



Grazing Regulates the Spatial Heterogeneity of Soil Microbial Communities Within Ecological Networks

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ABSTRACT

Grazing is a major driver of the composition of microbial communities, which play important roles in soil functioning. Mechanisms whereby grazing might regulate the spatial heterogeneity of microbial communities within ecological networks remain largely untested. We used network analysis to identify the impacts of increasing grazing intensity by livestock (cattle, sheep, goats), and native (kangaroos) and wild (rabbits) animals, on the spatial heterogeneity of the relative abundance of eight ecological clusters of co-occurring soil

microbial taxa: four from Grasslands and four from Forests. Grazing effects on microbial spatial heterogeneity were strongly nuanced and depended on (1) plant community type, (2) herbivore type and (3) microbial identity. Microbial within-site spatial heterogeneity was greater in Grasslands than in Forests, and most effects of grazing on microbial spatial heterogeneity were in Forests, effecting three of the four Forest clusters, but only one Grassland cluster. The associations between grazing intensity and microbial heterogeneity were driven indirectly by changes in the spatial heterogeneity of litter cover and soil pH. For Grasslands, we also detected a direct effect of grazing intensity on the heterogeneity of particular microbial groups. Our results indicate that increased grazing intensity will advantage some microbial clusters but disadvantage others. Together, our study provides evidence that grazing intensity regulates the abundance and spatial heterogeneity of microbial communities within ecological networks. Knowing the potential effects of herbivores on different

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microbial clusters can help us predict the likely effects of grazing on soil function. This has important implications for future sustainable management and conservation policies.

Key words: Livestock; Ecological clusters; Microbial communities; Fungi; Bacteria; Soil microbes; Landscape heterogeneity.

INTRODUCTION

Scientists have long realised the importance of landscape and resource heterogeneity in driving ecosystem productivity and stability (Shorrocks and Swingland 1990; Caldwell and Pearcy 1994). For example, soil heterogeneity affects above-ground plant nutrient uptake (de Kroon and Hutchings 1995) and below-ground root structure and growth rates (Jackson and Caldwell 1996). Similarly, variation in the distribution of soil moisture and vascular vegetation at scales ranging from individual organisms to entire communities contributes to this patchy distribution of soil nutrients (Jackson and Caldwell 1993), which generates and maintains plant community diversity and productivity (Reynolds and Haubensak 2009). Significant positive correlations have been identified between soil heterogeneity and plant community diversity and performance, such as recruitment and survival (Lundholm and Larson 2003; Davies and others 2005; Brandt and others 2013). Much less is known, however, about how and why microbial heterogeneity changes across environmental gradients, limiting our understanding of the potential implications of these changes for ecosystem functions. This is fundamental, as microbes are critically important regulators of ecosystem functions and services as broad as plant productivity, nutrient cycling, pollutant degradation and climate regulation (van Elsas and others 2012; Bardgett and van der Putten 2014; Delgado-Baquerizo and others 2016; Eldridge and Delgado-Baquerizo 2018).

Livestock grazing occupies 30% of the world's terrestrial land area and provides millions of people worldwide with milk, meat, fuel, fertiliser, security and transportation (Wirsenius and others 2010) and a fifth of the world's calories. Grazing, however, is an important driver of ecosystem change worldwide, and inappropriate grazing can cause substantial changes in soils and vegetation and therefore resource heterogeneity across multiple contexts and spatial scales (Schlesinger and Pilmanis 1998; Lin and others 2010; Ren and others

2015, Zhao and others 2011). The reduction in perennial grass cover by grazing, for example, can lead to replacement by large shrubs, changing the spatial scale of soil nutrients from relatively random to a scale aligned with the size of the shrubs (Schlesinger and Pilmanis 1998). While we have a relatively good understanding of how global change drivers influence the relative abundance of different microbial communities (Delgado-Baquerizo and others 2014; Maestre and others 2015), little is known about how these drivers affect the heterogeneity in microbial communities and whether this is consistent with the traditional models of disturbance demonstrated in vascular plant communities (for example, Schlesinger and others 1990; Adler and others 2001).

