

Fire and herbivory drive fungal and bacterial communities through distinct above- and belowground mechanisms



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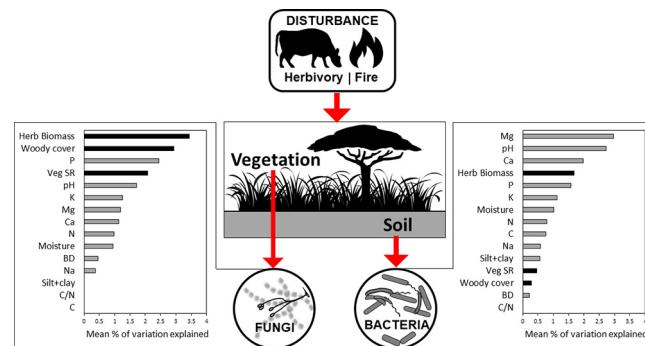
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HIGHLIGHTS

- Exclusion of ecological disturbances led to increases in woody cover.
- Changes in edaphic properties (Mg, pH, Ca) drove bacterial community structure.
- Changes of vegetation drove fungal community structure.
- Soil microbial communities in disturbance-driven grassy biomes are resistant to change.

GRAPHICAL ABSTRACT



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ABSTRACT

Fire and herbivory are important natural disturbances in grassy biomes. Both drivers are likely to influence belowground microbial communities but no studies have unravelled the long-term impact of both fire and herbivory on bacterial and fungal communities. We hypothesized that soil bacterial communities change through disturbance-induced shifts in soil properties (e.g. pH, nutrients) while soil fungal communities change through vegetation modification (biomass and species composition). To test these ideas, we characterised soil physico-chemical properties (pH, acidity, C, N, P and exchangeable cations content, texture, bulk density, moisture), plant species richness and biomass, microbial biomass and bacterial and fungal community composition and diversity (using 16S and ITS rRNA amplicon sequencing, respectively) in six long-term (18 to 70 years) ecological

Abbreviations: ASVs, amplicon sequence variants; KNP, Kruger National Park; LTERs, Long Term Ecological Research sites; OM, organic matter; SMBC, soil microbial biomass C; VIF, variable inflation factor; WPE, woody plant encroachment.

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research sites in South African savanna and grassland ecosystems. We found that fire and herbivory regimes profoundly modified soil physico-chemical properties, plant species richness and standing biomass. In all sites, an increase in woody biomass (ranging from 12 to 50%) was observed when natural disturbances were excluded. The intensity and direction of changes in soil properties were highly dependent on the topo-pedo-climatic context. Overall, fire and herbivory shaped bacterial and fungal communities through distinct driving forces: edaphic properties (including Mg, pH, Ca) for bacteria, and vegetation (herbaceous biomass and woody cover) for fungi. Fire and herbivory explained on average 7.5 and 9.8% of the fungal community variability, respectively, compared to 6.0 and 5.6% for bacteria. The relatively small changes in microbial communities due to natural disturbance is in stark contrast to dramatic vegetation and edaphic changes and suggests that soil microbial communities, having evolved with disturbance, are resistant to change. This represents both a buffer to short-term anthropogenic-induced changes and a restoration challenge in the face of long-term changes.

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1. Introduction

Grassland and savanna biomes together form the most abundant vegetation type globally, covering approximately 40.5% of the terrestrial area (White et al., 2000). Grassy biomes provide a wide variety of ecosystem goods and services, including wildlife habitat, livestock forage, regulation of fresh water and mineral fluxes, wood products, and socio-cultural services (Sandhage-Hofmann, 2016). Their sustainable use and management require the restoration and maintenance of critical ecological functions. Belowground microbial communities are a key component driving organic matter degradation, nutrient mineralization, and plant nutrient acquisition and growth in these biomes (Bardgett and van der Putten, 2014; Coleman et al., 2017; Wardle et al., 2004).

Fire and mammalian herbivory are among the most important drivers influencing the structure of grassy ecosystems (Archibald et al., 2005; Sankaran et al., 2008, 2005; van Langevelde et al., 2003) and likely their microbial components. Along with water and nutrient availability, fire and herbivory affect the patterns and processes that result in a range of distinct ecosystems, from forests to savannas to grasslands, within the same climate envelope. Fire and herbivory regimes have been deeply modified by human activity, resulting in changes in the overall structure of many African landscapes (Venter et al., 2017). Non-forest biomes in Africa have undergone a net 8% increase in woody plant cover over the past three decades, due to changes in herbivore densities and declines in burned area (Roques et al., 2001; Venter et al., 2018). This gradual woody plant encroachment (WPE) has been identified as a major concern across multiple biotic climate zones since the early 20th century, including grass-dominated biomes, and interactions between fire and herbivory are considered key for controlling WPE (Wilcox et al., 2018).

