Competition drives the response of soil microbial diversity to increased grazing by vertebrate herbivores

Article *in* Ecology · April 2017

DOI: 10.1002/ecy.1879

CITATIONS
3

READS
214

7 authors, including:

David J Eldridge
UNSW Sydney
220 PUBLICATIONS **6,283** CITATIONS
SEE PROFILE

Manuel Delgado-Baquerizo
University of Colorado Boulder
103 PUBLICATIONS **1,584** CITATIONS
SEE PROFILE

Ian Oliver
University of New England (Australia)
53 PUBLICATIONS **3,094** CITATIONS
SEE PROFILE

Kelly Hamonts
Western Sydney University
84 PUBLICATIONS **333** CITATIONS
SEE PROFILE

Ecology, 98(7), 2017, pp. 1922–1931

Competition drives the response of soil microbial diversity to increased grazing by vertebrate herbivores

David J. Eldridge,[1,2,8] Manuel Delgado-Baquerizo,[3] Samantha K. Travers,[2] James Val,[4] Ian Oliver,[5,6] Kelly Hamonts,[3] and Brajesh K. Singh[3,7]

[1]*Office of Environment and Heritage, c/o School of Biological, Earth and Environmental Sciences, University of New South Wales, Sydney, New South Wales 2052 Australia*
[2]*Centre for Ecosystem Science, School of Biological, Earth and Environmental Sciences, University of New South Wales, Sydney, New South Wales 2052 Australia*
[3]*Hawkesbury Institute for the Environment, Western Sydney University, Penrith, New South Wales 2751 Australia*
[4]*Office of Environment and Heritage, P.O. Box 363, Buronga, New South Wales 2739 Australia*
[5]*Office of Environment and Heritage, University of New England, P.O. Box U221, Armidale, New South Wales 2351 Australia*
[6]*School of Environmental and Rural Sciences, University of New England, Armidale, New South Wales 2350 Australia*
[7]*Global Centre for Land-Based Innovation, Western Sydney University, Penrith, New South Wales 2751 Australia*

Abstract. Scientists have largely neglected the effects of grazing on soil microbial communities despite their importance as drivers of ecosystem functions and services. We hypothesized that changes in soil properties resulting from grazing regulate the diversity of soil microbes by releasing/suppressing subordinate microbial taxa via competition. To test this, we examined how intensity of vertebrate herbivores influences the diversity and composition of soil bacteria and fungi at 216 soil samples from 54 sites across four microsites. Increasing grazing intensity reduced soil carbon, suppressing the dominant bacterial phylum Actinobacteria (indirectly promoting bacterial diversity) and increasing the dominant fungal phylum Ascomycetes (indirectly reducing fungal diversity). Our data provide novel evidence that grazing modulates the diversity and composition of soil microbes via increases or reductions in competition by dominant taxa. Our results suggest that grazing can potentially alter soil function by altering microbial community composition, providing a clear link between grazing management, carbon availability and ecosystem functions.

Key words: bacteria; competitive exclusion; fungi; grazing; herbivore activity; livestock; soil function; woodland.

Introduction

The livelihoods of many people depend on the goods and services provided by domestic livestock and wild herbivores. Livestock grazing is one of the most extensive land uses on Earth and provides meat, milk, fiber and hide, transport, dung for fuel and fertilizers, and the potential to accumulate capital for millions of people worldwide (Mannetje 2002). However, grazing is also a major disturbance that affects plant and animal communities, reduces ecosystem functions, and leads to the global degradation of soils (Diaz et al. 2013). The management of grazing is therefore critically important, allowing society to balance the competing needs of humans for food and fiber with the need to support essential ecosystem services and provide habitat for biota.

Grazing by livestock occupies about half of the land area of the globe, and has important implications for sustaining wildlife and human populations (Eldridge and Delgado-Baquerizo 2016). However, overgrazing by livestock has been reported to reduce plant diversity and biomass, and soil function (Cerdà and Lavee 1999, Eldridge et al. 2011, 2016*a*) and is probably the most pervasive and significant degrading process in rangelands. Little is known, however, about how soil microbial communities respond to grazing by domestic livestock and wild herbivores (Macdonald et al. 2015). Soil microbes are important components of healthy systems because they play critical roles in maintaining multiple ecosystem functions including litter decomposition, primary production, soil nutrient supply, and soil and plant fertility (van der Heijden et al. 2008, Bardgett and van der Putten 2014, Jing et al. 2015, Delgado-Baquerizo et al. 2016). Increasing our understanding about how grazing alters soil microbial communities is critical if humans are to maintain essential ecosystem functions that will ensure that we have the capacity to feed an ever expanding global population (Steinfeld et al. 2006).

Microbial diversity and composition have been shown to be strongly influenced by changes in environmental variables such as climate, vegetation, soil pH and carbon (Fierer et al. 2007, Siciliano et al. 2014, Tedersoo et al. 2014, Delgado-Baquerizo et al. 2016). Thus, changes in soil properties (e.g., carbon and pH) and biotic components (e.g., plant richness) resulting from grazing by

Manuscript received 13 December 2016; revised 5 March 2017; accepted 19 April 2017. Corresponding Editor: Matthias C. Rillig.
[8] E-mail: d.eldridge@unsw.edu.au

domestic livestock and wild herbivores may indirectly affect the diversity and composition of microorganisms. In particular, we suggest that grazing may alter the diversity of soil fungi and bacteria, consistent with the principle of "competitive exclusion," which occurs when competition among species results in the elimination of one species from a given habitat because species compete for the same resources or niche, and one will be at least slightly more efficient at exploiting these resources than the other. This gives the more efficient species a competitive advantage and greater fitness, leaving the subordinate species to either decline or evolve (Hibbing et al. 2010). The principle of competitive exclusion has been used widely to explain how herbivore impacts might lead to increases in vascular plant diversity. Grazing-induced herbivory can reduce the biomass of dominant, highly productive and often nutrient-poor grasses and forbs. This promotes the competitive release of smaller, ephemeral plant species that would normally be outcompeted, resulting in increased plant richness (Milchunas et al. 1988, Noy-Meir et al. 1989, Collins et al. 1998, Frank 2005). We invoke the principle of competitive exclusion for explaining processes in microbial communities for three reasons. First, competitive exclusion has been reported previously for microbial communities. For example, Pérez-Valera et al. (2017) showed that wildfire, a substantial driver of ecosystem disturbance, resulted in the competitive exclusion of bacteria via closely related species competing and excluding one another. Second, microbes are highly resource selective, using similar and specific resources such as carbon or nitrogen, or by more rapidly using up existing resources. Availability of these specific resources drives the relative abundance of specific taxa (Cherif and Loreau 2007), similar to processes operating in vascular plants. Thus changes in these resources should alter their relative abundance and therefore function. Finally, the potential pool of microbes and microbial competitors is vast, such that small changes in environmental conditions such as soil pH would be expected to result in substantial changes in microbial community composition (Trivedi et al. 2016).