Grazing has also been shown to regulate the diversity and community composition of soil microbial communities in drylands by altering soil chemistry and the relationships among dominant and subordinate microbial taxa (Eldridge and others 2016). Grazing could alter microbial heterogeneity by modifying the distribution of important environmental predictors such as soil pH (Fierer and others 2007), nutrients (Delgado-Baquerizo and others 2017) or plant community attributes such as cover and size (Delgado-Baquerizo and others 2018a, b, c), directly, by herbivory, or indirectly, by altering soil surface morphology (Tongway and Smith 1989). Trampling of vegetation can lead to reductions in plant cover, changes in litter distribution and reductions in resource connectivity (Schlesinger and others 1990; Eldridge and others 2017a, b), which might also alter microbial community composition. Grazing can also alter nutrient pools *via* deposits of urine and faeces (Noble and Tongway 1986), particularly in areas where animals congregate such as night camps or where herbivores such as rabbits (Snedden 1991) or horses (Lamoot and others 2003) use latrines for territory marking. Given the close links among microbial composition, plants and soils, these grazing-induced impacts can lead to changes in the heterogeneity of soil microbes. However, unlike herbivore effects on plant and soil heterogeneity (for example, Adler and others 2001), we know relatively little about how grazing affects the heterogeneity of microbial communities. This lack of knowledge hampers our ability to recommend effective grazing management strategies that lead to better management of terrestrial ecosystems.

Soil microbes live within complex ecological networks, forming ecological clusters of strongly co-occurring species (Delgado-Baquerizo and oth-

ers 2018a, b, c) that share common environmental preferences. Here, we examine how grazing by livestock (cattle, sheep) and native herbivores (kangaroos), and soil and vegetation heterogeneity, influences the heterogeneity of different clusters of co-occurring microbial communities in two contrasting vegetation communities: Forests and Grasslands. Though the links between soil, grazing and vascular plant heterogeneity are well known, how and why the variation in these drivers might affect microbial community heterogeneity remain untested. The link between grazing, environmental heterogeneity and microbial heterogeneity is a key knowledge gap in our understanding of microbial responses to environmental drivers. An improved understanding of the response of microbes to resource heterogeneity generated by grazing will help us to understand whether microbes respond to increased grazing in a similar way to vascular plant communities.

METHODS

Study Area

Our study is based on an ongoing field survey where we sought to understand the impacts of grazing in three of the most common forested communities, and one grassland community, in eastern Australia. The 54 forest sites (hereafter 'Forests') comprised three communities characterised by the dominant trees Blackbox (*Eucalyptus largiflorens*), Red gum (*Eucalyptus camaldulensis*) and White cypress pine (*Callitris glaucophylla*), with 18 sites in each community. Sites spanned the distribution of each community either north–south (Blackbox, Cypress pine) or north, east and west (Red gum). The 30 grassland sites (hereafter 'Grasslands') were sampled in open grassy plains adjacent to riparian areas dominated by snow grasses (*Poa* spp.), wallaby grasses (*Rhytidospermum* spp.) and sedges (*Carex* spp.; Eldridge and others 2019, Appendix S2). Forest sites were sampled in spring and summer 2014 and Grassland sites in spring 2016. The different structure of Grasslands and Forests required slightly different sampling designs.

At each of the 54 Forest sites, we established a 100-m transect and placed a large quadrat (5 m by 5 m) at the 0, 50 and 100 m positions. Within this quadrat, we centrally located a small quadrat (0.5 by 0.5 m). At the 0, 50 and 100 m positions, we then selected four patches: the nearest grass, shrub and tree (and a bare or open patch near these three patches) and collected a sample of soil from the top

5 cm at each of the four patches. This yielded 216 soil samples (54 sites by 4 patches). Within the small quadrats, we counted the dung and pellets of livestock (sheep/goats), kangaroos (*Macropus* spp.) and rabbits (*Oryctolagus cuniculus*) and also counted sheep/goat, kangaroo and cattle dung in the three large quadrats. Dung and pellet counts have been used widely to estimate the abundance of large herbivores, including kangaroos (Johnson and Jarman 1987; Marques and others 2001). For cattle, we counted dung events rather than individual fragments; that is, we considered a number of small fragments to have originated from one dung event, if the fragments were within an area of a few metres. We used previously developed algorithms (Eldridge and others 2017a, b) to calculate the total oven-dried mass of dung per hectare per herbivore for each of the 54 sites based on the density of different pellets recorded in the field. This total oven-dried mass (kg ha^{-1}) of dung was used as our measure of grazing intensity (Figure S1).