The importance of fire and herbivory as major drivers of above-ground structure and functioning in African ecosystems raises the question of how these drivers affect soil microorganisms and biogeochemical cycles. A considerable body of literature has explored the mechanisms through which fire- or herbivory-induced changes in vegetation and soil affect microbial communities (Bardgett et al., 1998; Bardgett and Wardle, 2003; Neary et al., 1999; Pellegrini et al., 2019; Wardle et al., 2004). No clear picture has, however, emerged from studies of long-term effects of fire (both prescribed and wildland fires) and herbivory on microbial communities, nor on how regional (e.g. biome) or local (e.g. riparian zone) contexts modify these effects. Fire and herbivory may affect soil microorganisms through several direct and indirect mechanisms. Herbivory directly affects belowground organisms by (1) returning nutrients via faeces and urine in a form more available for plant and microbe uptake compared to the original plant matter; (2) increasing soil bulk density through trampling; and (3) reducing litter input due to the removal of aerial plant parts (Bardgett et al., 1998; Bardgett and Wardle, 2003; Harrison and Bardgett, 2008; Wardle et al., 2004). Fire directly affects belowground communities through a temporary increase in soil temperature, volatilization of minerals and inputs of pyrogenic carbon and ash (Andersson et al., 2004; Certini, 2005; Keeley, 2009; Neary et al., 1999).

Fire and herbivory also indirectly affect soil microbial communities through their impact on the vegetation, both by altering plant fitness and modulating plant community composition. Vegetation modification resulting from herbivory and fire include changes in total biomass production, concentrations of secondary metabolites, resource allocation patterns, and root exudation (Augustine and McNaughton, 1998; Bardgett and Wardle, 2003; Neary et al., 1999). These changes alter carbon (C) inputs to the soil, and can influence soil microorganisms through competition for nutrients and mutualisms (Bever, 2003; Carney and Matson, 2006; Millard and Singh, 2010; Pressler et al., 2019). Whether disturbance-induced change in vegetation increases or decreases soil microbial biomass and diversity depends on (1) the intensity of the disturbance and how it affects the dominant plants (compensatory growth vs. impairment of plant productivity), (2) the ecosystem productivity, and (3) how closely the plants and microorganisms associate (with greater impacts likely for mycorrhiza and parasites). Finally, disturbances can also indirectly affect soil microorganisms through modification of soil properties over long time scales, as differences in plant residue quality and quantity, root exudates and adsorption, and soil trampling can result in profound modification of soil physico-chemical properties (Crocker, 2015; Hart et al., 2005).

Fungi and bacteria have very different requirements and habitats within soil profiles (Baldock and Skjemstad, 2000; Chenu et al., 2002; Gadd, 2007). Fungi are obligatory aerobes and heterotrophs, mainly involved in litter degradation, and are largely restricted to superficial horizons of soil profiles. Mycorrhizal fungi form symbioses with vegetation and are consequently closely affected by change in their host populations (Antunes and Koyama, 2017; Read, 1991). Bacteria have metabolisms that are more versatile. They can adapt to oxic and anoxic environments, and while they are mostly heterotrophs (i.e. rely on labile substrates), some of them are autotrophic. On the other hand, bacteria are less tolerant of acidic conditions than fungi and prefer a pH close to neutrality (Paul, 2014). Edaphic variables, including pH, control bacterial community composition at the global scale (Bahram et al., 2018; Fierer et al., 2009; Fierer and Jackson, 2006; Kaiser et al., 2016; Lauber et al., 2009), while fungi follow similar biogeographic patterns as plants and animals, and are more affected by climatic factors (Bahram et al., 2018; Tedersoo et al., 2014).

To investigate how fire and herbivory affect soil microbial communities in African savannas and grasslands, we studied both bacterial and fungal communities at a range of sites with fire and herbivory treatments that span decades. We hypothesized that soil bacterial communities change through disturbance-induced shifts in soil properties (e.g. pH, nutrients) while soil fungal communities change through vegetation modification (biomass and species composition). We tested these hypotheses by characterising soil, vegetation and soil microbial communities at six experimental areas covering a wide range of topo-pedo-climatic contexts. We expect that the amplitude of the effect of fire and herbivory on soil microbes will be larger in the sites where the ecosystem productivity allows a larger modification of vegetation and consequently of soil properties.

2. Materials and methods

2.1. Approach

We studied four field experiments at Long Term Ecological Research sites (LTers) (described in Table 1), two in the Savanna biome and two in the Grassland biome of South Africa. The two savannas were semi-arid, one having slightly higher annual rainfall (hereafter "moist savanna") than the other (hereafter "dry savanna"). The grasslands were either montane (moist, nutrient-poor leached soils, hereafter "high altitude grassland") or lowland (relatively dry, nutrient rich soils, hereafter "low altitude grassland"; Table 1). Both savanna LTers had distinct nutrient-poor upslopes (hereafter, "crest" zone) and relatively nutrient-enriched down-slopes, which transitioned to riparian zones of perennial rivers (hereafter, "riparian" zone). Stratification of the upper slopes and riparian zones provided an additional two experimental areas, which allowed for determining the importance of local-scale variations due to the proximity of a river. The six experimental areas are hereafter referred as "sites".

2.2. Site descriptions

The moist and dry savanna sites were located in the Kruger National Park (KNP). Each had exclosures which exclude all herbivores larger than ~5 kg over an area of 70 ha and 42 ha, respectively. Adjacent unfenced areas (25 and 36 ha for the moist and dry savanna sites, respectively) are open to a full suite of herbivores indigenous to the area. Each exclosure and unfenced area is divided into burn- and no-burn sections. The fenced areas, unfenced area and burn and no-burn areas all extend from the riparian zone to adjacent upslopes. Prescribed burning occurred between June and October approximately once every five years, with four burn events between the erection of the exclosures and sampling (2002, 2007, 2012 and 2017). Complete ecological and edaphic descriptions of all sites have been previously published (Paterson and Steenkamp, 2003a, 2003b; Siebert et al., 2010; Siebert and Eckhardt, 2008). Within each site, we sampled four treatments on the same soil type: (1) no herbivory, no fire; (2) no herbivory plus fire; (3) herbivory no fire; (4) herbivory plus fire. We randomised sampling by creating 10 random GPS points per treatment constrained to

polygons stratified to a single soil form using QGIS (reference. 3.08, GNU General Public License). The sampling was done between February and April 2018.

