*grazing (*p-value* < 0.05)).

There were 72 fungi ASVs identified as indicators for differences between Canopy and Open sites and an additional 38 indicator ASVs for Grazing-Ungrazed differences in Autumn. The number of indicator ASVs differed only slightly for the Spring data; 62 Canopy-Open indicators and 35 Grazing-Ungrazed indicators.

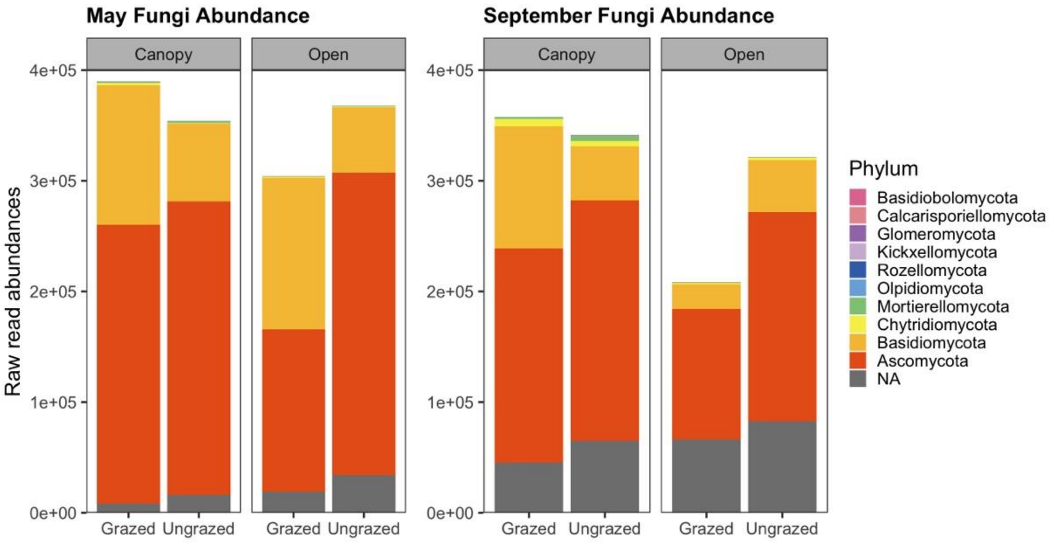

Figure 1. The raw read abundances of fungal Amplicon Sequence Variants (ASV) identified to Phylum across microsites and grazing treatment between sampling periods in May and September in 2023 at Koonamore, South Australia.

Bacteria and archaea community

There were 8412 ASVs (of which 8367 were bacterial and 45 archaeal) identified across 24 Autumn-sampled sites; the most abundant Phyla were Actinobacteria by majority, followed by Chloroflexi, Proteobacteria, Acidobacteria, Gemmatimonadetes, Cyanobacteria, and Firmicutes. The only recorded archaeal Phyla were Thaumarchaeota and Euryarchaeota. Bacterial abundance was higher under Canopy and higher in the Grazed areas compared to Ungrazed (**Figure 3**). The multivariate response differed between Canopy and Open sites (p-value = 0.02) as well as between the Ungrazed and Grazed sites (p-value = 0.006).

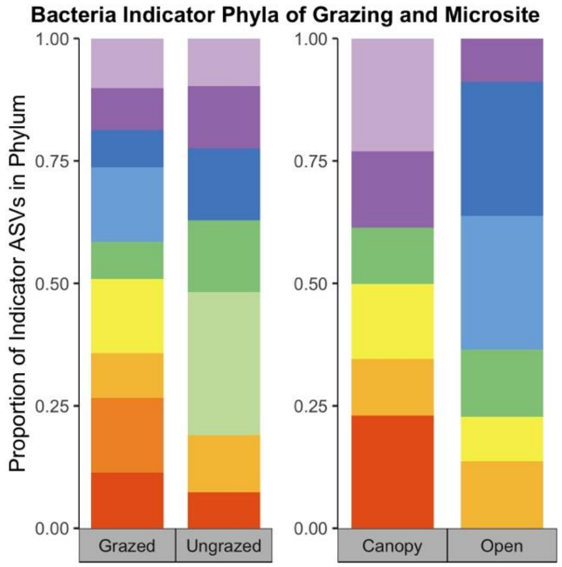

Figure 2. The proportion of bacterial Indicator species classified into Phylum plotted as a proportion of the total assigned indicator species in both grazing and microsites.