For each of the 30 Grassland sites, we established a 200-m transect running parallel to and 10-m away from riparian areas lining small intermittent stream channels. This was to concentrate on areas that are grazed by different herbivores, particularly horses. At each site, we placed five large (5 × 5 m) quadrats at 0 m, 50 m, 100 m, 150 m and 200 m and collected surface soil from the centre of the quadrat to a depth of 5 cm, a layer of soil that supports most of the biological activity in the surface. We counted kangaroo and rabbit pellets in each quadrat and collected all horse dung visible on the soil surface and weighed it on-site using spring balances. Sampling dung with different sized quadrats allowed us to adequately count the large number of small pellets of rabbits and kangaroos with 0.25 m² quadrats in Forests, but also to capture large patches of horse or cattle dung in the 25 m² quadrats in both Forests and Grasslands. Further, a larger (25 m²) quadrat was used to assess plants and litter in Grasslands because individual grassland plants often exceed 1 m in diameter. We followed a similar procedure used for Forests, drying, weighing and calculating the oven-dry mass of rabbits and kangaroo faecal pellets and ten units of horse dung in order to calibrate field measurements. At the 54 Forest sites, we calculated total plant cover and litter cover within 0.5 × 0.5 m quadrats situated within each of the four patch types. At the Grassland sites, plant cover and litter cover were assessed in the five equidistant quadrats (Grasslands) (Figure S2). In general, the heterogeneity in litter cover was greater, plant cover

lower, and soil pH and N similar for Grasslands compared to Forests (Table S1).

Molecular and Soil Analyses

Soil genomic DNA was extracted from 0.5 g of defrosted soil samples that were stored at -20°C , using the DNeasy Powersoil[®] DNA Isolation Kit (QIAGEN Inc., Valencia, CA) according to the manufacturer's instructions. Amplicons targeting the bacterial 16S rRNA gene (341F-805R, Herlemann and others 2011) and the fungal ITS region (FITS7-ITS4R, Ihrmark and others 2012) were sequenced at the Western Sydney University NGS facility (Sydney, Australia) using Illumina MiSeq 2×301 bp (bacteria) or 2×280 bp (fungi) paired end sequencing (Appendix S3). The operational taxonomic unit (OTU) abundance tables were rarefied to an even number of sequences per sample (10,851 and 20,797 sequences for bacteria and fungi, respectively; the minimum number of sequences for a soil sample). Alpha diversity metrics were then calculated using MOTHUR (Schloss and others 2009). Total soil nitrogen (N) was measured using high-temperature combustion in an oxygen stream using a LECO CNS-2000 CNS Analyser (LECO Corporation, St Joseph, MI, USA), and pH was measured in 1:5 soil–water extracts. We did not use carbon in our models because it was highly correlated with N ($r = 0.96$, $P < 0.001$).

Statistical Analyses

We used correlation network ('co-occurrence networks') analysis to identify ecological clusters ('modules') of strongly associated bacteria and fungi according to Delgado-Baquerizo and others (2018a, b, c). In brief, we calculated all pairwise Spearman's (ρ) rank correlations between all taxa (% relative abundance), focussing exclusively on positive correlations because they provide information on species that may respond similarly to soil, plant, climatic, and grazing conditions (Barberán and others 2012). We considered a co-occurrence to be robust if the Spearman's correlation coefficient was greater than 0.25 and P less than 0.01 (see Barberán and others 2012 for a similar approach). This cut-off has a biological meaning, because we only focus on taxa that are significantly strongly co-occurring, which are therefore more likely to interact with each other within a given plant community. The network was visualised with the interactive platform Gephi 0.9.2 (Bastian and others 2009). Default parameters (network resolution = 2.0 in all cases) were then used with the

Gephi interactive platform to identify ecological clusters of the most strongly correlated microbial taxa. We then computed the relative abundance of each ecological cluster by averaging the standardised relative abundances (z -scores) of the taxa from each ecological cluster. Standardising the data allowed us to exclude any effect of merging data from different groups, for example, fungus vs bacterium.

We then used structural equation modelling (SEM) to build a system-level understanding of the effects of mean levels of grazing (livestock and kangaroos) and the heterogeneity in plants (plant cover, litter cover) and soils (pH, N) on the heterogeneity in microbial clusters. Our aim was not to compare databases of Forests and Grasslands directly, but to evaluate the role of grazing in controlling microbial heterogeneity in two independent systems and databases. Heterogeneity of predictor variables was calculated as the coefficient of variation (CV%) in plant and soil attributes and heterogeneity in ecological clusters calculated as the CV of relative abundance. Our aim was to include different forest types so that we could maximise our chances of obtaining wide gradients of within-site microbial and soil heterogeneity, which we then relate to different environmental conditions. Our *a priori* model (Appendix S4) predicted that increased grazing, and heterogeneity in the soils and plants, would have direct effects on ecological clusters, separately, and that there would be a number of indirect effects, where grazing indirectly influences ecological clusters of microbes by affecting the heterogeneity of either plants (Adler and others 2001) or soils (Brandt and others 2013). Grazing is known to have direct effects on microbes (Eldridge and others 2017a, b) and is likely to have indirect effects by altering soil pH or litter cover (Delgado-Baquerizo and others 2018a, b, c), thereby altering the balance of microbes with copiotrophic and oligotrophic lifestyles (Fierer and others 2007).