The low altitude grassland site was at the Ukulinga Research Farm (University of KwaZulu-Natal; Table 1). The experiment involves a combination of burning and simulated herbivory treatments (mowing and removal of the cut material). In the mowing treatment, the material is removed from the treatment plots. All treatments are replicated three times in a randomised block design. Plot sizes are 18.3 × 13.7 m spaced by 4 m corridors. We selected the following burning treatments for sampling: no burn, biennial burn, triennial burn, with factorial combinations of either no mowing or two cuts per year (one early December and one end of February). Burns are applied in the first week of August. The site was sampled at the beginning of December 2017. More details about the experimental site, design and treatments can be found in Fynn et al. (2004) and Knapp et al. (2006).

The high-altitude grassland site was on the Brotherton plateau at Cathedral Peak in uKhahlamba-Drakensberg Park (Table 1). Burn treatments have been applied to 25 × 25-m plots since 1980, replicated three times in a randomised block design. The treatments inside the trial are protected from herbivores, and wild herbivores graze the surroundings at low density. Three treatments were sampled: no burn, annual autumn and biennial autumn. To include the herbivore effect, we also sampled areas outside the trial: three plots in the firebreak zone (with annual burning) and three plots outside the firebreak zone, which burns every two years in Ezemvelo KZN Wildlife management fires. This field experiment did not include an herbivory with no burn treatment. The site was sampled at the end of January 2018. More details about the experimental site and design can be found in Manson et al. (2007).

2.3. Sampling design and physico-chemical analysis

At each sampling point ($n = 10$ per treatment for savanna sites and $n = 6$ per treatment for grassland sites), five bulk soil cores were collected using an auger (20-cm length, 5-cm diameter) after removing surface debris, homogenised in-field and split into two subsamples. The first subsample was immediately stored on ice, then kept in the freezer and processed within one week for DNA extraction. The second

Table 1

Pedo-topo-climatic context, vegetation, common herbivores and establishment dates of the Long-Term Ecological Research sites (LTers). Abbreviations: Mean annual precipitations (MAP); mean annual temperatures (MAT); soil types according the World Reference Base (WRB); large stock unit (LSU).

	Grassland		Savanna	
	High altitude	Low altitude	Moist	Dry
LTers	Brotherton	Ukulinga	Nkuhlu	Letaba
Established	1980	1950	2002	2002
MAP (mm)	1075	820	560	400
MAT (°C)	15	16	22	23
Altitude (m)	1890	840	Riparian: 200 Crest: 240	Riparian: 253 Crest: 280
Location	29.00°S 29.00°E	29.67°S 30.40°E	24.99° S 31.77°E	23.75°S 31.44°E
Lithology ^a	Basalt overlying cave sandstone	Colluvium shale with intrusions of dolerite	Grey migmatite and gneiss of the Nelspruit Suite, Swazian Erathem	Grey biotite gneiss and migmatite of the Makhutsi Gneiss, Swaziland Erathem
Soil (WRB) ^b	Rhodic Acrisols	Dystric Regosols	Riparian and crest: Eutric Regosols	Riparian and crest: Ferric Luvisols
Vegetation type ^c	uKhahlamba Basalt Grassland (Gd7)	KwaZulu-Natal Hinterland Thornveld (SVs3)	Granite Lowveld (SV13)	Lowveld Rugged Mopaneveld (SVmp 6)
Common herbivores	<i>Alcelaphus buselaphus caama</i> , <i>Damaliscus pygargus phillipsi</i> , <i>Ourebia ourebi</i> , <i>Pelea capreolus</i> , <i>Redunca arundinum</i> , <i>Redunca fulvorufa</i> , <i>Tragelaphus oryx</i>	n/a	<i>Aepyceros melampus</i> , <i>Giraffa camelopardalis</i> , <i>Hippopotamus amphibius</i> , <i>Kobus ellipsiprymnus</i> , <i>Loxodonta africana</i> , <i>Pedetes capensis</i> , <i>Syncerus caffer</i> , <i>Tragelaphus strepsiceros</i>	
Animal densities ^{d,e}	55 ha LSU ⁻¹	Simulated (mowing)	30 ha LSU ⁻¹	20 ha LSU ⁻¹

^a SOTER database (Engelen and Dijkshoorn, 2013).

^b IUSS Working Group WRB (2015).

^c Mucina and Rutherford (2006).

^d Rowe-Rowe (1986).

^e Smit et al. (2013).

subsample was air-dried, sieved at 2-mm and used for soil physico-chemical analyses. Bulk density samples were taken at 8 to 13 cm depth using a 100-cm³ stainless steel sample ring (Eijelkamp Soil and Water, Netherlands). Field water content was measured by weighing the bulk density sample before and after drying at 105 °C according to the standardized method ISO 11465 (1993).

