Plant Soil
https://doi.org/10.1007/s11104-018-3617-6

REGULAR ARTICLE

Functional groups of soil fungi decline under grazing

David J. Eldridge · Manuel Delgado-Baquerizo

Received: 30 November 2017 / Accepted: 27 February 2018

Abstract
Background and aims Fungi play vital roles in organic matter decomposition, and mineralisation of phosphorus and nitrogen, are significant plant and animal pathogens, and major mutualistic symbionts with the roots of higher plants. Despite their importance, relatively little is known about the effects of livestock grazing on different functional groups of fungi.
Methods We used structural equation modelling to examine how grazing by domestic livestock and native herbivores, and aridity, plant cover and soil carbon influenced four functional groups of soil fungi (ectomycorrhizal fungi, arbuscular mycorrhizal fungi, dung saprobes, plant pathogens) from three microsites (tree, shrub, grass) at 54 woodland sites across 0.4 million km^2 of dryland in eastern Australia.
Results Structural equation modelling showed that aridity influenced fungi indirectly by affecting different herbivores and by changing plant cover, which had different effects on different fungal groups. Rabbit grazing had a direct negative effect on ectomycorrhizal and arbuscular mycorrhizal fungi, most likely by disrupting hyphal networks through soil disturbance. Increased cattle grazing was directly positively associated with fungal dung saprobe abundance, and indirectly, negatively associated with dung saprobes by suppressing the positive effects of soil carbon. Sheep had direct and indirect negative effects on the abundance of plant pathogens.
Conclusions Grazing was always an important predictor of the relative abundance of all fungal groups, either directly or indirectly. Thus, overgrazing is likely to have substantial effects on a range of important soil processes controlled by these microorganisms. Overall, our work indicates that increasing grazing, linked to on-going land use intensification to support a growing global population, will have major impacts on fungal functional groups.

Keywords Fungi · Livestock · Grazing · Drylands · Soil function · Saprobes · Mycorrhizal fungi

Responsible Editor: Thom W. Kuyper.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11104-018-3617-6) contains supplementary material, which is available to authorized users.

D. J. Eldridge (✉)
Centre for Ecosystem Science, School of Biological, Earth and Environmental Sciences, University of New South Wales, Sydney, NSW 2052, Australia
e-mail: d.eldridge@unsw.edu.au

M. Delgado-Baquerizo
Cooperative Institute for Research in Environmental Sciences, University of Colorado, Boulder, CO 80309, USA

M. Delgado-Baquerizo
Departamento de Biología y Geología, Física y Química Inorgánica, Escuela Superior de Ciencias Experimentales y Tecnología, Universidad Rey Juan Carlos, Calle Tulipán Sin Número, 28933 Móstoles, Spain

Introduction

Microbes are the most abundant and ubiquitous organisms on Earth, and fungi are one of the most important

Published online: 09 March 2018

groups of microbes. In terrestrial ecosystem, fungal communities play important roles in multiple ecosystem functions involving the decomposition of organic matter (mineralizing organic phosphorus and nitrogen) and the release of CO_2, and in some biomes, can contribute more than 80% of microbial biomass (Joergensen and Emmerling 2006). The hyphae of mycorrhizal fungi also enhance the stability of soil surface aggregates (Rillig and Mummey 2006). Fungal (mycorrhizal) associations with some plants lead to greater uptake of P and increases in net primary productivity (Treseder and Lennon 2015). Most of these functions are driven by well-defined functional groups of fungi such as symbionts (mycorrhizal fungi), pathogens or saprobes (decomposers) (Rillig and Mummey 2006; Treseder and Lennon 2015).

Despite their ecosystem importance, we know relatively little about the extent to which fungi respond to major environmental drivers such as climate and land use change. Two of the most important drivers of ecosystem functions in drylands are overgrazing by livestock and increased aridity resulting from global climate change (Maestre et al. 2016; Eldridge et al. 2016a). Two other major drivers, not considered here, are N-deposition and woody encroachment (Maestre et al. 2016). Grazing provides millions of peoples and their cultures worldwide with essential goods and services, but overgrazing can cause substantial negative impacts on ecosystem functions (Steinfeld et al. 2006; Eldridge et al. 2016b). Aridity, the second significant global change driver, is a proxy of potential changes that are likely to occur as Earth experiences drier, hotter climates (Maestre et al. 2016). Increasing aridity will reduce the efficiency with which plants and microbes perform essential processes such as the organic matter mineralisation (Maestre et al. 2016), and is predicted to decouple N and P cycling across large areas of dryland globally (Delgado-Baquerizo et al. 2013). Both grazing and aridity are expected to increase under a changing climate and this will likely influence fungi and the processes they control (Maestre et al. 2015). Grazing would increase because climate change would make less land available for grazing, placing increased pressure on a smaller amount of land. Understanding how major environmental drivers such as aridity and grazing might influence fungal communities is critically important in order to predict and manage any potential long-term consequences on soil biogeochemical cycles (Treseder and Lennon 2015), predict how ecosystem services might change in the face of a drying climate, and determine any consequences of these effects on societal well-being.

Herein, we develop a system-level understanding of the direct and indirect effects of grazing, by livestock (cattle, sheep/goats) and free-ranging herbivores (kangaroos, rabbits) on four functional groups of fungi, ectomycorrhizal fungi, arbuscular mycorrhizal fungi, dung saprobes and plant pathogens across an area of 0.4 million km^2 of eastern Australia. These four groups of fungi are important because they play important roles in nutrient acquisition, decomposition, microbial symbioses and pathogenesis (Treseder and Lennon 2015).