Herein, we posit that the effects of grazing on soil microbial diversity and composition are analogous to the effects of grazing on the aboveground vascular plant community, i.e., by altering species composition via changes in soil properties. We expected that grazing would release subordinate microbial taxa (bacteria and fungi) from competitive exclusion by altering the relative abundance of the dominant microbial taxa in dryland soils, ultimately increasing their diversity. Specifically, we predicted that this mechanism would operate via an effect of grazing on soil carbon and pH, likely due to grazing disturbance (McSherry and Ritchie 2013), altering the relationships among microbes favored by different nutritional strategies, i.e., high or low carbon environments (sensu Trivedi et al. 2012, Maestre et al. 2015). We applied a systems-based approach, using structural equation modelling (SEM), to examine the direct and indirect effects of grazing on above- and belowground communities, via vascular plant cover and species richness, soil pH and soil carbon.

To test our hypothesis, we examined the effects of increasing intensity of grazing by domestic livestock (cattle, sheep/goats) and wild herbivores (kangaroos and rabbits) on microbial community composition in three woodland communities across an area of 0.3 million km^2 in eastern Australia. Because grazing effects are most noticeable in water-limited environments, any grazing effects on microbial communities would likely be felt most strongly in drylands. Drylands are the largest biome on Earth, occupying about 45% of the area of the globe and supporting 38% of its human population (Maestre et al. 2016). Many of these drylands rely on livestock grazing or the harvesting of wild herbivores to support their livelihoods, either under traditional or commercial management systems. Overall, we know very little about how microbial communities respond to grazing and the likely functions that are altered when environments are overgrazed. This lack of knowledge hampers our ability to manage drylands effectively. The need for such information will become more pressing as we move to a world where the climate is drier, more variable, and the effects of grazing, particularly overgrazing, are more pronounced.

Methods

Study sites and design

The study was undertaken in three woodland communities in semi-arid eastern Australia (see Appendix S1). The sites were characterized by the dominant trees Blackbox (*Eucalyptus largiflorens*), River red gum (*Eucalyptus camaldulensis*) and White cypress pine (*Callitris glaucophylla*). In each community we examined 18 sites (total N = 54) which spanned the distribution of the community, with six sites in the north, south, and central regions of each community (north, east, and west for River red gum).

At each site we established a 100 m transect. At three points along this transect (0, 50, 100 m), we: (1) selected the nearest of three patch types (grass, shrub, tree) and then selected a bare patch (open) central to these; (2) established a large quadrat (5 × 5 m) within which was nested a small quadrat (0.5 × 0.5 m) along the transect. We collected soil from each of the four patches (grass, tree, shrub, open) from each of the three small quadrats and pooled the quadrat samples at the site level to give a total of 216 soil samples (Appendix S2). Soil was collected from the uppermost 5 cm layer where we would expect biological activity and nutrients to be greatest. Full floristic surveys, including cover and estimated abundance, were conducted in 0.5 m^2 circular plots located within each patch type. These data were used to obtain a value of total plant richness and average plant cover for each microsite per site.

Within the small quadrats we counted the dung/pellets of livestock (sheep/goats), macropods (kangaroos; *Macropus* spp.) and lagamorphs (European rabbit; *Oryctolagus cuniculus*). Additional counts of sheep/goats, kangaroos, and cattle dung were also made in 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 et al. 2001). For cattle, we counted dung events rather than individual fragments, i.e., we considered a number of small fragments to have originated from one dung event, if the fragments were within an area of a few meters. We used algorithms, developed previously for the study area, to calculate the total oven-dried mass of dung per hectare per herbivore based on the number of pellets recorded in the field. This total oven dried mass of dung was used as our measure of recent grazing intensity (Eldridge et al. 2016*b*). The total cross-sectional area of livestock tracks crossing the transect at each site was used as our measure of historic livestock grazing.

Molecular analyses

Soil DNA was extracted from 0.5 g of defrosted soil samples using the Powersoil DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, California, USA) according to the manufacturer's instructions. Amplicons targeting the bacterial 16S rRNA gene (341F-805R, Herlemann et al. 2011) and the fungal ITS region (FITS7-ITS4R, Ihrmark et al. 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. 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 et al. 2009).

Microbial sequencing

After visual assessment of the quality of all Illumina R1 and R2 reads using FastQC (Andrews 2010), low quality regions ($Q < 20$) were trimmed from the 5′ end of the sequences (1 bp from R1 and 22 bp from R2 for primer set 341F/805R; 5 bp from R1 and 55 bp from R2 for primer set FITS7-ITS4R) using SEQTK (https://github.com/lh3/seqtk). The paired ends were subsequently joined using FLASH (Magoc and Salzberg 2011). Primers were removed from the resulting sequences using SEQTK and a further round of quality control was conducted in MOTHUR (Schloss et al. 2009) to discard short sequences (<380 bp for primer set 341F-805R; <150 bp for primer set FITS7-ITS4R), as well as sequences with ambiguous characters or more than 8 homopolymers. Operational taxonomic units were built at 97% sequence similarity using UPARSE (Edgar 2013). Singletons were discarded, as well as chimeric sequences identified by the UCHIME algorithm using the recommended SILVA gold 16S rRNA gene or UNITE reference databases for bacteria and fungi, respectively (Edgar et al. 2011). Operational taxonomic unit abundance tables were constructed by running the usearch_global command (http://www.drive5.com/). Taxonomy was assigned to OTUs in MOTHUR using the naïve Bayesian classifier (Wang et al. 2007) with a minimum bootstrap support of 60% and the Greengenes database version 13_8 (McDonald et al. 2012) for bacteria or the dynamic UNITE version 6 dataset (Kõljalg et al. 2013) for fungi.

Laboratory analyses and aridity

Total carbon was measured using high temperature combustion in an oxygen stream using a LECO CNS-2000 CNS Analyser (LECO Corporation, St Joseph, Michigan, USA) and pH measured in 1:5 soil-water extracts. We measured the concentrations of four enzymes (β-glucosidase, β-D-cellobiosidase, *N*-acetyl-β-glucosaminidase, phosphatase) that are proxies for carbon, nitrogen and phosphorus degradation, respectively. Enzyme activities were measured by fluorometry using 1.00 g of soil, as described in Bell et al. (2013). The FAO Aridity Index (AI) was used because we would expect it to be a significant driver of microbial communities (Maestre et al. 2015). Aridity was calculated as 1 − AI, where AI = precipitation/potential evapotranspiration using FAO's global aridity map (http://data.fao.org/en/map).

Statistical analyses

We used SEM (Grace 2006) to test whether the competitive exclusion principle, linked to changes in soil properties as a consequence of grazing, was an important driver of microbial richness in terrestrial ecosystems. We specifically used SEM so that we could evaluate both the direct and indirect effects of our endogenous variables grazing, vascular plant cover and richness, soil pH, soil carbon, aridity, and different microsites (tree, shrub, grass) and the two dominant fungal and bacterial phyla on bacterial and fungal community richness based on the number of OTUs and to estimate the strengths of these multiple effects. This is particularly important in grazing studies because grazing has both direct effects on microbial communities, for example, by influencing soil chemistry through additions of dung and urine, or indirectly, by removal of plant species and plant biomass (herbivory).