Hypothesised pathways in our *a priori* model were compared with the variance–covariance matrix of our data in order to calculate an overall goodness-of-fit using the χ^2 statistic. The goodness-of-fit test estimates the likelihood of the observed data given the *a priori* model structure. Thus, high probability values indicate that models are highly plausible causal structures underlying the observed correlations. Before fitting empirical data to our *a priori* model, we examined the univariate correlations among all variables and standardised (z -transformed) the data. The stability of the resultant models was evaluated as described in Reisner and others (2013). Analyses were performed using the

AMOS 22 (IBM, Chicago, IL, USA) software. After fitting our empirical data to the a priori model (Appendix S4), we interpreted a good model fit as one with a low χ^2 , high Goodness of Fit Index [GFI] and high Normal Fit Index [NFI]).

RESULTS

We identified eight ecological clusters formed by bacterial and fungal taxa strongly co-occurring within the microbial correlation network (Figure 1A). Four of these ecological clusters (C0, C4, C5 and C6) were found almost exclusively in Forest sites and the other clusters (C1, C2, C3 and C7) exclusively in Grasslands (Figure 1B). Overall, the ecological clusters were formed by phylotypes belonging to similar phyla, suggesting that any effects of grazing on microbial clusters are not phylogenetically conserved; that is, clusters are unrelated to a single group of organisms, but affect phylotypes from multiple phyla (Figure 2A). A list including the membership of each soil phylotype within ecological clusters can be found in Appendix S5. Four bacterial and fungal phyla (Actinobacteria, Ascomycota, Proteobacteria and Acidobacteria) contributed between 40 and 80% of the relative abundance of all ecological clusters. Forests and Grasslands differed slightly in their composition, with Actinobacteria relatively more abundant in Forests, and Verrucomicrobia found almost exclusively in Grasslands (Figure 2A). Basidiomycota

were relatively more abundant in ecological cluster C6 and Ascomycota more abundant in ecological cluster C7 (Figure 2A). For fungal lifestyles, ectomycorrhizal fungi were relatively abundant across all ecological clusters, particularly in Grasslands, yet dominated the Forest ecological cluster C6 (Figure S6). In Forest sites, ecological clusters C4 and C6 were less variable (lower heterogeneity) than ecological clusters C0 and C5 (Figure 2B). Heterogeneity of Grassland clusters was greater than 70% (Figure 2B).

Given the strong differences in the relative abundance of ecological clusters between the Grasslands and Forests (ANOVA; $P < 0.001$), we analysed our dataset independently for both ecosystem types in downstream analyses.

We then used SEM to examine potential direct and indirect associations between grazing and microbial heterogeneity *via* changes in the spatial heterogeneity of key environmental factors such as soil pH and N, and plant cover and litter cover. We found that the effects of grazing on within-site spatial heterogeneity of the relative abundance of ecological clusters were driven by different herbivores and environmental drivers, with four major effects. First, any effects of grazing intensity on microbial heterogeneity were indirectly regulated *via* changes in the spatial heterogeneity of plants (plant cover, litter cover) or soil chemistry (pH, N; Table S1). Second, the effects of variation in plants, soils and grazing intensity differed among the