Methods

Study sites and design

This study was carried out at 54 sites across 0.4 million km^2 of dryland in eastern Australia. We had 18 sites in each of three woodland communities dominated by either Blackbox (*Eucalyptus largiflorens*), River red gum (*Eucalyptus camaldulensis*) or White cypress pine (*Callitris glaucophylla*). We established a 100 m transect. Then at the 0 m, 50 m and 100 m positions we: (1) established a large quadrat (5 × 5 m) within which was nested (2) a smaller quadrat (0.5 × 0.5 m). These two quadrats were used to count the dung of all herbivores in order to assess grazing intensity. At each of the 0, 50 and 100 m marks we selected the nearest of three patch types (tree, shrub, grass) and an adjacent open patch (open) where we placed a circular 0.5 m^2 quadrat (Fig. 1). We used this quadrat size to assess the cover and abundance of all plants, by species, 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. For trees and shrubs, the 0.5 m^2 quadrats were placed in the centre of the canopy, but for grasses, around individual butts or clumps of grasses. For trees, we then sampled soil from the uppermost 5 cm of the surface of each of the three trees using a 48 mm diameter soil core, took three replicate samples, bulked them and selected a subsample of about 300 g which was stored under refrigeration before being transported back to the laboratory. The depth of 5 cm was chosen because we expected it to coincide with the zone of maximum biological activity and soil carbon concentrations. The procedure was repeated for the other three microsites. All samples were taken from the centre of the tree and shrub canopies and adjacent to perennial grass butts. Thus, in total we had 216 soil samples across the three communities.

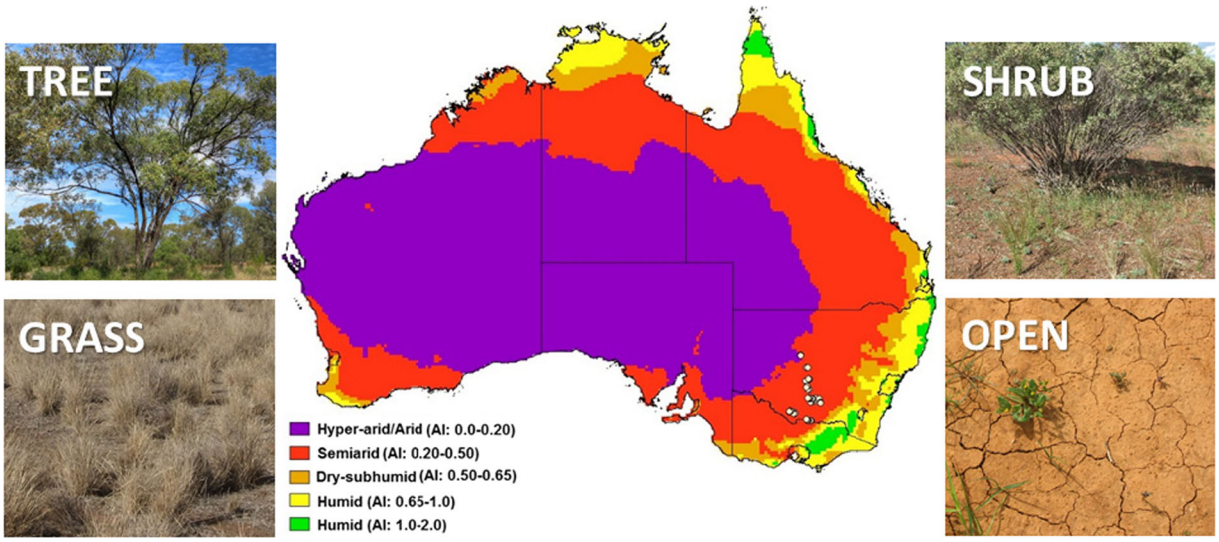

Fig. 1 Location of the study area and study sites (white circles) in eastern Australia and images of the four microsites within which microbes were assessed

To assess grazing intensity, we identified and counted the dung of all herbivores within 25 m^2 (cattle, sheep/goat, kangaroo) and 0.25 m^2 (kangaroo, rabbit, sheep/goat) quadrats that were placed along the transect. Dung counts have been used widely to estimate the abundance of large herbivores including kangaroos (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 metres. At ten sites we counted, collected, dried and weighed the dung from 10 large quadrats to obtain the relationship between dung counts and dry mass for each herbivore. This relationship was then used to calculate the total per hectare oven-dried mass of dung of each herbivore type as our measure of recent grazing intensity. Where dung from the same herbivore was assessed in both the large and small quadrats, we derived an average mass per hectare based on both quadrats for that herbivore type. We were unable to discriminate between sheep, goat and in very few cases deer dung, or between European rabbit and European hare dung. To assess historic grazing intensity, we measured the width and depth of tracks that sheep and cattle use when travelling to water. These data were then used to derive a total cross-sectional area of livestock tracks for each site.

Molecular analyses and sequencing

Soils collected in the field were stored in a commercial freezer for 2 days and transported to the laboratory using dry ice. A subsample of 50 g from each site was immediately frozen and stored at -35 °C for 2 weeks before DNA was extracted. We extracted DNA from 0.5 g of defrosted soil samples from each site using the Powersoil® DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA, USA) according to the manufacturer's instructions. We sequenced the fungal ITS2 gene with the FITS7-ITS4R primer set (Ihrmark et al. 2012) sequenced at the Western Sydney University NGS facility (Sydney, Australia) using Illumina 2 × 280 bp (fungi) paired end sequencing. We used the ITS2 region to obtain relative abundance of major fungal groups. This approach, therefore, might have underestimated the relative abundance of arbuscular mycorrhizal fungi. The OTU abundance tables were rarefied to an even number of sequences per sample (20,797; the minimum number of sequences for a soil sample).