Soil pH and exchangeable acidity were measured in 1-M KCl and 1-M K₂SO₄ soil extracts respectively, while plant available ions were measured by inductively coupled plasma spectrometry (Thermo iCAP 6000 Series Spectrometer, Thermo Fisher Scientific, Surrey, UK) after extraction of soil in 1% (w/v) citric acid (P, K, Ca, Mg and Na) as per standard soil testing methods (Soil Science Society of South Africa, 1990). Subsamples of soil were ground into a fine powder using a ball mill and analysed for total C and N via Dumas combustion using a Flash 2000 organic elemental analyser (Thermo Scientific, Bremen, Germany) and in-house standards (Merck gel and plant leaf material). Soil particle size distribution was measured by laser diffraction using a Malvern Mastersizer and Hydro 2000G (Malvern Panalytical, Almelo, Netherlands), after removing the OM with 6% H₂O₂ and dispersion using 5% sodium hexametaphosphate.

Microbial biomass C was estimated from the UV absorbance of extracts of fumigated and unfumigated soils (modified from Turner et al., 2001). Briefly, frozen soil samples were thawed and incubated at 40% water holding capacity, 25 °C and in the dark for 7 days. The UV absorbance of filtered (Whatman no. 42) 0.5 M K₂SO₄ extracts of fumigated and unfumigated soils were measured at 280 nm. The following equation was used to convert to soil microbial biomass C: SMBC (µgC g⁻¹dw) = 34,735 x (increase in UV280 after fumigation).

2.4. Plant biomass and composition

We employed plant-sampling methods used routinely and historically for each site. Standing herbaceous plant biomass (g m⁻²) was measured at the peak of the growing season (February in grassland sites, March in savanna sites). All herbaceous biomass was clipped in five to ten 0.5 m² quadrats and eight 1 m² quadrats per treatment, in the high and low altitude grassland site, respectively. These samples were oven-dried at 60 °C to constant weight and weighed. For the high altitude grassland site, measurements from a disc pasture meter (30 drops per treatment) that was calibrated using clipped quadrats was used. In the savanna sites, herbaceous biomass was estimated using a disc pasture meter (15 drops per treatment) calibrated according to Zambatis et al. (2006). The percentage woody cover at each sampling point was calculated using satellite imagery as described by Venter et al. (2018). For a detailed summary of remote sensing methods, see Supplementary Material A. Herbaceous and woody plant species composition in the grassland sites was measured using a line-point-intercept method (Levy bridge; Levy and Madden, 1993), with plant species identified at 10 descending points spaced 25 cm apart (200 points per treatment). Plant species composition in the savanna sites was measured using five replicate 1 m² quadrats at each subsample point.

2.5. High-throughput sequencing of bacterial and fungal ribosomal markers and data processing

DNA was isolated from 0.5 g of frozen soil using the PowerSoil DNA isolation kit (MO BIO Laboratories, Carlsbad, CA) according to the manufacturer's recommendations. DNA samples were sent to the Biology Department at Montclair State University (USA) for sequencing on the Illumina MiSeq platform (Illumina Inc., San Diego, CA, USA). PCR amplification of the bacterial and fungal markers was performed as described in the Illumina Metagenomic Sequencing Library Preparation with the adaption of using the I-5™ Hotstart DNA Polymerase from Molecular Cloning laboratories (South San Francisco, CA 94080), with PCR primers sequences targeting the 16S rRNA gene V3 and V4 regions

(Illumina_16S_341F and Illumina_16S_805R) and the ITS region of the rRNA operon (ITS_fwd_7 and ITS_rev_6).

Fungal sequence data were processed using the QIIME2 v2019.4.0 pipeline (Bolyen et al., 2019). Briefly, forward and reverse sequences were merged (min. overlap = 20 bp) and sequences that did not perfectly overlap removed. Merged reads were quality filtered and denoised with DADA2 (Callahan et al., 2016) plugin in QIIME2. Reads were truncated to 240 bp. Taxonomy was assigned using the QIIME2 q2-feature-classifier (Bokulich et al., 2018) classify-sklearn naïve Bayes taxonomy classifier against the Unite V8 (ITS) (Unite community, 2019) reference taxonomy for 97% identity. Putative fungal functional groups were identified using FUNGuild (Nguyen et al., 2016). Bacterial reads were processed using facilities provided by the University of Cape Town's ICTS High Performance Computing team: hpc.uct.ac.za. Illumina MiSeq read quality assessment and taxonomic profiling were performed using a custom Nextflow pipeline, available at: <https://github.com/h3abionet/16S-rDNA-dada2-pipeline>. Quality was assessed with FastQC (Andrews, 2010) and MultiQC (Ewels et al., 2016), reads were truncated at 265 bp and the first 10 bp from the start of the forward and reverse reads were trimmed. Default settings were used for the remainder of the pipeline, which uses the DADA2 method to group reads into ASVs. Taxonomic assignment was performed against the RefSeq-RDP 16S database (v3 May 2018). After quality filtering and chimera removal, amplicon sequencing yielded a total of 10,285,016 bacterial 16S rRNA genes (average of 42,854 ± 32,386 per sample) and 25,211,240 fungal ITS (average of 131,309 ± 77,172 per sample) high-quality sequences. Bacterial and fungal α-diversity were estimated by calculating observed richness and Shannon diversity index per site. For further analyses, rare microbial taxa were removed, keeping only ASVs that had at least 10 counts in at least 10% of samples, or a total relative abundance of >0.001 of the total number of reads.