We first developed an a priori model (Appendix S3) based on the known effects and relationships among the main drivers and our bacterial and fungal exogenous variables, and standardized (z-transformed) the endogenous variables, where necessary, after examining their distribution and testing their normality. The three

microsites were treated as categorical exogenous variables with two levels (0 or 1). This approach allowed us to compare the effect of a particular microsite (e.g., Tree) on richness or the relative abundance of the main bacterial or fungal phylum compared with the average of the remaining microsites (e.g., open areas + grasses + shrubs). We used open areas as a procedural control, so this microsite was not explicitly considered in our models. In all of our models, grazing was depicted as a composite variable that represented the combined effects of recent livestock (cattle dung, sheep/goat dung) and other herbivore (kangaroo dung, rabbit dung) grazing and historic livestock grazing (cattle and sheep/goat tracks). The use of composite variables does not alter the underlying SEM model, but collapses the effects of multiple, conceptually related variables into a single combined effect, aiding the interpretation of model results (Grace 2006). We also calculated the standardized total effects (STE) of grazing on bacterial and fungal richness, and the relative abundance of the two most abundant taxa in Bacteria (Phylum Actinobacteria, Class α-Proteobacteria) and fungi (Phyla Ascomycetes and Basidiomycetes). The STEs are the sum of all direct and indirect pathways from grazing to the exogenous variable, and provide a measure of the net influence that the five attributes comprising the composite variable "grazing" have on our exogenous variables and most abundant bacterial/fungal phyla. Importantly, it allows us to identify which of the five grazing attributes contribute most to bacterial/fungal richness or relative abundance. We found that the relationships between bacterial/fungal richness and the relative abundance of Actinobacteria/α-Proteobacteria and Ascomycetes/Basidiomycetes, respectively, were best described by second-order polynomials (Fig. 1). To introduce polynomial relationships into our models, we constructed a composite model, as described earlier, using both the relative abundance and the square of relative abundance of the four main bacteria or fungi, in our models.

Our *a priori* model was compared with the variance-covariance matrix of our data to allow us to estimate 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 these models are highly plausible causal structures underlying the observed

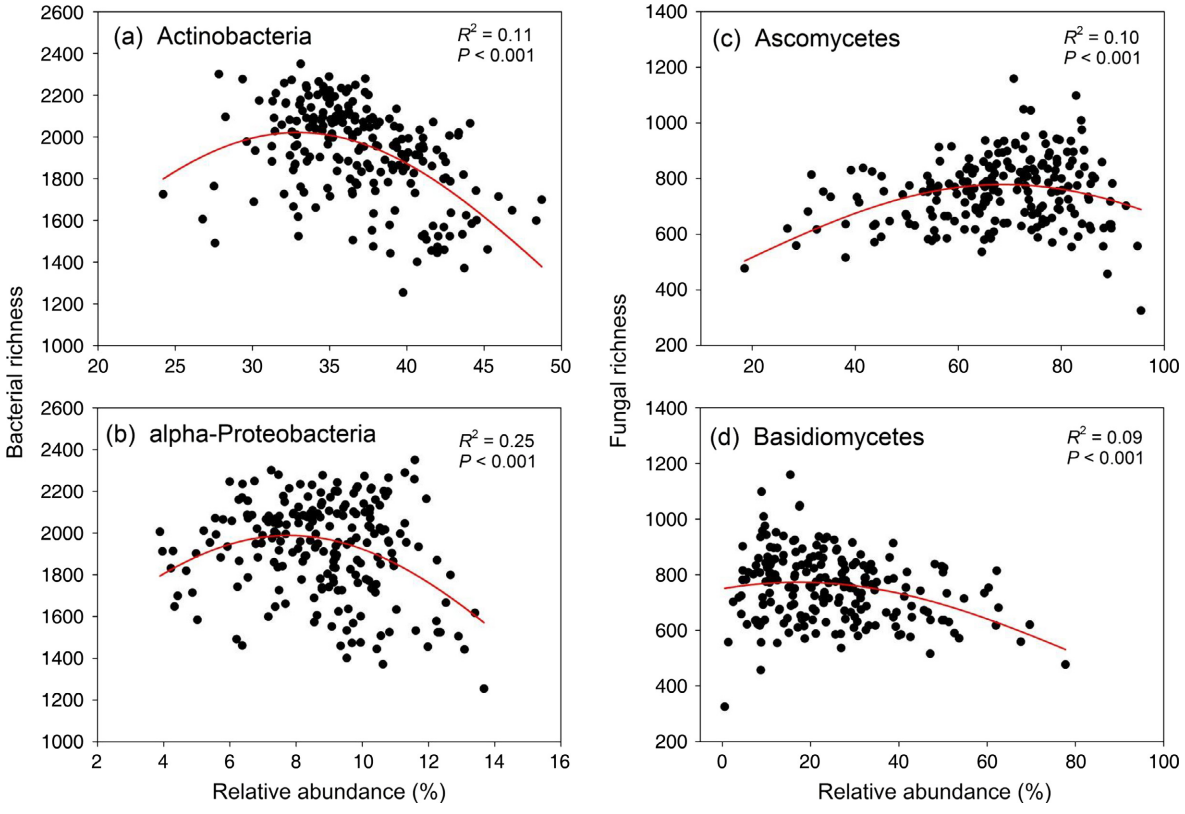

FIG. 1. Plots of relative abundance of the two most common bacterial groups: phylum Actinobacteria and Class α-Proteobacteria, and the most common fungal phyla (Ascomycetes, Basidiomycetes) in relation to bacterial and fungal operational taxonomic unit richness, respectively. The solid line represents the fitted linear regression for the quadratic model. The best fit for quadratic *cf.* linear models was determined using the Akaike information criterion (AIC). Models with an ΔAIC > 2 were considered to be different, with a better model having a lower AIC (see Delgado-Baquerizo et al. 2016 for a similar approach). Akaike information criterion for quadratic cf. linear relationships for each panel are as follows: (a) 2,849.47 vs. 2,867.91; (b) 2,882.96 vs. 2,900.95; (c) 2,620.53 vs. 2,636.10; and (d) 2,624.83 vs. 2,628.97. [Color figure can be viewed at wileyonlinelibrary.com]

correlations. Models, those with low χ^2, high Goodness of Fit Index, high Normal Fit Index and low root mean error of approximation (RMSEA < 0.05) were interpreted as showing the best fit to our data. Because some variables were not normal, we confirmed the fit of the model using the Bollen-Stine bootstrap test (the model has a good fit when 0.10 < bootstrap $P \leq 1.00$; Schermelleh-Engel et al. 2003). Analyses were performed using the AMOS 22 (IBM, Chicago, Illinois, USA) software.