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 (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 (Magoč 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 (<150 bp for primer set FITS7-ITS4R), as well as sequences with ambiguous characters or more than eight homopolymers. Operational

Taxonomic Units (OTUs) 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 UNITE reference database (Edgar et al. 2011). OTU 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 dynamic UNITE version 6 dataset (Kõljalg et al. 2013) for fungi.

We categorized fungi into functional groups using FUNGuild (Nguyen et al. 2016). We only used those taxa for which a unique role was identified in FUNGuild. We focused on four fungal functional groups (ectomycorrhizal fungi, arbuscular mycorrhizal fungi, dung saprobes and plant pathogens) because information on these groups is widely available. The relative abundance of each functional group was calculated as the sum of the relative abundance of all taxa (OTUs) sharing that particular functional group. This allowed us to examine the distribution of functional groups in relation to grazing, microsites (tree, shrub, grass), soil carbon, plant cover and aridity. The contribution of different fungal families to each functional group is shown in Fig. 2.

We assessed total soil carbon using high temperature combustion (LECO CNS-2000 CNS Analyser, LECO Corporation, St Joseph, MI, USA). We calculated a measure of aridity (Aridity) as 1-AI, where AI = the FAO Aridity Index, calculated as precipitation/potential evapotranspiration (http://data.fao.org/en/map). We used Aridity in our models because it has been shown to be a useful tool to account for spatial differences in the data among sites (Delgado-Baquerizo et al. 2013) and is a major driver of microbial communities (Maestre et al. 2015).

Statistical analyses

We first used Random Forest modelling (Breiman 2001) to identify the major environmental predictors of the relative abundance of the four fungal functional groups. Random forest extends standard classification and regression tree (CART) methods by creating a collection of classification trees with binary divisions (Wei et al. 2010). The fit of each tree is assessed using randomly selected cases, which are withheld during its construction (out-of-bag or OOB cases). The importance of each predictor variable is determined by evaluating the decrease in prediction accuracy (i.e. increase in the mean square error

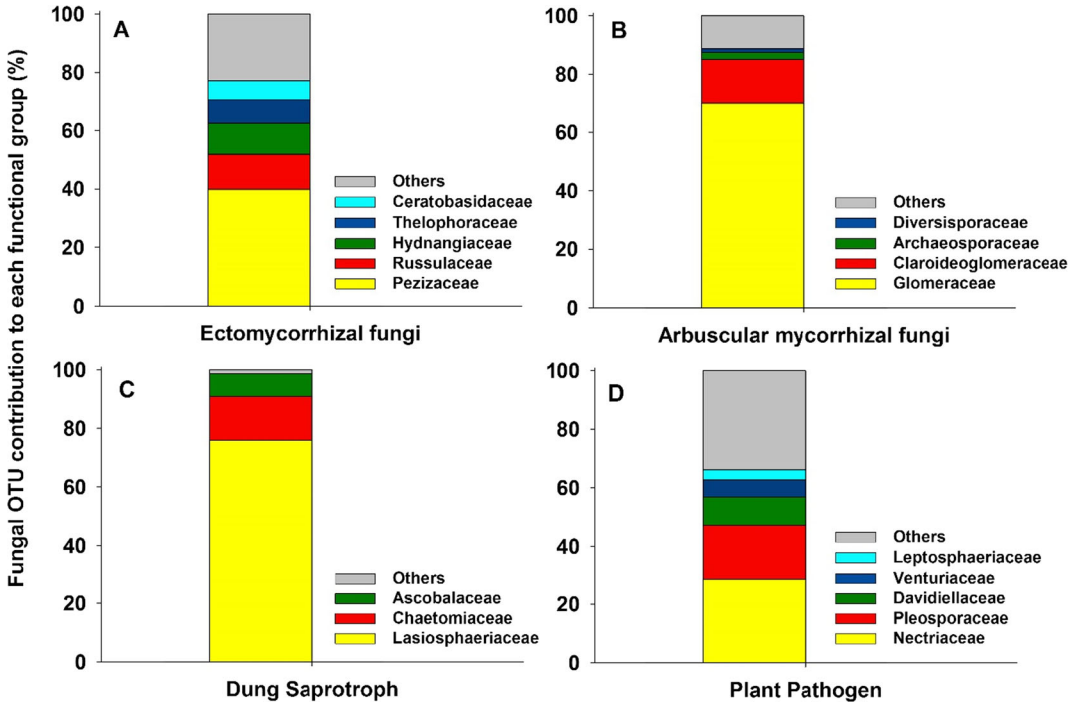

Fig. 2 Fungal OTU contribution to each functional group (%) for (**a**) ectomycorrhizal fungi, (**b**) arbuscular mycorrhizal fungi, (**c**) dung saprobes and (**d**) plant pathogens. Within a functional group, families are listed from top to bottom

between observations and OOB predictions) when the data for that predictor are randomly permuted. The final measure of importance is determined by the average decline across all trees (Wei et al. 2010).

Our analyses included climate (aridity), recent grazing (cattle, sheep/goat, rabbit, kangaroo), historic grazing (livestock tracks), plant microsite (tree, shrub, grass), groundstorey plant cover, and soil carbon as predictors. These analyses were conducted using the rfPermute package (Archer 2016) of the R statistical software (http://cran.r-project.org/). The mean and variation in these attributes are shown in Supplementary Material Table S1.