2.6. Statistical analyses

All downstream statistical analyses were performed using R software (R Core Team, 2018). The main packages used were phyloseq (McMurtrie and Holmes, 2013), vegan (Oksanen et al., 2007), multcomp (Hothorn et al., 2016) and lme4 (Bates et al., 2007).

To assess the effects of fire, herbivory and their two-way interactions on soil, vegetation, α-diversity parameters and fungal guild composition per site, we used linear models, ANOVAs and generalized linear models (poisson regression for count data). The soil and vegetation variables were z-transformed to standardize and thus enable comparison of linear model estimates. For the linear models, the assumptions of normality and homogeneity of variances of the residuals were checked both visually and with the Shapiro-Wilk and Levene's tests. When the differences were significant, they were further analysed using a post hoc Tukey's Honest Significant Difference (HSD) test.

Differences in microbial β-diversity were examined using Bray-Curtis dissimilarities calculated from standardized (i.e., proportions) and square-root transformed ASV abundances (Hellinger transformation). The heterogeneity of variance between groups was tested using the function *betadisper* in the vegan package. The significance of the experimental factors (fire, herbivory, their interaction) on the bacterial and fungal communities in the different sites was tested using PERMANOVA (*adonis* function - vegan package) with 99,999 permutations.

The relationship between soil properties and microbial β-diversity was assessed using redundancy analysis (RDA) using the vegan R package. The selection of the 'best' explanatory environmental variables for each site was done by forward selection using the *ordistep* function (Blanchet et al., 2008). In order to avoid collinearity, the sum of the percentages of silt and clay was used as soil texture variable, as in Delgado-Baquerizo et al. (2019). Collinearity among the selected explanatory variables was tested by checking the variable inflation factor (VIF) of the selected variables using the *vif.cca* function from vegan. The VIF

values of the best predictors of microbial community structure were all below 10 as recommended by Montgomery and Peck (1992), and most of them below 3. We assume that none of these variables are redundant and that the regression coefficients are not excessively inflated due to multicollinearity in the model (James et al., 2013).

3. Results

3.1. Disturbance effects on soil and vegetation

The six sites had distinct soil properties, with clear differences between all sites, particularly the grassland versus savanna sites (Table S1). The two grassland soils were loams, the high-altitude grassland having the highest clay (22%), C (8.5%) and soil moisture (92% dry weight), but the lowest pH (4.4) and highest acidity (4.7 cmol kg⁻¹). The low altitude grassland was intermediate, with 13% clay, 43% silt, 3% C, 25% soil moisture, a pH of 4.5 and an acidity of 2.2 cmol kg⁻¹. The two savanna sites were sandy (80% sand) with low C concentration and soil moisture (~1% and 6%, respectively) and higher pH than the grassland sites (5.7). In both savanna sites, soil nutrients (N, C, Mg, Na, P) were higher in the riparian zone, while Ca was only higher in the riparian zone of the moist site and K in the riparian zone of the dry site. As expected, the woody cover was also consistently higher in riparian zones of both savanna sites, while plant species richness was highest on the upland crest (Table S1).

Fire and herbivory influenced most of the measured soil physico-chemical properties, but the influences differed in direction (increase or decrease) and magnitude between sites (Fig. 1, Table S1). Compared to the treatment without disturbance, fire increased soil pH except in the moist savanna crest site. Fire and herbivory affected soil C and N concentration in site- and zone-dependent ways: fire increased soil C and N in the absence of herbivory in the high-altitude grassland site, but decreased total N concentration in the low altitude grassland. In the dry savanna, the disturbances did not affect soil C at the crest but herbivory decreased soil C and N in the riparian zone. In the moist savanna, fire increased soil C and N in the absence of herbivory in the riparian zone, and when combined with herbivory in the crest zone. Herbivory decreased available cation concentration at all sites, while the effect of fire varied depending on the site.

Fire and herbivory profoundly modified the vegetation relative to treatments without disturbance. In all sites, a significant increase in woody biomass was observed when disturbances were excluded (Fig. 2, Table S1). The effect of the disturbances on the herbaceous

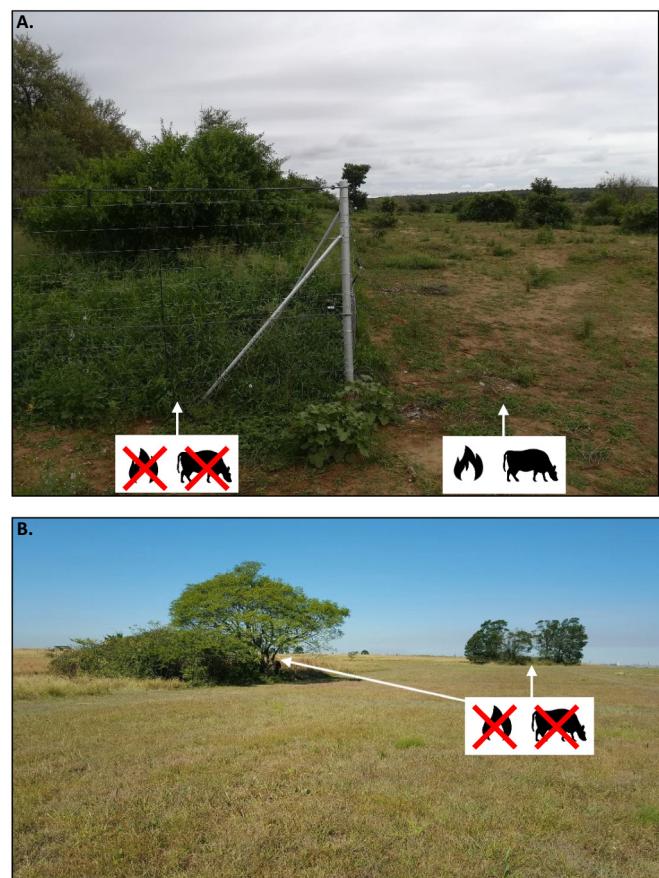


Fig. 2. (A) Difference between the treatment without disturbance and the treatment with fire and herbivory in the riparian zone of the dry savanna site; (B) Low altitude grassland, showing the woody encroachment in the treatments without disturbance compared to the adjacent treatments.