Finally, to test for a link between changes in microbial community diversity and composition with ecosystem functions, we then assessed the correlation among the concentrations of the four enzymes (β-glucosidase, β-D-cellobiosidase, *N*-acetyl-β-glucosaminidase and phosphatase) and the relative abundance (based on OTUs) of the dominant phylum: Actinobacteria and Ascomycetes using Spearman's ρ.

Results

Actinobacteria was the dominant bacterial phylum (mean ± SE relative abundance: 37% ± 0.2%) followed by Class α-Proteobacteria (12% ± 0.2%). Less common taxa were Chloroflexi (5% ± 0.1%) and Acidobacteria (5% ± 0.4%). Similarly, Ascomycetes (68% ± 1.0%) was by far the most dominant fungal phylum followed by Basidiomycetes (24.6% ± 1.0%; Appendix S4).

Our structural equation models explained 56% and 32% of the variance in the alpha diversity (i.e., OTU richness) of bacteria (Fig. 2) and fungi (Fig. 3), respectively. These models supported the hypothesis that the competitive exclusion principle linked to grazing is driving the diversity of both bacteria and fungi. In both models, increases in the two dominant bacterial (α-Proteobacteria and Actinobacteria) and fungal (Ascomycetes and Basidiomycetes) taxa were associated with a relatively strong suppression of bacterial and fungal richness, respectively.

Our models also show that grazing-induced reductions in soil carbon provided the key mechanism behind the release of subordinate bacteria and fungi from competitive exclusion. For example, grazing suppressed the positive effect of soil carbon on Actinobacteria, resulting in increased bacterial richness (Fig. 2), thus releasing the suppression of bacteria subordinates. Grazing also enhanced bacterial richness by directly increasing plant richness, or via an alternative pathway that involved suppressing the negative effects of plant cover on plant richness.

Grazing also had an indirect negative effect on fungal richness by suppressing the negative effect of soil carbon

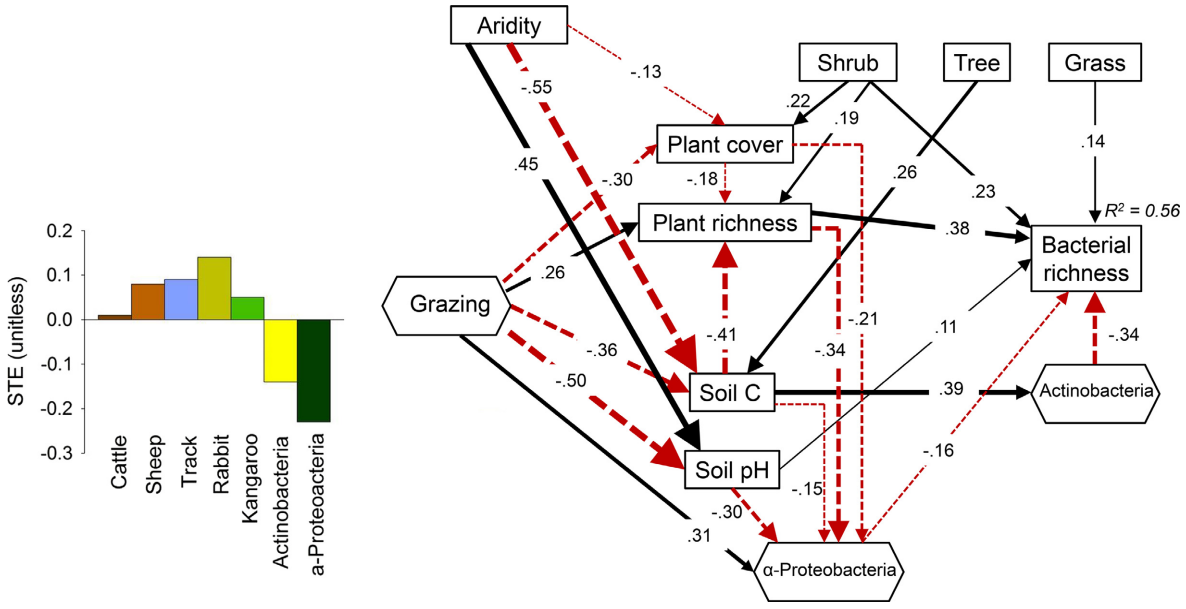

Fig. 2. Structural equation modelling of the direct and indirect effects of grazing, aridity, plant cover, plant richness, soil carbon, soil pH, and the three vegetated microsites on total bacterial richness (based on operational taxonomic units) and the relative abundance of Actinobacteria and α-Proteobacteria, the two most abundant bacterial phyla. Grazing is a composite variable comprising recent grazing by all herbivores, and historic grazing by livestock. Actinobacteria and α-Proteobacteria are presented as composite variables comprising relative abundance and relative abundance^2 to account for the unimodal relationship between relative abundance and bacterial richness. Standardized path coefficients, superimposed upon the arrows, are analogous to partial correlation coefficients, and indicative of the effect size of the relationship. Continuous and dashed arrows indicate positive and negative relationships, respectively. The width of arrows is proportional to the strength of path coefficients. The proportion of variance in bacterial richness is presented above the response variable. Only significant path coefficients are presented. The inset histogram shows the standardized total effects (the sum of direct plus indirect effects) of grazing (cattle, sheep, rabbit, kangaroo, tracks), relative abundance of Actinobacteria and α-Proteobacteria on bacterial richness. $\chi^2 = 0.65$, df = 19, $P = 1.0$, root mean error of approximation = 0, Bootstrapped $P = 1.0$. [Color figure can be viewed at wileyonlinelibrary.com]