We then used Structural Equation Modelling (SEM; Grace 2006) to further clarify the direct and indirect effect of grazing, climate and plant and soil variables on the relative abundance of the four fungal groups. Structural equation modelling requires the development of an a priori model of the hypothesised effects and relationships among the main drivers and fungal group (Supplementary Material Fig. S1). In our case we predicted direct and indirect effects of grazing, climate and plant and soil carbon on the relative abundance of the four fungal groups. Assessing the direct and indirect effects is important because grazing effects on fungi can be direct, by altering chemistry (Eldridge et al. 2016a) via dung or urine deposition, or indirect, by altering plant community composition. All of our models included the five measures of grazing. Because grazing was assessed at the site level rather than the microsite level, any level of grazing for a particular herbivore at a particular site was the same for tree, shrub, grass and open. In our models, the values of the main drivers endogenous (predictor) variables were standardized (z-transformed), where necessary, after examining their distribution and testing their normality. The three microsites were treated as categorical variables of two levels (0 or 1). This approach allowed us to compare the effect of a particular microsite (e.g. Tree) on the relative abundance of each fungal group. Open areas were used as a procedural control. Thus this microsite was not explicitly considered in our models.

We compared the a priori model with the variance-covariance matrix of our data in order 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 correlations. Models, those with low χ^2, high Goodness of Fit Index [GFI], high Normal Fit Index [NFI] and low Root Mean Error of Approximation (RMSEA<0.05) were interpreted as showing the best fit to our data. We also used the Bollen-Stine bootstrap test to improve goodness of fit when variables were not normal. A model with a good fit is represented as $0.10 <$ bootstrap $P \leq 1.00$ (Schermelleh-Engel et al. 2003). Analyses were performed using the AMOS 22 (IBM, Chicago, IL, USA) software.

Data availability All data used in this study are publicly available in Figshare https://figshare.com/s/******.

Results

Random Forest analyses showed that grazing factors were always important predictors of the relative abundance of all fungal functional groups (Fig. 3). All grazing predictors were important predictors of ectomycorrhizal fungi, and recent grazing by cattle, rabbits and kangaroos, and historic livestock grazing (intensity of livestock tracks) were important predictors of arbuscular mycorrhizal fungi. Historic grazing (livestock tracks) and rabbit grazing intensity were important predictors of dung saprobes, and all grazing predictors, other than historic livestock grazing, were good predictors of the relative abundance of plant pathogens. The standardized total effects of grazing indicated that grazing effects on all fungal functional groups were generally negative (Fig. 4).

Increasing intensity of cattle grazing and historic grazing by livestock had a positive effect on the relative abundance of dung saprobes. Conversely, sheep and rabbit grazing had negative direct effects on the relative abundance of dung saprobes (Fig. 5c). Sheep had a negative effect on the relative abundance of plant pathogens both directly, and indirectly, by reducing plant cover (Fig. 5d).

Grazing effects on fungal functional groups were mediated by aridity in two ways. First, locations with higher aridity tended to have a greater intensity of sheep grazing, but lower levels of kangaroo and cattle grazing (Fig. 5a-d). Second, aridity moderated the effects of plants on functional groups of fungi. For example, tree cover suppressed the negative effect of increasing aridity on ectomycorrhizal and arbuscular mycorrhizal fungi (Fig. 5a, b). Aridity also reduced the positive effect of