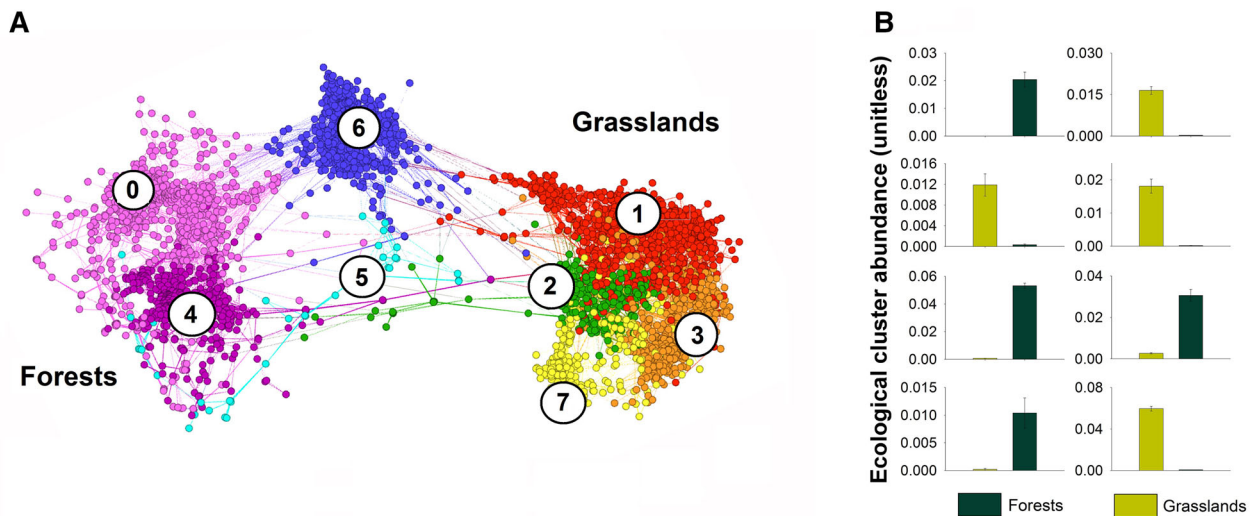


Figure 1. (A) Correlation network for Forest and Grassland sites analysed together, and histograms of the relative abundance of different ecological clusters. Links between nodes are based on correlations and illustrate the potential interaction among taxa. (B) Ecological cluster abundances for Grasslands and Forests for each of the eight nodes (0–7) from A.

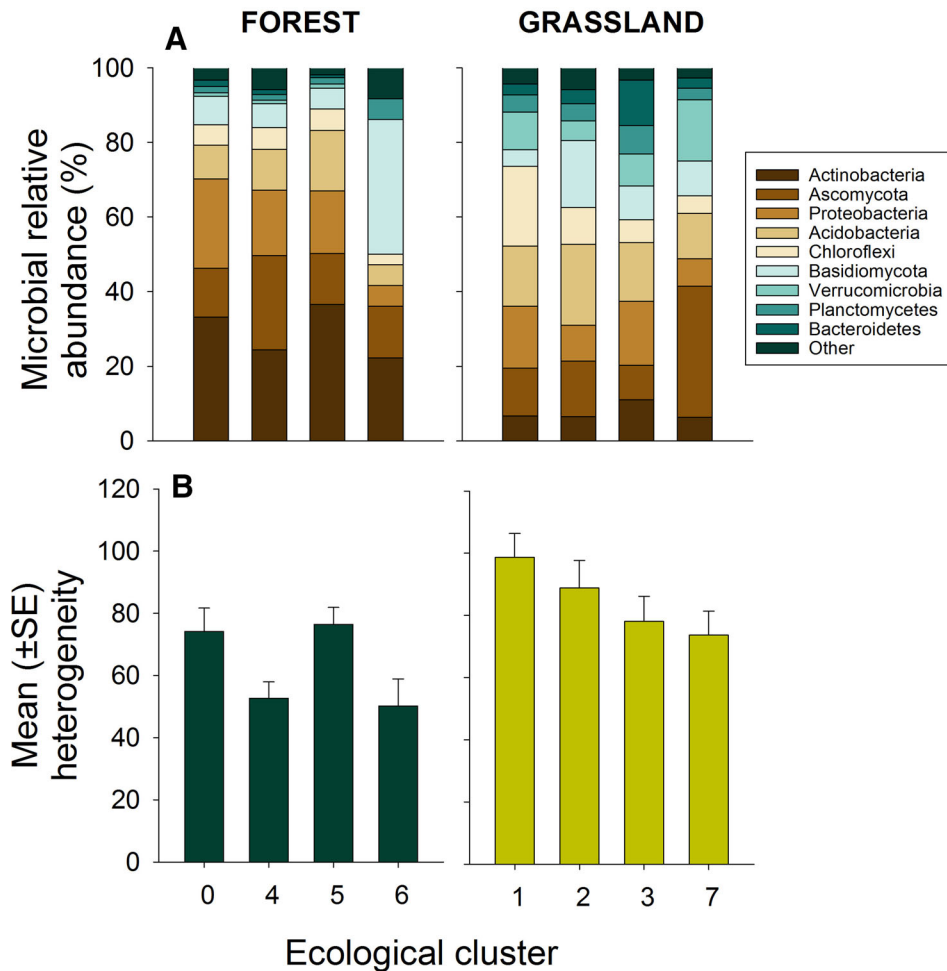


Figure 2. (A) Relative abundance (%) of the major bacterial and fungal phyla and (B) mean (\pm SE) heterogeneity (CV%) of the relative abundance of microbial communities for the eight ecological clusters.