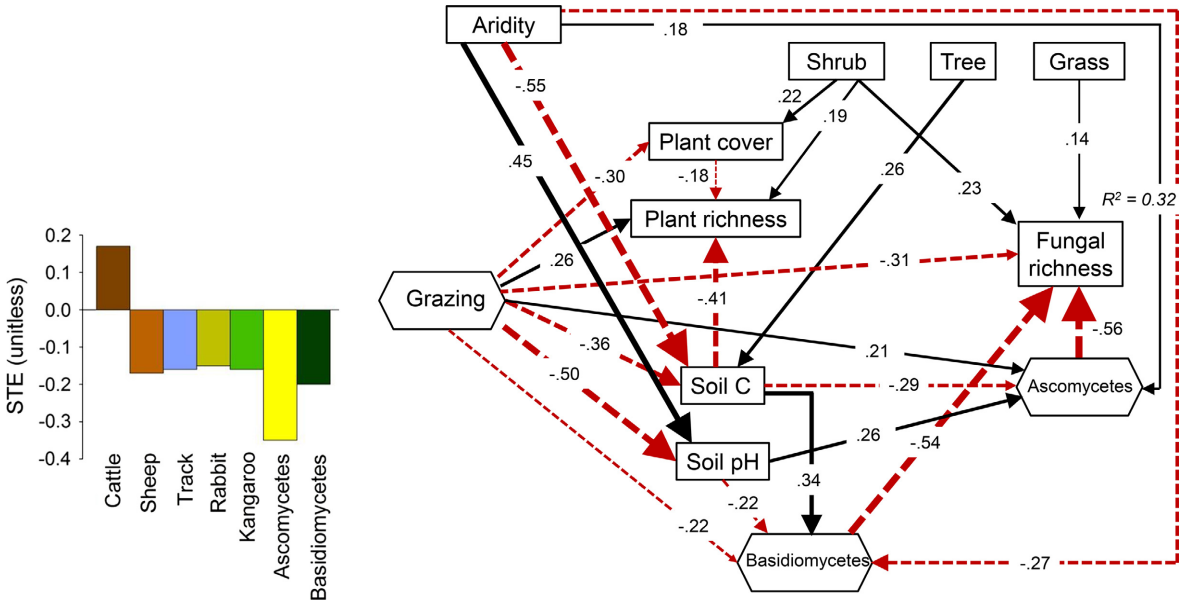

FIG. 3. Structural equation modelling of the direct and indirect effects of grazing, aridity, plant cover, plant richness, soil carbon, soil pH, and the three vegetated microsites on total fungal richness (based on operational taxonomic units) and the relative abundance of Ascomycetes and Basidiomycetes, the two most abundant fungal phyla. Grazing is a composite variable comprising recent grazing by all herbivores, and historic grazing by livestock. Ascomycetes and Basidiomycetes are presented as composite variables comprising relative abundance and relative abundance^2 to account for the unimodal relationship between relative abundance and bacterial richness. Standardized path coefficients, superimposed upon the arrows, are analogous to partial correlation coefficients, and indicative of the effect size of the relationship. Continuous and dashed arrows indicate positive and negative relationships, respectively. The width of arrows is proportional to the strength of path coefficients. The proportion of variance in bacterial richness is presented above the response variable. Only significant path coefficients are presented. The inset histogram shows the standardized total effects (the sum of direct plus indirect effects) of grazing (cattle, sheep, rabbit, kangaroo, tracks), relative abundance of Ascomycetes and Basidiomycetes on bacterial richness. $\chi^2 = 0.65$, df = 19, $P = 1.0$, root mean error of approximation = 0, Bootstrapped $P = 1.0$ [Color figure can be viewed at wileyonlinelibrary.com]

on Ascomycetes, the dominant fungal phylum. However, grazing also had a negative indirect effect on fungal richness by reducing soil pH, which promoted the relative abundance of Basidiomycetes, the second most dominant fungal phylum.

The STE (i.e., sum of indirect and direct effects from SEM) of grazing was positive for bacterial richness but negative for fungi (Figs. 2, 3). The total positive effects of grazing on bacterial richness were related mainly to rabbit grazing, and to a lesser extent, historic (livestock tracks) and sheep grazing (Fig. 2), while the negative total effects of grazing on fungi were attributable to all grazing predictors, except cattle grazing (Fig. 3). Overall, the standardized total effects show that the dominant and codominant bacterial and fungal taxa reduced bacterial and fungal richness.

Finally, we found multiple positive relations between microbial richness and individual functions (i.e., enzyme activities and carbon degradation assays (Table 1). Thus, fungal richness was positively correlated with phosphatase (Table 1). Moreover, there was a positive relationship between bacterial richness and β-D-cellobiosidase ($P = 0.01$), and Actinobacteria was positively correlated with both β-glucosidase and phosphatase. The relative abundance of Ascomycetes was negatively related to phosphatase, and Basidiomycetes positively related to both phosphatase and *N*-acetyl-β-glucosaminidase.

DISCUSSION

To our knowledge, this study provides the most novel evidence that the competitive exclusion principle, largely applicable to aboveground vascular plant communities, also applies to soil microbial communities and drives the impacts of grazing intensity on microbial richness in terrestrial ecosystems. In particular, increasing grazing intensity reduced the positive effect of soil carbon on Actinobacteria, thereby promoting a richer bacterial community by releasing subordinate (i.e., less dominant) bacteria from competitive exclusion. Conversely, but also supported by the competitive exclusion principle, grazing increased the suppressive effect of Ascomycetes, the dominant fungal phylum, on fungal richness, either directly or via reductions in soil carbon, with both mechanisms promoting competitive exclusion and reducing fungal richness. Grazing also reduced the suppressive effect of Basidiomycetes on fungal richness, and suppressed the positive effect of soil pH on Ascomycetes, both of which led to small overall increases in fungal

TABLE 1. Spearmans correlations (ρ) among the four enzymes, fungal and bacterial richness (based on OTUs) and the relative abundance of Proteobacteria, Acidobacteria, Chloroflexi, Actinobacteria, Ascomycetes and Basidiomycetes.

Soil enzymes	PHOS	CB	BG	NAG
Bacterial richness		0.30		
Fungal richness	0.16			
α-Proteobacteria	−0.18			
Acidobacteria	−0.36		−0.26	−0.18
Chloroflexi		0.24		
Actinobacteria	0.29	−0.16	0.20	
Ascomycetes	−0.14			
Basidiomycetes	0.15			0.15

Notes: OTUs, operational taxonomic units; BG, β-glucosidase; CB, β-D-cellobiosidase; NAG, *N*-acetyl-β-glucosaminidase; PHOS, phosphatase. Only significant correlations following Bonferroni post-hoc corrections are shown.

richness. However, supporting our main hypothesis, the net effect of these two processes was a reduction in fungal richness, as indicated by the standardised total effects. Changes in richness were associated with changes in soil carbon, which largely drive soil microbial communities in drylands (Maestre et al. 2015), reinforcing the notion that grazing has substantial impacts on belowground processes via changes in key soil properties. The results of this study provide important information for managers on how grazing, microbial communities and ecosystem functions are related in drylands. This is particularly important as we move towards a drier planet where soil carbon is predicted to be lower (Kirschbaum 1995, Delgado-Baquerizo et al. 2013), and where it will be increasingly difficult to support a growing global human population.

Grazing releases bacterial subordinates and increase their diversity via competitive exclusion

The relative abundance of Actinobacteria and α-Proteobacteria, the dominant bacterial phyla in global drylands (Maestre et al. 2015), declined under increasing grazing intensity via reductions in soil carbon (Fig. 2). Direct effects of livestock trampling on soil carbon are well known from global studies (e.g., Steffens et al. 2008, McSherry and Ritchie 2013). Similarly, indirect grazing-induced carbon depletion via vegetation removal (herbivory) has been demonstrated in Mediterranean systems (Yates et al. 2000, Martinez-Mena et al. 2002). Actinobacteria and α-Proteobacteria have recently been considered to follow a copiotrophic (*k*-strategist) life strategy (Leff et al. 2015), which may explain their negative response to declining soil carbon linked to grazing. In particular, reductions in the relative abundance of the copiotrophic Actinobacteria may promote a greater relative abundance of other less dominant microbial taxa such as, Acidobacteria and Chloroflexi, which have functionally distinct life strategies (oligotrophs or *r*-strategists) and tend to be associated with low carbon environments (Trivedi et al. 2012, Maestre et al. 2015). Grazing-induced increases in relative abundance of these oligotrophic taxa may also relate to a relatively greater contribution of recalcitrant carbon to total carbon pools (Trivedi et al. 2011). We found no direct effects of increasing carbon on bacterial or fungal richness, but a strong indirect negative effect of carbon on bacteria, mediated by plant richness (Fig. 2), providing support for our hypothesis of release from competitive exclusion by the more abundant Actinobacteria and α-Proteobacteria.