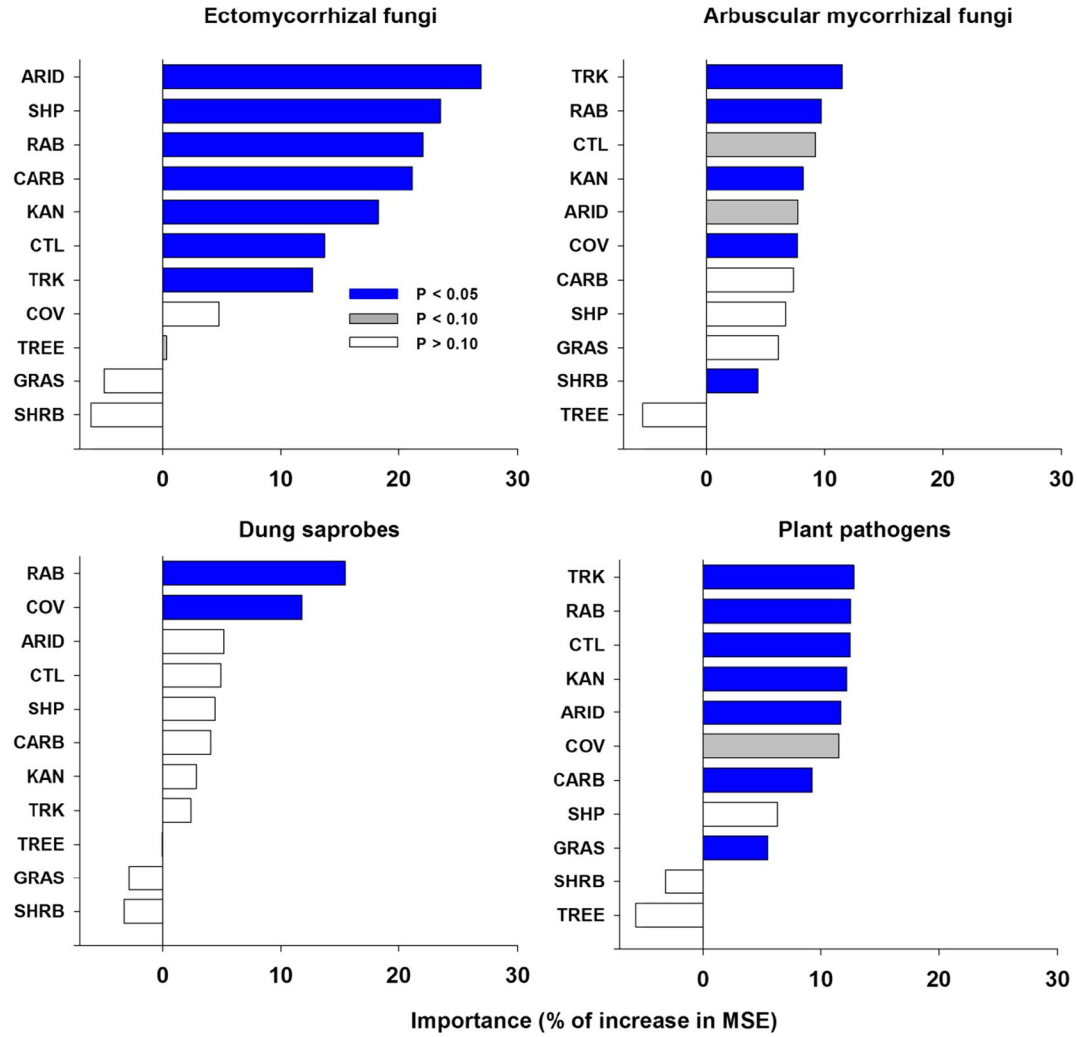

Fig. 3 Results from a Random Forest analyses showing the importance of the climatic (aridity: ARID), plant (plant cover: COV, tree: TREE, shrub: SHRB, grass: GRAS), soil (carbon: CARB) and grazing (cattle: CTL, sheep: SHP, track: TRK, rabbit: RAB, kangaroo: KAN) predictors on the four fungal functional groups. The MSE (mean squared error) is a measure of the quality of the estimator. Different colours indicate different levels of significance. Darkest colour (blue) $P < 0.05$, lighter colour (light grey) $P < 0.10$, no colour $P > 0.10$

grasses on saprobes and plant cover on pathogens (Fig. 5). Other grazing effects were independent of aridity, such as those from rabbits and historic grazing. Increasing rabbit grazing had a direct negative effect on the relative abundance of ectomycorrhizal and arbuscular mycorrhizal fungi (Figs. 5a, b).

Discussion

There is abundant work demonstrating that current and historic livestock grazing, and grazing by rabbits have strong, typically negative, effects on soil health and community composition of groundstorey plants (e.g. Eldridge et al. 2016a). The results of the current study suggest that grazing intensity is a major driver of the relative abundance of key functional groups of fungi, with potential implications for ecosystem functioning, and consistent with our understanding of grazing-induced responses of vascular plant communities.

The positive effect of increasing intensity of cattle grazing on dung saprobes is well known, with abundant evidence that cattle manure is a rich habitat for fungi (Dickinson and Underhay 1977). However, the

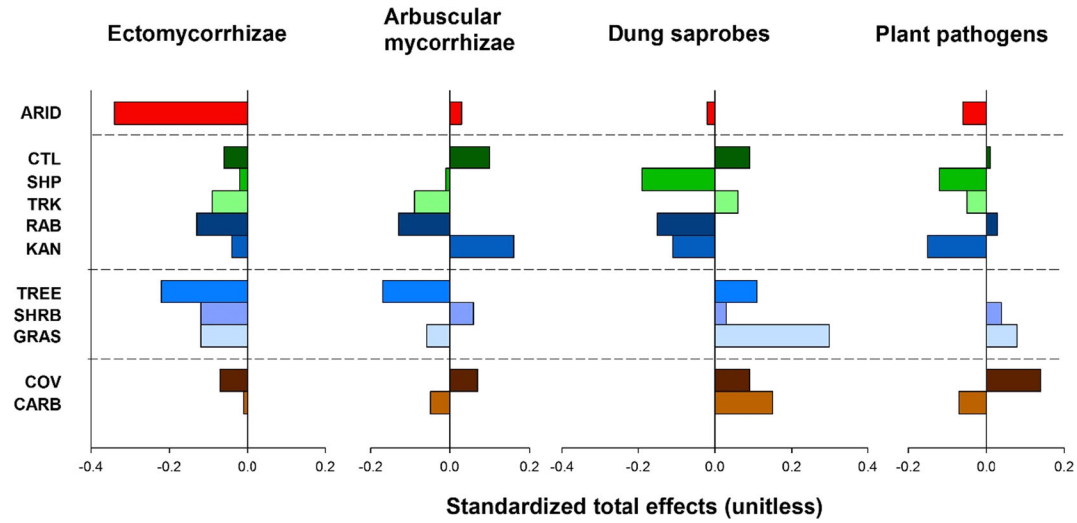

Fig. 4 Standardised total effects (STE: sum of direct plus indirect effects) derived from the structural equation modelling) of aridity (ARID), cattle (CTL), sheep (SHP), track (TRK), rabbit (RAB), kangaroo (KAN), tree (TREE), shrub (SHRB), grass (GRAS), plant cover (COV) and soil carbon (CARB)