biomass and vegetation species richness differed by site. Herbaceous plant species richness was not affected by fire, but decreased and increased with herbivory in the high-altitude grassland and savanna sites, respectively. Herbaceous biomass increased with fire, and decreased with herbivory. Both disturbances increased the soil microbial

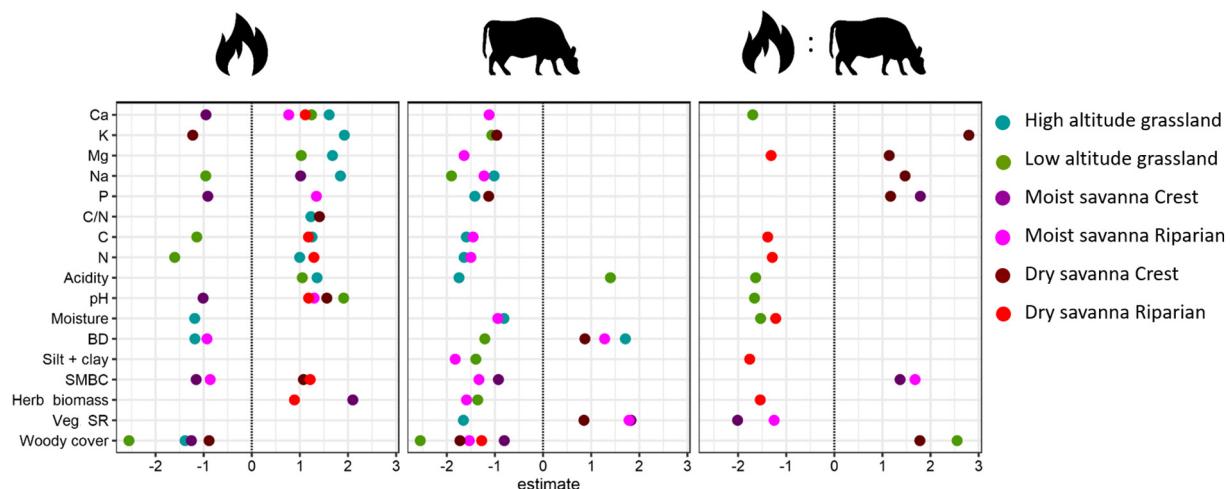


Fig. 1. Least square estimates of the linear model testing the effect of fire (flame icon), herbivory (rhino icon), and their interaction on the z-transformed soil and vegetation parameters for the six studied sites. Only the significant effects ($p < .05$) are presented. Available elements (Ca, Mg, Na, K, P); total C, N concentration and their ratio (C, N and C/N); pH KCl (pH); field moisture (Moisture); bulk density (BD); silt+clay fraction; soil microbial biomass C (SMBC); herbaceous biomass (Herb biomass); vegetation species richness (Veg SR); woody cover. High and low altitude grasslands are in blue and green, respectively. Moist and dry savanna are in purple and red, respectively, dark and light for crest and riparian zones, respectively.

biomass C in the moist savanna riparian site and decreased it in the dry savanna sites (Fig. 1, Table S1).

3.2. Disturbance effects on soil microbial community composition

We obtained a total of 164,942 (1205 ± 921 per sample) bacterial and 86,998 (1097 ± 447 per sample) fungal ASVs. The dominant bacteria phyla were Actinobacteria (relative abundance between 20 and 31% depending on the site), Acidobacteria (between 14 and 40%), Proteobacteria (between 16 and 26%) and Verrucomicrobia (between 3 and 27%). The dominant fungal phyla were Ascomycota (between 32 and 82%) and Basidiomycota (between 12 and 52%) (Fig. S1).

Shifts in α -diversity across the different treatments were observed (Table 2), but in different intensities and directions depending on the site. Fire and herbivory modified fungal and bacterial richness in all sites, but the Shannon index only on the low altitude grassland for fungi (significant increase of the index with disturbances) and the moist savanna crest for bacteria (significant decrease of the index when only fire is applied). The taxonomical parsing of fungal ASVs by ecological guilds (Fig. 3), revealed a negative impact of disturbances on fungal symbiotrophs as well as saprotrophs-symbiotrophs, especially in the grasslands.

In all sites, disturbances explained significant portions of variation in both bacteria and fungi β -diversity (Table 3). Fire and herbivory explained 6.0 and 5.6%, respectively, of the variation in bacterial communities and 7.5 and 9.8% in fungal communities (average R^2 of the 6 sites per disturbance and organism). The interaction between fire and herbivory also shaped both bacterial (except in the case of dry savanna) and fungal communities. The effect differed between sites, fire being the main driver in the moist savanna crest and the high-altitude grassland, and herbivory in the others.