ecological clusters. For example, increasing heterogeneity in litter cover was associated with reductions in the heterogeneity of ecological cluster C4 (Figure 3A) but increases in ecological cluster C5 (Figure 3B). Sites with a more variable plant cover were associated with a less variable ecological cluster C2 (Figure S7), and sites with more variable soil pH were associated with a less variable ecological cluster C6 (Figure 3C). Increased intensity of livestock grazing was negatively correlated with ecological clusters C7 directly (Figure 3D) and C5 indirectly (Figure 3B). Increasing kangaroo grazing intensity was positively correlated with heterogeneity of C6 (both directly and indirectly; Figure 3C).

Third, grazing had substantial effects on the heterogeneity of microbial communities. Increasing intensity of livestock grazing suppressed the negative effect of increased heterogeneity of litter on

ecological cluster C4 (Figure 3A) but had the opposite effect on ecological cluster C5 (Figure 3B). Thus, increasing grazing promoted a more variable community of ecological cluster C4 but a more homogeneous community of ecological cluster C5. Similarly, increasing intensity of kangaroo grazing suppressed the negative effect of a more variable distribution of soil pH on ecological cluster C6 (Forest; Figure 3C) and suppressed the positive effect of a more variable cover of litter on ecological cluster C7 (Grassland, Figure 3D). Finally, grazing had no significant direct or indirect effects on the heterogeneity of ecological clusters C0 (Forest), C1, C2 or C3 (Grasslands; Figure S7).

Although not the main goal of this work, we also detected associations between grazing intensity and the average relative abundance of ecological clusters within the ecological network. Our results were relatively similar to those for environmental