negative effects of sheep and rabbit grazing intensity on dung saprobes was unexpected and might at first seem counterintuitive. However, we argue that increased intensity of sheep grazing, and substantial rabbit activity tend to break up cattle dung pads into smaller pieces of dung that are then redistributed over larger areas by livestock and rabbits. The scattering of cattle dung would be expected to be greater where cattle are concentrated and where disturbance by sheep and rabbit grazing is greater. In fact, part of the negative effect of grazing by rabbits on dung saprobes was driven, indirectly, via reductions in soil carbon, a surrogate of organic matter that is derived from plant detritus, frass, and fragmented dung deposits. We found that the standardised total effects (STEs) of recent cattle grazing and historic grazing on dung saprobes (i.e. the sum of all direct and indirect pathways from these measures of grazing to the fungal category) were positive (Fig. 4). This likely relates to the large surface area of cattle dung, which is a good fungal medium (Srivastava et al. 2010). Cattle dung is readily colonised by fungi (Yokoya et al. 1991), particularly when wet, and we have observed substantial growth of fungi on cattle dung after rainfall (Eldridge et al. 2016a). Cattle frequently deposit dung across a large area due to their habit of defecating while moving, so that the effect of dung on fungal communities likely extends over an area that is larger than the area occupied by dung deposits. The positive effect of increasing cattle intensity is particularly remarkable as we did not sample under dung, indicating that the effects of cattle intensity likely spread distance from the actual site of deposition. Our STEs also indicate that increasing plants (tree, shrub, grasses, plant cover) were associated with increases in plant pathogens. Plant density is known to be a major driver of plant pathogens because different plants provide a range of habitats for different pathogens. A richer and denser plant community would be expected to have a greater root biomass (De Deyn et al. 2011), support a greater range of plant root types, with a wider spectrum of root exudates (Berg and Smalla 2009) and thus a greater range of resources for fungi (Lamb et al. 2011). By reducing plant cover, therefore, grazing by specific herbivores would likely reduce the relative abundance of plant pathogens.

Soil disturbance by rabbits when they excavate plant roots or construct underground colonies (warrens) likely explains part of the negative effects of rabbits on ectomycorrhizal and arbuscular mycorrhizal fungi, potentially because rabbits would physically truncate hyphal networks when digging their burrows. Grazing by cattle and other large herbivores has been shown to reduce the length of fungal hyphae (Miller et al. 1995; Van der Heyde et al. 2017), indirectly, via removal of plant material, therefore disrupting of host-specific associations, or by increasing soil P (Cournane et al.

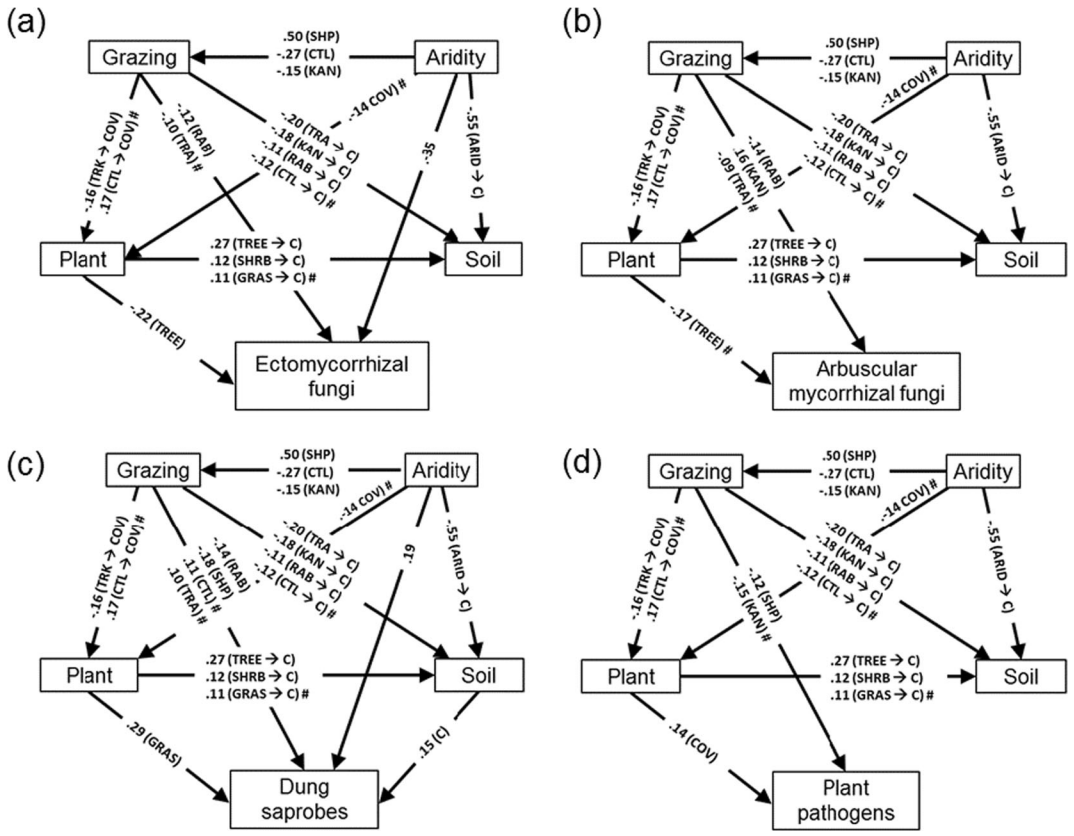

Fig. 5 Structural equation models describing the effects of multiple drivers Grazing (cattle, sheep/goats, rabbits, kangaroos, historic grazing), Plants (cover, microsite [tree, shrub, grass]), Aridity and Soil (carbon) on the relative abundance of 1) ectomycorrhizal fungi, (**b**) arbuscular mycorrhizal fungi, (**c**) dung saprobes, and (**d**) plant pathogens. Arrows indicate the direction of the effect. The numbers adjacent to arrows, path coefficients, which are analogous to partial correlation coefficients, indicative the effect size of the relationship and may be positive or negative. Significant ($P < 0.05$) pathways are shown; # indicates pathways significant at $P < 0.10$. The arrows (path coefficients) indicate the direction and magnitude of the effect of one variable on another. For example, for ectomycorrhizal fungi, grazing by rabbits reduces the relative abundance of ectomycorrhizal fungi (−0.12 RAB; Fig. 3d). Similarly, in all models, increasing levels of historic grazing are associated with reduced soil carbon (−0.20 TRA → C)