3.3. Relationship between soil, vegetation and microbial community composition

The set of explanatory variables that best predicted the observed changes in bacterial and fungal community composition varied depending on the site (pedo-climatic context), and the microbial community considered (bacteria or fungi, Fig. S2). The average importance of each parameter was calculated separately for bacteria and fungi (Fig. 4). The predictors explaining the highest percentage of variation (Fig. 4) were Mg > pH > Ca > herbaceous biomass for bacteria (explaining 3.0, 2.7, 2.0 and 1.7% of the variation, respectively), and for fungi herbaceous biomass > woody cover > P > vegetation species richness (explaining 3.4, 2.9, 2.4 and 2.1% of the variation, respectively).

4. Discussion

The direction and magnitude of fire and herbivory effects on soil microbial biomass and diversity were not consistent between sites. This suggests that one cannot easily predict the effects of fire and herbivory without knowing the site-specific climatic and topographic context. Indeed, no consensus exists in the literature regarding the effect of fire and herbivory on microbial biomass and communities either. Differences in biome (Dooley and Treseder, 2012), soil fertility (Bardgett and Wardle, 2003), fire regimes and grazing intensity (Bardgett et al., 1998; Zhao et al., 2017) are known to contribute to these observed discrepancies, but no generalizable pattern emerges. Investigators have found increases, decreases, or no effects of mammalian grazing on microbial biomass carbon, depending on the ecosystem and its abiotic context (Bardgett and Wardle, 2003; Hamilton et al., 2008). Fire mostly decreased soil microbial biomass, abundance, richness, evenness, and diversity according to meta-analysis (Dooley and Treseder, 2012;

Table 2

Fungal and bacterial α -diversity (total species richness, and Shannon index) in the six sites. Values are means and \pm standard deviation per treatment. Treatments: + and - when fire (🔥) and herbivory (🐂) were applied or excluded, respectively. The small letters and bold characters represent the significant difference between treatments, as measured by two-way ANOVA and generalized linear model, for Shannon and richness, respectively, and post-hoc Tukey HSD test. The letters are separate for the different organisms and metrics.

Site	Treatment		Fungi		Bacteria	
	🔥	🐂	Richness	Shannon	Richness	Shannon
High Altitude Grassland	+	+	1120 ± 281^c	5.4 ± 0.5	1375 ± 812^b	6.5 ± 0.5
	+	-	1066 ± 562^b	5.5 ± 0.5	1324 ± 484^a	6.5 ± 0.4
	-	-	763 ± 93^a	4.8 ± 0.2	1318 ± 433^a	6.6 ± 0.4
Low Altitude Grassland	+	+	1039 ± 182^b	5.3 ± 0.4^b	1231 ± 554^a	6.3 ± 0.6
	+	-	1072 ± 194^b	5.2 ± 0.3^b	1599 ± 482^c	6.6 ± 0.3
	-	+	1287 ± 276^c	5.4 ± 0.8^b	1388 ± 155^b	6.5 ± 0.1
	-	-	787 ± 215^a	4.1 ± 0.8^a	1655 ± 419^d	6.7 ± 0.3
Moist Savanna Crest	+	+	1729 ± 152^b	5.7 ± 0.4	2129 ± 932^c	7 ± 0.4^{ab}
	+	-	1648 ± 262^a	5.7 ± 0.3	1005 ± 199^a	6.4 ± 0.2^a
	-	+	1792 ± 299^c	5.7 ± 0.4	1696 ± 573^b	6.8 ± 0.3^{ab}
	-	-	1701 ± 354^b	5.8 ± 0.2	2705 ± 2079^d	7.1 ± 0.5^b
Moist Savanna Riparian	+	+	1527 ± 214^a	5.6 ± 0.3	1241 ± 201^a	6.7 ± 0.2
	+	-	1838 ± 393^b	5.7 ± 0.3	2103 ± 931^d	7.1 ± 0.4
	-	+	1527 ± 409^a	5.5 ± 0.4	1906 ± 1409^c	6.9 ± 0.4
	-	-	1851 ± 152^b	5.9 ± 0.2	1490 ± 616^b	6.6 ± 0.6
Dry Savanna Crest	+	+	1002 ± 310^b	5.3 ± 0.5	1168 ± 264^b	6.5 ± 0.3
	+	-	880 ± 154^a	5.2 ± 0.4	1119 ± 620^a	6.4 ± 0.8
	-	+	1018 ± 217^b	5.3 ± 0.3	1199 ± 232^b	6.6 ± 0.2
	-	-	988 ± 284^b	5.4 ± 0.2	1456 ± 441^c	6.8 ± 0.3
Dry Savanna Riparian	+	+	899 ± 302^a	5.1 ± 0.4	1568 ± 459^b	6.8 ± 0.4
	+	-	1264 ± 122^b	5.4 ± 0.3	2013 ± 1966^c	7 ± 0.5
	-	+	1367 ± 415^c	5.2 ± 0.4	1592 ± 572^b	6.8 ± 0.3
	-	-	898 ± 367^a	5.2 ± 0.2	1299 ± 226^a	6.7 ± 0.2