Grazing supresses fungal subordinates and reduces their diversity via competitive exclusion

Grazing showed both direct and indirect effects on fungal richness Fig. 3). Noticeably, grazing intensity enhanced the relative abundance of Ascomycetes, or suppressed the negative effect of soil carbon on the dominant Ascomycetes. These results are consistent with the results of previous studies (e.g., Zechmeister-Boltenstern et al. 2015) finding this fungal phylum to favour resource-poor environments (i.e., soil carbon). By promoting the relative abundance of Ascomycetes, grazing intensity indirectly led to reductions in fungal richness via the process of competitive exclusion. It is important to note, however, that we also detected some indirect positive effects of grazing on fungal richness. For example, grazing reduced the suppressive effect of Basidiomycetes on fungal richness, and reduced the positive effect of soil pH on the dominant Ascomycetes, thus leading to slight increases in fungal richness. Despite these small positive and indirect effects of grazing on fungal richness, however, the net effect of grazing was to reduce fungal diversity. Our results are consistent with the expected response of oligotrophic taxa to reduced soil carbon and with results from global studies (e.g., Maestre et al. 2015) and meta-analyses (e.g., Manzoni et al. 2012, Angel et al. 2013).

Our models also indicate that increased grazing intensity reduced bacterial richness by increasing soil pH, which also increased the relative abundance of Ascomycetes but reduced Basidiomycetes. More acidic soils could impose a physiological constraint on some soil fungi (Maestre et al. 2015), favouring taxa with pH optima closer to alkaline or ameliorating harsh acidic conditions and enhancing niches for bacteria (Tedersoo et al. 2014), resulting in greater bacterial richness.

Mediating effects of plant cover and richness on the response of bacteria and fungi to grazing intensity

Plant richness increased directly with increased grazing intensity, but also indirectly via reductions in plant cover. Both of these pathways were highly correlated with increasing bacterial richness, consistent with global studies (Mariadassou et al. 2015, Delgado-Baquerizo et al. 2016, though see Prober et al. 2015). For fungal richness, however, we failed to detect significant vascular

plant effects on fungal richness. Rather, all effects were via changes in soil carbon or pH (Fig. 3).

Nitrogen inputs from dung and urine could account for part of the direct effect of grazing on fungal richness (Baron et al. 2002). Greater nitrogen could inhibit enzymes needed to decompose recalcitrant carbon, thereby reducing overall microbial activity (Gallo et al. 2014). More nitrogen could release microbes from the need to decompose recalcitrant carbon, shifting their focus to more labile forms. Nitrogen deposition can also alter the composition of Basidiomycetes (Edwards et al. 2011) by reducing the transcriptional genes encoding enzymes that depolymerise cell wall lignin (Eisenlord et al. 2013). The most parsimonious explanation, however, is that nitrogen addition would likely shift the microbial composition from oligotrophs to copiotrophs (Trivedi et al. 2012), which have high nitrogen demands (Ramirez et al. 2012).

Niche complementarity may account for the strong plant-bacterial richness relationship. A richer plant community would be expected to have a greater root biomass (De Deyn et al. 2011), and thus a greater volume of the soil influenced by rhizosphere relationships. A richer plant community should support a greater range of plant root types, a wider spectrum of root exudates (Bezemer et al. 2006, Berg and Smalla 2009) and therefore a greater range of resources and microhabitats for bacteria (Lamb et al. 2011). A more diverse plant community will also produce more varied litter with different decay times, different chemistries (e.g., C:N ratios, phenol, cellulose and lignin concentrations; Bardgett et al. 1998) and therefore a more varied substrate quality supporting a richer bacterial community (e.g., Osanai et al. 2013). Reductions in plant cover are therefore likely to alter bacterial community composition favoring subordinates (e.g., Acidobacteria, Chloroflexi) and therefore declining functions associated with our proxies of carbon, nitrogen, and phosphorus cycling (Table 1).

Implications for ecosystem functioning

Our results have clear implications for ecosystem functioning. Changes in the microbial composition and diversity linked to grazing may alter the multiple soil ecosystem functions they provide to humans. The diversity of soil microbes was positively related to multiple soil functions. These included phosphatase, an enzyme in microbes and plants that mineralizes soil phosphorus from organic matter and makes it available to plants, β-D-cellobiosidase, β-glucosidase and *N*-acetyl-β-glucosaminidase. Any grazing-induced reductions in fungal richness, or the release of subordinate bacteria via suppression of Actinobacteria, therefore, can reduce the bioavailability of phosphorus and carbon for plants and microbes (Zhang et al. 2015). Our results suggest, therefore, that overgrazing may promote considerable negative feedbacks on important functions that are mediated by soil microbes such as nutrient cycling and litter decomposition which are critical for food and fiber production (Delgado-Baquerizo et al. 2016, Trivedi et al. 2016).

Concluding Remarks

Our data provide the most novel evidence that grazing modulates the diversity and composition of soil microbes via shifts in competitive exclusion of dominant microbes. At the core of our results is the pivotal role of soil carbon. The effect of increasing intensity of grazing is to alter soil carbon, changing the competitive abilities of different microbial phyla. This means that grazing has the potential to alter soil function by altering microbial community composition, providing a clear link between the management of domestic and native herbivores, carbon availability and ecosystem functions. Assessment of the microbial community response to overgrazing may provide, therefore, an early warning sign of impending changes in ecosystem functions that are not apparent in more traditional forms of livestock degradation.

Acknowledgments

We thank a large number of people for help with project management, survey, and analysis particularly Mark Peacock, Ross McDonnell and Sarah Carr (NSW National Parks and Wildlife Service), the Office of Environment and Heritage (OEH), and the River Red Gum Scientific Advisory Committee for project guidance, and Forest Corporation NSW and landholders for allowing access to field sites. Paul O'Keefe, Anthony Collins, Charlie Carruthers and Vandandorj Sumaya assisted with field data collection. We particularly thank Sarah Carr and Greg Summerell for their ongoing support.

Literature Cited

Andrews, S. 2010. FastQCA quality control tool for high throughput sequence data. http://www.bioinformatics.babraham.ac.uk/projects/fastqc

Angel, R., Z. Pasternak, M. I. M. Soares, R. Conrad, and O. Gillor. 2013. Active and total prokaryotic communities in dryland soils. FEMS Microbiology Ecology 86:130–138.