2011), similar to the effects of rabbits. Thus, grazing might decouple the symbiotic relationship between plants and fungi by releasing the dependency of plants on mycorrhizal fungi to obtain soil P.

Identity of tree community regulates the abundance of ectomycorrizal and arbuscular mycorrhizal fungi

Our models indicated a weak, but significant negative relationship between the presence of microsites dominated by trees and the relative abundance of both ectomycorrhizal fungi (path coefficient [PC] = − 0.22; Fig. 5a) and arbuscular mycorrhizal fungi (PC = −0.17; Fig. 5b). These results are consistent with the Random Forest analyses, which revealed that tree cover was not a predictor of the relative abundance of ectomycorrhizal fungi (Fig. 3). This was an unexpected result, which required further analysis of our dataset. Interestingly, the relative abundance of ectomycorrhizal fungi was community dependent and substantially greater beneath *Eucalyptus camaldulensis* trees, but values were very low for *Eucalyptus largiflorens* and *Callitrus glaucophylla* (Fig. 6). Previous studies have shown a strong relationship between ectomycorrhizal fungi and *Eucalyptus camaldulensis* (Ducousso et al. 2012). On the contrary, *Callitris glaucophylla* is known to form associations with arbuscular mycorrhizal fungi but not ectomycorrhizal fungi, further supporting our result (Reiter et al. 2013). The soils associated with *Eucalyptus largiflorens* are also known to contain very high levels of available P (~ 58 ppm, Olsen P; Eldridge et al. 2016a), potentially releasing the dependency of these

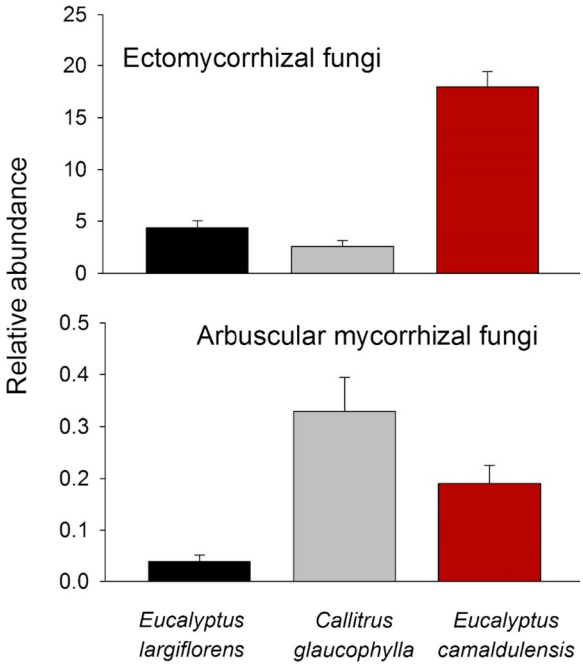

Fig. 6 Relative abundance of ectomycorrhizal fungi and arbuscular mycorrhizal fungi in relation to three tree species. Within a functional group, the relative abundance was significantly different among the three tree species

trees on mycorrhizal associations. The negative effects could have resulted from the presence of other trees in the vicinity of our focal trees; species that are not known to support ectomycorrhizal fungi, such as *Callitris glaucophylla* (Reiter et al. 2013). Another potential mechanism could be that greater tree cover causes shading of understory arbuscular mycorrhizal taxa, reducing the carbon available to support arbuscular mycorrhizal symbioses.

Conclusions

Our work has shown that increasing grazing by domestic livestock, feral goats and rabbits has major impacts on four functional groups of soil fungi, with implications for ecosystem functions in terrestrial ecosystems at regional scales. These results reinforce existing global concern over high levels of livestock grazing and soil disturbance by feral animals such as the European rabbit. Land managers should aim to keep populations of free-ranging wild herbivores such as feral goats and rabbits at low levels. Successful pastoralists use strategies such as low-risk (conservative) stocking practices and aim to destock early when droughts are imminent in order to reduce the pressure on plants and soils. Ultimately the challenge is to balance the competing needs of human populations that derive a living from grazing, with the need to maintain stable and productive ecosystems and habitat for native animals.

Acknowledgements We thank James Val and Samantha Travers for all aspects of field work, Marta Ruiz-Colmenero, James Glasier and staff from OEH, Umwelt and Ecology Australia for assistance with some data collection and management, and Sarah Carr and Ian Oliver for project management. We thank two anonymous reviewers and Dr. Thomas Kuyper, Section Editor, for their positive comments that have helped improve the manuscript.