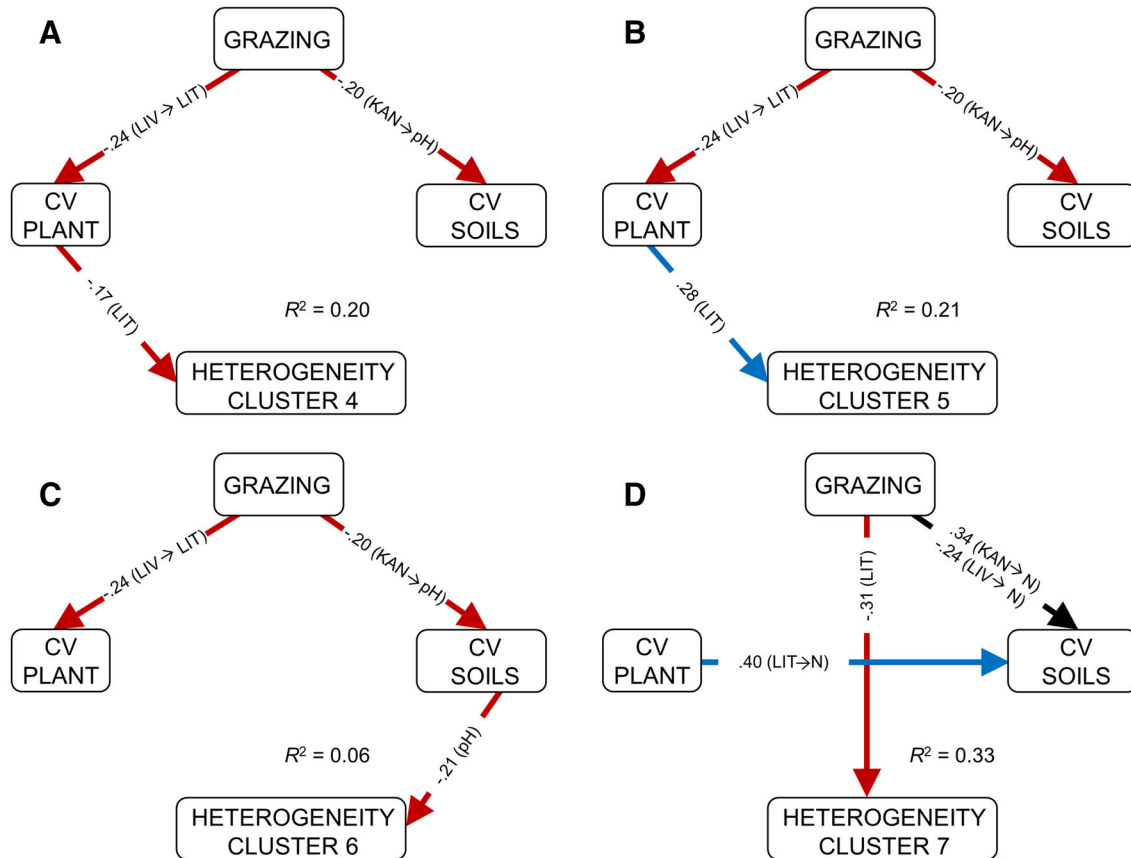


Figure 3. Structural equation models for the within-site heterogeneity (CV%) in the relative abundance of ecological clusters C4, C5 and C6 (Forest sites) and C7 (Grassland site) in relation to livestock (LIV) and kangaroo (KAN) grazing, and the within-site heterogeneity (CV%) in plant cover (COV), litter cover (LIT), soil pH (pH) and soil nitrogen (N). Standardised path coefficients, embedded within the arrows, are analogous to partial correlation coefficients and indicate the effect size of the relationship. Red, blue and black arrows indicate negative, positive and mixed relationships, respectively. The proportion of variance explained (R^2) is shown in each figure. Only significant pathways are shown in the models. Model fit: Forest sites: $\chi^2 = 0.44$, $df = 1$, $P = 0.51$, NFI = 0.965; Grassland sites: $\chi^2 = 0.52$, $df = 1$, $P = 0.47$, NFI = 0.923.

heterogeneity in that effects of grazing were indirectly mediated by changes in average levels (rather than spatial heterogeneity) of soil and plant attributes, and the results varied among different ecological clusters. For example, both livestock and kangaroos were associated with the relative abundance of ecological cluster C0 (Figure S8a), positively associated with the relative abundance of C5 and C6 via changes in lower soil pH (Figs. S8c and S8d). Kangaroos were also associated with a reduction in the relative abundance of ecological cluster C5 (Figure S8c), and increases in the relative abundance of ecological cluster C4 *via* reduced litter cover levels (Figure S8b). As with heterogeneity data, there were no grazing effects on the ecological clusters C1, C2, C3, but a direct, and therefore unexplained negative effect of kangaroos on ecological cluster C7 (Figure S9).

DISCUSSION

Our work provides evidence that grazing intensity regulates the spatial heterogeneity and relative abundance of ecological clusters of microbial communities within ecological networks directly, but also indirectly, *via* changes in the heterogeneity or absolute values of important plant and soil attributes. Moreover, our results indicate that any effects of increasing grazing intensity on ecological clusters vary among ecosystems (Grasslands *cf.* Forests), herbivores, and the identity of microbial clusters. Most of the effects of livestock were in Forest systems where increased grazing intensity promoted either a more heterogeneous or homogeneous community of microbes, depending on the identity of the cluster, by altering the heterogeneity of either litter cover levels or soil pH. The hetero-

generality of microbial clusters within Grassland sites, which were more variable in their relative abundance of microbial clusters, was also less influenced by grazing intensity. Our results are consistent with the notion that increased livestock grazing alters the heterogeneity of microbial communities, analogous to the known effects of livestock grazing on the heterogeneity of vascular plant communities (Nunes and others 2018) and soil nutrient pools (Schlesinger and Pilmanis 1998), with important implications for the management of terrestrial ecosystems globally.

One of the major findings of our work is that the effects of grazing on microbial spatial heterogeneity were context dependent. First, our results varied between plant communities, with grazing intensity associated with multiple changes in spatial heterogeneity of all but one Forest cluster, but only one Grassland cluster. Thus, increased heterogeneity in Grasslands may have buffered any effects of grazing. Greater microbial heterogeneity in Grasslands could relate to the substantially greater plant cover, double the levels of soil N, and three times the level of grazing intensity as Forests. The effects of grazing on heterogeneity are also known to depend on the distribution of herbivores, particularly how herbivores interact with vegetation structure (Adler and others 2001). Livestock in Grasslands are free-ranging and tend to graze along linear riparian areas, which is very different from grazing in Forest sites, where herbivores are restricted to paddocks and centred on human-constructed watering points (Andrew 1988). The substantially greater mass of dung in Grasslands may also account for the greater relative abundance of saprotrophic genera such as *Trichoderma* spp. and animal pathogens such as *Metarhizium* in ecological cluster C7 (Figure S6).