Bardgett, R. D., and W. H. van der Putten. 2014. Belowground biodiversity and ecosystem functioning. Nature 515:505–511.

Bardgett, R. D., D. A. Wardle, and G. W. Yeates. 1998. Linking above-ground and below-ground interactions: How plant responses to foliar herbivory influence soil organisms. Soil Biology and Biochemistry 30:1867–1878.

Baron, V. S., E. Mapfumo, A. C. Dick, M. A. Naeth, E. K. Okine, and D. S. Chanasyk. 2002. Grazing intensity impacts on pasture carbon and nitrogen flow. Journal of Range Management 55:535–541.

Bell, C. W., B. E. Fricks, J. D. Rocca, J. M. Steinweg, S. K. McMahon, and M. D. Wallenstein. 2013. High-throughput fluorometric measurement of potential soil extracellular enzyme activities. Journal of Visualized Experimentation 81; doi: 10.3791/50961

Berg, G., and K. Smalla. 2009. Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. FEMS Microbiology Ecology 68:1–13.

Bezemer, T. M., C. S. Lawson, K. Hedlund, A. R. Edwards, A. J. Brook, J. M. Igual, S. R. Mortimer, and W. H. van der Putten 2006. Plant species and functional group effects on

abiotic and microbial soil properties and plant-soil feedback responses in two grasslands. Journal of Ecology 94:893–904.
Cerdà, A., and H. Lavee. 1999. The effect of grazing on soil and water losses under arid and Mediterranean climates. Implications for desertification. Pirineos 153–154:159–174.
Cherif, M., and M. Loreau. 2007. Stoichiometric constraints on resource use, competitive interactions, and elemental cycling in microbial decomposers. American Naturalist 169:709–724.
Collins, S. L., A. K. Knapp, J. M. Briggs, J. M. Blair, and E. M. Steinauer. 1998. Modulation of diversity by grazing and mowing in native tallgrass prairie. Science 280:745–747.
De Deyn, G. B., H. Quirk, and R. D. Bardgett. 2011. Plant species richness, identity and productivity differentially influence key groups of microbes in grassland soils of contrasting fertility. Biology Letters 7:75–78.
Delgado-Baquerizo, M., F. T. Maestre, D. J. Eldridge, and B. K. Singh. 2016. Microsite differentiation drives the abundance of soil ammonia oxidizing bacteria along aridity gradients. Frontiers in Microbiology 7: DOI: 10.3389/fmicb.2016.00505
Delgado-Baquerizo, M. F., et al. 2013. Decoupling of soil nutrient cycles as a function of aridity in global drylands. Nature 502:672–676.
Diaz, S., A. Purvis, J. H. C. Cornelissen, G. M. Mace, M. J. Donoghue, R. M. Ewers, P. Jordano, and W. D. Pearse. 2013. Functional traits, the phylogeny of function, and ecosystem service vulnerability. Ecology and Evolution 3:2958–2975.
Edgar, R. C. 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nature Methods 10:996–998.
Edgar, R. C., B. J. Haas, J. C. Clemente, C. Quince, and R. Knight. 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27:2194–2200.
Edwards, I. P., D. R. Zak, H. Kellner, S. D. Eisenlord, and K. S. Pregitzer. 2011. Simulated atmospheric n deposition alters fungal community composition and suppresses ligninolytic gene expression in a northern hardwood forest. PLoS ONE 6: doi.org/10.1371/journal.pone.0020421
Eisenlord, S. D., Z. Freedman, D. R. Zak, K. Xue, Z. L. He, and J. Z. Zhou. 2013. Microbial mechanisms mediating increased soil c storage under elevated atmospheric N deposition. Applied Environmental Microbiology 79:1191–1199.
Eldridge, D. J., and M. Delgado-Baquerizo. 2016. Continental-scale impacts of livestock grazing on ecosystem supporting and regulating services. Land Degradation and Development. https://doi.org/10.1002/ldr.2668
Eldridge, D. J., A. G. B. Poore, M. Ruiz-Colmenero, M. Letnic, and S. Soliveres. 2016*a*. Ecosystem structure, function and composition in rangelands are negatively affected by livestock grazing. Ecological Applications 26:1273–1283.
Eldridge, D. J., M. Delgado-Baquerizo, S. K. Travers, J. Val, and I. Oliver. 2016*b*. Do grazing intensity and herbivore type affect soil health?: insights from a semi-arid productivity gradient. Journal of Applied Ecology 54:976–985.
Eldridge, D. J., J. Val, and A. I. James. 2011. Abiotic effects predominate under prolonged livestock -induced disturbance. Austral Ecology 36:367–377.
Fierer, N., M. A. Bradford, and R. B. Jackson. 2007. Toward an ecological classification of soil bacteria. Ecology 88:1354–1364.
Frank, D. A. 2005. The interactive effects of grazing ungulates and aboveground production on grassland diversity. Oecologia 143:629–634.
Gallo, E. L., K. A. Lohse, C. M. Ferlin, T. Meixner, and P. D. Brooks. 2014. Physical and biological controls on trace gas fluxes in semi-arid urban ephemeral waterways. Biogeochemistry 121:189–207.
Grace, J. B. 2006. Structural equation modelling and natural systems. Cambridge University Press, Cambridge, UK.
van der Heijden, M. G. A., R. D. Bardgett, and N. M. van Straalen. 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecology Letters 11:296–310.
Herlemann, D. P. R., M. Labrenz, K. Jurgens, S. Bertilsson S, J. J. Waniek, and A. F. Andersson. 2011. Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. ISME Journal 5:1571–1579.
Hibbing, M. E., C. Fuqua, M. R. Parsek, and S. B. Peterson. 2010. Bacterial competition: surviving and thriving in the microbial jungle. Nature Reviews Microbiology 8:15–25.
Ihrmark, K., et al. 2012. New primers to amplify the fungal ITS2 region – evaluation by 454-sequencing of artificial and natural communities. FEMS Microbiology Ecology 82:666–677.
Jing, X., N. J. Sanders, Y. Shi, H. Y. Chu, A. T. Classen, K. Zhao, L. Chen, Y. Shi, Y. Jiang, and J. S. He. 2015. The links between ecosystem multifunctionality and above- and belowground biodiversity are mediated by climate. Nature Communications 6:Article 8159.
Johnson, C. N., and P. J. Jarman. 1987. Macropod studies at Wallaby Creek. VI: A validation of the use of dung-pellet counts for measuring absolute densities of populations of macropodids. Australian Wildlife Research 14:139–146.
Kirschbaum, M. U. F. 1995. The temperature-dependence of soil organic-matter decomposition, and the effect of global warming on soil organic-C storage. Soil Biology and Biochemistry 27:753–760.
Kõljalg, U., et al. 2013. Towards a unified paradigm for sequence-based identification of fungi. Molecular Ecology 22:5271–5277.
Lamb, E. G., N. Kennedy, and S. D. Siciliano. 2011. Effects of plant species richness and evenness on soil microbial community diversity and function. Plant and Soil 338:483–495.
Leff, J. W., et al. 2015. Consistent responses of soil microbial communities to elevated nutrient inputs in grasslands across the globe. Proceeding of the National Academy of Sciences USA 112:10967–10972.
Macdonald, C. A., M. J. Crawley, D. J. Wright, J. Kuczynski, L. Robinson, R. Knight, W. A. Al-Soud, S. J. Sørensen, Y. Deng, J. Zhou, and B. K. Singh. 2015. Identifying qualitative effects of different grazing types on below-ground communities and function in a long-term field experiment. Environmental Microbiology 17:841–854.
Maestre, F. T., et al. 2015. Increasing aridity reduces soil microbial diversity and abundance in global drylands. Proceeding of the National Academy of Sciences USA 112:15684–15689.
Maestre, F. T., D. J. Eldridge, S. Soliveres, S. Kéfi, M. Delgado-Baquerizo, M. A. Bowker, P. García-Palacios, J. Gaitán, A. Gallardo, R. Lázaro, and M. Berdugo. 2016. Structure and functioning of dryland ecosystems in a changing world. Annual Reviews of Ecology, Evolution and Systematics. https://doi.org/10.1146/annurev-ecolsys-121415-032311
Magoc, T., and S. L. Salzberg. 2011. FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics 27:2957–2963.
Mannetje, L. T. 2002. Advances in grassland science. Netherlands Journal of Agricultural Science 50:195–221.
Manzoni, S., J. P. Schimel, and A. Porporato. 2012. Responses of soil microbial communities to water stress: results from a meta-analysis. Ecology 93:930–938.
Mariadassou, M., S. Pichon, and D. Ebert. 2015. Microbial ecosystems are dominated by specialist taxa. Ecology Letters 18:974–982.
Marques, F. F. C., S. T. Buckland, D. Goffin, C. E. Dixon, D. L. Borchers, B. A. Mayle, and A. J. Pease. 2001. Estimating deer abundance from line transect surveys of dung: sika