References

Andrews S (2010) FastQC: a quality control tool for high throughput sequence data. Available online at: http://www.bioinformatics.babraham.ac.uk/projects/fastqc

Archer E (2016) rfPermute. Permutation P-values for Random Forest Importance Metrics. V2.1.5

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

Breiman L (2001) Random forests. Mach Learn 45:5–32

Cournane FC, McDowell R, Littlejohn R, Condron L (2011) Effects of cattle, sheep and deer grazing on soil physical quality and losses of phosphorus and suspended sediment losses in surface runoff. Agric Ecosyst Environ 140:264–227

De Deyn GB, Quirk H, Bardgett RD (2011) Plant species richness, identity and productivity differentially influence key groups of microbes in grassland soils of contrasting fertility. Biol Lett 7:75–78

Delgado-Baquerizo M, Maestre FT, Gallardo A, Bowker MA, Wallenstein MD, Quero JL et al (2013) Decoupling of soil nutrient cycles as a function of aridity in global drylands. Nature 502:672–676

Dickinson CH, Underhay VHS (1977) Growth of fungi in cattle dung. Trans Br Mycol Soc 69:473–477

Ducousso M, Duponnois R, Thoen T, Prin Y (2012) Diversity of ectomycorrhizal fungi associated with eucalyptus in Africa and Madagascar. Int J For Res 2012:1–10. https://doi.org/10.1155/2012/450715.ID450715

Edgar RC (2013) UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat Methods 10:996–998

Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R (2011) UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27:2194–2200

Eldridge DJ, Delgado-Baquerizo M, Travers SJ, Val J, Oliver I (2016) Do grazing intensity and herbivore type affect soil health? Insights from a semi-arid productivity gradient. J Appl Ecol 54:976–985

Eldridge DJ, Poore AGB, Ruiz-Colmenero M, Letnic M, Soliveres S (2016b) Ecosystem structure, function and composition in

rangelands are negatively affected by livestock grazing. Ecol Appl 36:1273–1283

Grace JB (2006) Structural equation modelling and natural systems. Cambridge University Press, UK

Ihrmark K, Bodeker ITM, Cruz-Martinez K, Friberg H, Kubartova A, Schenck J et al (2012) New primers to amplify the fungal ITS2 region - evaluation by 454-sequencing of artificial and natural communities. FEMS Microbiol Eco 82:666–677

Joergensen RG, Emmerling C (2006) Methods for evaluating human impact on soil microorganisms based on their activity, biomass, and diversity in agricultural soils. J Plant Nutr Soil Sci 169:295–309

Kõljalg U, Nilsson RH, Abarenkov K, Tedersoo L, Taylor AFS, Bahram M et al (2013) Towards a unified paradigm for sequence-based identification of fungi. Mol Ecol 22:5271–5277

Lamb EG, Kennedy N, Siciliano SD (2011) Effects of plant species richness and evenness on soil microbial community diversity and function. Plant Soil 338:483–495

Maestre FT, Delgado-Baquerizo M, Jeffries TC, Eldridge DJ, Ochoa V, Gozalo B et al (2015) Increasing aridity reduces soil microbial diversity and abundance in global drylands. Proc Natl Acad Sci U S A 112:15684–15689

Maestre FT, Eldridge DJ, Soliveres S, Kéfi S, Delgado-Baquerizo M, Bowker MA et al (2016) Structure and functioning of dryland ecosystems in a changing world. Annu Rev Ecol Evol Syst 47:215–237

Magoč T, Salzberg SL (2011) FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics 27:2957–2963

Marques FFC, Buckland ST, Goffin D, Dixon CE, Borchers DL, Mayle BA et al (2001) Estimating deer abundance from line transect surveys of dung: sika deer in southern Scotland. J Appl Ecol 38:349–363

Miller RN, Reinhardt DR, Jastrow JD (1995) External hyphal production of vesicular–arbuscular mycorrhizal fungi in pasture and tallgrass prairie communities. Oecologia 103:17–23

Nguyen NH, Song Z, Bates ST, Branco S, Tedersoo L, Menke J et al (2016) FUNGuild: an open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecol 20:241–248

Reiter N, Lawrie A, Walsh N (2013) The mycorrhizal associations of *Borya mirabilis*, an endangered Australian native plant. Muelleria 31:81–88

Rillig MC, Mummey DL (2006) Mycorrhizas and soil structure. New Phytol 171:41–53

Schermelleh-Engel K, Moosbrugger H, Müller H (2003) Evaluating the fit of structural equation models: Tests of significance and descriptive goodness-of-fit measures. Method Psych Res Online 8:23–74

Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA et al (2009) Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 75:7537–7541

Srivastava R, Aragno M, Sharma AK (2010) Cow dung extract: a medium for the growth of pseudomonads enhancing their efficiency as biofertilizer and biocontrol agent in rice. Indian J Microbiol 50:349–354

Steinfeld H, Gerber P, Wassenaar T, Castel V, Rosales M, de Haan C (2006) Livestock's long shadow. Environmental Issues and Options. FAO: Rome

Treseder, K.K., Lennon, J.T., 2015. Fungal traits that drive ecosystem dynamics. Microbiol Mol Biol Rev 79, 243–262

Van der Heyde M, Ohsowski B, Abbott LK, Hart M (2017) Mycorrhizal fungus responses to disturbance are context-dependent. Mycorrhiza 27:431–440

Wang Q, Garrity GM, Tiedje JM, Cole JR (2007) Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol 73:5261–5267

Wei C-L, Rowe GT, Escobar-Briones E, Boetius A, Soltwedel T, Caley MJ et al (2010) Global patterns and predictions of seafloor biomass using random forests. PLoS One 5:e15323

Yokoya K, Kai H, Koga T, Aibe T (1991) Nitrogen mineralization and microbial populations in cow dung, dung balls and underlying soil affected by paracoprid dung beetles. Soil Biol Biochem 23:649–653