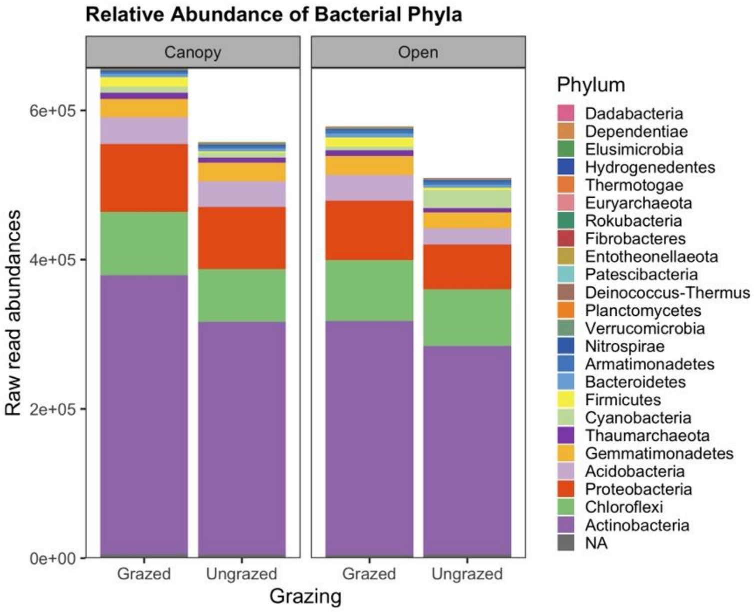

Figure 3. The raw read abundances of bacterial Amplicon Sequence Variants (ASV) identified to Phylum across microsites and grazing treatment in May 2023 at Koonamore, South Australia.

There were 61 fungi ASVs identified as indicators for differences between Canopy and Open sites and an additional 88 indicator ASVs for Grazing-Ungrazed differences in Autumn (**Figure 2**).

Discussion

This study's cross-fence comparison revealed significant differences between livestock Grazed and long-term Ungrazed soil microbiomes, with distinct variation in responses between fertile island soils and soils in open spaces. The fertile island effect was evident for bacterial taxa, with abundances higher in Canopy soils in both Grazed and Ungrazed soils compared to Open soils. Higher abundances of soil bacteria and fungi in fertile islands is well documented throughout both Australian and worldwide drylands, associated with increased substrates and ameliorated conditions under perennial vegetation (Ding and Eldridge 2021; Noy-Meir 1973). However, the distinct fertile island effect was not consistently found in both Grazed and Ungrazed soils for fungal taxa in this study. Specifically, fungal abundances in Ungrazed conditions did not consistently show a fertile island pattern, with Open soil fungal abundance higher than Canopy in May; however in September, a slight fertile island effect could be seen. Compared to the pronounced Canopy-to-Open differences in Grazed soils, it is hypothesised that this response may be due to physical disturbance of biological soil crusts by livestock. Previous studies indicate that fungi are more sensitive to physical disturbance than bacteria and that ungulates can adversely impact Australian dryland soils (Eldridge and Delgado-Baquerizo 2018; Eldridge et al. 2016; Zhang et al. 2016).

In addition to abundance differences, there are significant community composition differences between Grazed and Ungrazed soils that are hypothesised to result in differences in soil function. Bacteria were more abundant in Grazed areas, with that higher abundance mostly found in Canopy soils. Fungi were more abundant in grazed Canopy soils but considerably less abundant in open Grazed areas compared to the Ungrazed overall. For bacterial communities, the most abundant taxa remain similar across grazing treatments and between Canopy and Open, however differences emerge in taxa that form smaller proportions of the overall community. Taxa from the bacterial phylum Firmicutes were more abundant and indicative of Grazed soils, while taxa from phylum Cyanobacteria were more abundant and uniquely indicative of Ungrazed soils. These abundance and community composition shifts may impact the known spatial partitioning of ecosystem services such as nutrient cycling in arid ecosystems (Macdonald et al. 2015). While this study has identified multiple significant compositional and abundance differences, limited knowledge of the functional classifications of these taxa prevents interpretation of the processes leading to these differences without further soil functional analyses.

This study highlights the significant spatial heterogeneity of soil microbiomes, influenced not only by perennial vegetation but also by disturbance. These findings underscore the importance of considering small-scale variation to comprehend the broader impacts of disturbance and recovery in arid ecosystems.

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