deer in southern Scotland. Journal of Applied Ecology 38:349–363.
Martinez-Mena, M., J. Alvarez Rogel, V. Castillo, and J. Albaladejo. 2002. Organic carbon and nitrogen losses influenced by vegetation removal in a semiarid Mediterranean soil. Biogeochemistry 61:309–321.
McDonald, D., M. N. Price, J. Goodrich, E. P. Nawrocki, T. Z. DeSantis, A. Probst, G. L. Andersen, R. Knight, and P. Hugenholtz. 2012. An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. ISME Journal 6:610–618.
McSherry, M. E., and M. E. Ritchie. 2013. Effects of grazing on grassland soil carbon: a global review. Global Change Biology 19:1347–1357.
Milchunas, D. G., O. E. Sala, and W. K. Lauenroth. 1988. A generalized-model of the effects of grazing by large herbivores on grassland community structure. American Naturalist 132:87–106.
Noy-Meir, I., M. Gutman, and Y. Kaplan. 1989. Responses of Mediterranean grassland plants to grazing and protection. Journal of Ecology 77:290–310.
Osanai, Y., D. S. Bougoure, H. L. Hayden, and M. J. Hovenden. 2013. Co-occurring grass species differ in their associated microbial community composition in a temperate native grassland. Plant and Soil 368:419–431.
Pérez-Valera, E., M. Goberna, K. Faust, J. Raes, C. García, and M. Verdú. 2017. Fire modifies the phylogenetic structure of soil bacterial co-occurrence networks. Environmental Microbiology 19:317–327.
Prober, S. M., et al. 2015. Plant diversity predicts beta but not alpha diversity of soil microbes across grasslands worldwide. Ecology Letters 18:85–95.
Ramirez, K. S., J. M. Craine, and N. Fierer. 2012. Consistent effects of nitrogen amendments on soil microbial communities and processes across biomes. Global Change Biology 18:1918–1927.
Schermelleh-Engel, K., H. Moosbrugger, and H. Müller. 2003. Evaluating the fit of structural equation models: tests of significance and descriptive goodness-of-fit measures. Methods of Psychological Research Online 8:23–74.
Schloss, P. D., et al. 2009. Introducing MOTHUR: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Applied Environmental Microbiology 75:7537–7541.
Siciliano, S. D., et al. 2014. Soil fertility is associated with fungal and bacterial richness, whereas pH is associated with community composition in polar soil microbial communities. Soil Biology and Biochemistry 78:10–20.
Steffens, M., A. Kolbl, K. U. Totsche, and I. Kogel-Knabner. 2008. Grazing effects on soil chemical and physical properties in a semiarid steppe of Inner Mongolia (PR China). Geoderma 143:63–72.
Steinfeld, H., P. Gerber, T. Wassenaar, V. Castel, M. Rosales, and C. de Haan. 2006. Livestock's long shadow. Environmental issues and options. FAO, Rome, Italy.
Tedersoo, L., et al. 2014. Global diversity and geography of soil fungi. Science 346:1078.
Trivedi, P., M. Delgado-Baquerizo, I. C. Anderson, and B. K. Singh. 2016. Response of soil properties and microbial communities to agriculture: implications for primary productivity and soil health indicators. Frontiers in Plant Science 7. https://doi.org/doi.org/10.3389/fpls.2016.00990
Trivedi, P., Z. L. He, J. D. Van Nostrand, J. Zhou, G. Albrigo, and N. Wang. 2011. Effect of Huanglongbing on the structure and functional diversity of microbial communities associated with citrus rhizosphere. Phytopathology 101: S178.
Trivedi, P., Z. L. He, J. D. Van Nostrand, J. Zhou, G. Albrigo, and N. Wang. 2012. Huanglongbing alters the structure and functional diversity of microbial communities associated with citrus rhizosphere. ISME Journal 6:363–383.
Wang, Q., G. M. Garrity, J. M. Tiedje, and J. R. Cole. 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Applied Environmental Microbiology 73:5261–5267.
Yates, C. J., D. A. Norton, and R. J. Hobbs. 2000. Grazing effects on plant cover, soil and microclimate in fragmented woodlands in south-western Australia: implications for restoration. Austral Ecology 25:36–47.
Zechmeister-Boltenstern, S., K. M. Keiblinger, M. Mooshammer, J. Penuelas, A. Richter, J. Sardans, and W. Wanek. 2015. The application of ecological stoichiometry to plant-microbial-soil organic matter transformations. Ecological Monographs 85:133–155.
Zhang, K. R., H. S. Dang, Q. F. Zhang, and X. L. Cheng. 2015. Soil carbon dynamics following land-use change varied with temperature and precipitation gradients: evidence from stable isotopes. Global Change Biology 21:2762–2772.

Supporting Information

Additional supporting information may be found in the online version of this article at http://onlinelibrary.wiley.com/doi/10.1002/ecy.1879/suppinfo

Data Availability

Data available from the Dryad Digital Repository: http://dx.doi:10.5061/dryad.94kq1