Our results also varied with the identity of the specific clusters. For example, for the Forest communities where grazing effects were most apparent, increasing livestock grazing increased the heterogeneity in the relative abundance of ecological cluster C4, reduced cluster C5, but had no effects on clusters C0 or C6. Livestock grazing has been shown to reduce the variability in litter cover (Daryanto and Eldridge 2010), most likely due to scattering by hoof action and subsequent movement by wind or water (Li and others 2009), effectively reducing a larger number of small litter patches to a few large patches (Daryanto and Eldridge 2010). Changes in litter distribution are also associated with reductions in soil surface health by reducing sites for the infiltration of water and potential microsites for seedling establishment.

Litter cover is linked to soil organic matter and plant productivity (Tongway and Smith 1989) and would therefore be expected to promote a greater relative abundance of copiotrophic microbes that prefer more fertile niches with higher levels of soil carbon than oligotrophs. For example, ecological cluster 4, which was negatively associated with litter, indirectly driving grazing effects, included phylotypes from genus *DA101* and *Geodermatophilus obscurus* that are dominant taxa found in drylands and low-productivity systems worldwide (Delgado-Baquerizo and others 2018a, b, c) and commonly associated with oligotrophic lifestyles (Bergmann and others 2011). It also contained *Rubrobacter*, a thermophilic genus that has been isolated from arid soils in Australia (Holmes and others 2000), and which would explain the negative relationship with litter cover (Figure 3A). *Candidatus koribacter* and *Candidatus solibacter*, which were abundant in ecological cluster 5, have been shown to have an affinity with relatively acidic, nutrient-poor soils (Jeanbille and others 2015). This would explain the positive relationship of this cluster with litter cover, primarily from trees, whereby microbial respiration and additions of organic acids would reduce soil pH (for example, Finzi and others 1998). Of special note, ecological cluster 5 included globally distributed dominant taxa such as genus *C. solibacter*, *Mycobacterium*, *Nocardioidea*, *Bradyrhizobium*, *Phenyllobacterium* and *Rhodoplanes*, which have been reported to be highly associated with acid conditions in soils across the globe (Delgado-Baquerizo and others 2018a, b, c).

The relationship between livestock and litter contrasts with that of kangaroos, which were associated with reduced soil pH. For example, increasing kangaroo grazing corresponded to increased heterogeneity of ecological cluster 6 directly, and indirectly, *via* the suppressive effects of increasing variability in soil pH (Figure 3C). Not only was kangaroo grazing associated with more homogeneous pH, but also reduced average soil pH levels (Figure S8). The direct positive association of Actinobacteria with increased kangaroo grazing and the negative relationship between kangaroos and soil pH likely relate to an association between greater kangaroo grazing intensity in the eastern part of the study area where the soils tend to have slightly lower pH values, thereby representing an associational effect with pH. Our pH results are consistent with evidence showing that soil pH is a major driver of microbial communities (Lauber and others 2009), but also suggest that other factors, such as the spatial heterogeneity of resource availability, influence microbial heterogeneity in

terrestrial environments. Ecological cluster 6 contained a mixture of bacteria, principally Actinobacteria, and basidiomycotal fungi. Actinobacteria play important roles as symbionts and pathogens in plant-associated microbial communities and are known to be suppressed by increasing pH. The Actinobacterial genus *Solirubrobacterales*, which was an abundant component of ecological cluster 6, has secondary metabolites and antibodies that allow it to withstand water loss and desiccation and survive in areas of high radiation (Rampelotto and others 2013).

Taken together, our findings provide novel evidence that grazing regulates the spatial heterogeneity of microbial communities by altering the spatial heterogeneity of fundamental environmental drivers of soil microbes such as soil pH and resource availability. However, our results also indicate that grazing effects on the spatial heterogeneity of soil microbes are context dependent and vary between herbivores and ecosystems types (that is, Forest or Grassland) and with the identity of microbial clusters. Thus, unlike vascular plant communities where grazing typically homogenises plant communities (Adler and others 2001), microbial responses to grazing are strongly nuanced, with a mixture of negative and positive effects, mediated indirectly by changes in litter cover and soil pH, which are known to be strong drivers of microbial function. A one-size-fits-all approach to grazing and microbial community structure is therefore not supported by our analyses.

Global demand for livestock products is likely to double by 2050 (Rojas-Downing and others 2017) to feed a growing global human population, placing greater stress on an already declining agricultural land base. This is likely to have important implications for microbial communities and the processes that they mediate. A knowledge of the potential effects of different herbivores on different ecological clusters of microbes can help us to improve our capacity to predict how land use change and intensification associated with grazing might lead to changes in microbes and therefore soil function.

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DATA AVAILABILITY

Data are available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.94kq1>.

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