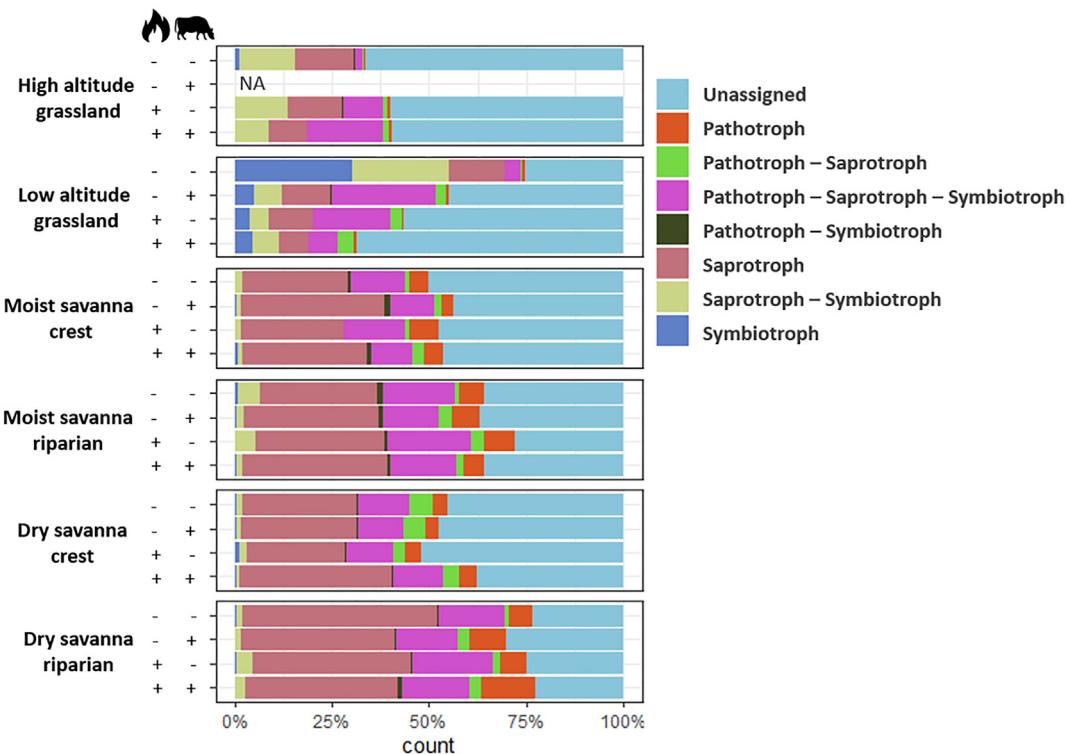


Fig. 3. Analysis of fungal guilds with FUNGuild per site and treatment. X-axis gives counts of the fungal trophic modes as relative abundances [%]. Y-axis shows treatments per site: + and – when fire (🔥) and herbivory (🐂) were applied or excluded, respectively. NA stands for 'not available'.

Pressler et al., 2019). Apart from being variable between sites, the changes induced by fire and herbivory were relatively small in comparison to the differences between the sites, especially considering the long-term duration of the experimental manipulations. This suggests that the microbial communities exhibit a degree of resistance to change (see below).

In our sites, the consequences of the disturbances on soil physico-chemical parameters were highly variable (different magnitudes and directions), depending on the site ecology. Given that soil physico-chemical characteristics are important determinants of soil

microorganisms, it is not surprising that we also found variable microbial responses. The variability of soil physico-chemical responses to fire and herbivory has been highlighted by several reviews and meta-analyses (Augustine and McNaughton, 1998; Certini, 2005; González-Pérez et al., 2004; McSherry and Ritchie, 2013; Wardle et al., 2004; Zhou et al., 2017). The effect of fire on the soil C and N in the surface layers may range from almost total consumption of the soil organic matter (SOM) to increases of up to 30% (González-Pérez et al., 2004; Pellegrini et al., 2018; Raison, 1979; Wan et al., 2001). A review on the effect of fire on soil aggregation also revealed that the patterns observed

Table 3

Impact of fire (🔥), herbivory (🐂) and their two-way interaction on bacterial and fungal β -diversity. Values present the output of the PERMANOVA on the Bray-Curtis distance matrix of Hellinger-transformed abundance data in the six sites.

Site	Bacteria			Fungi					
	F.Model	R ²	Pr(>F)	F.Model	R ²	Pr(>F)			
Dry savanna Crest	🔥	1.5	0.04	0.017	*	1.4	0.04	0.012	*
	🐂	1.5	0.03	0.024	*	2.1	0.06	0.001	***
	🔥:🐂	1.3	0.03	0.083		1.5	0.04	0.003	**
Dry savanna Riparian	🔥	1.9	0.04	0.002	**	2.0	0.06	0.006	**
	🐂	4.1	0.10	0.001	***	5.5	0.16	0.001	***
	🔥:🐂	1.3	0.03	0.087		1.5	0.04	0.043	*
Moist savanna Crest	🔥	2.9	0.06	0.001	***	3.1	0.08	0.001	***
	🐂	1.6	0.03	0.019	*	2.0	0.05	0.002	**
	🔥:🐂	1.8	0.04	0.006	**	1.8	0.04	0.001	***
Moist savanna Riparian	🔥	1.7	0.04	0.005	**	1.8	0.05	0.007	**
	🐂	2.3	0.05	0.001	***	4.4	0.13	0.001	***
	🔥:🐂	1.6	0.04	0.012	*	1.4	0.04	0.035	*
High altitude Grassland	🔥	3.9	0.12	0.001	***	3.7	0.13	0.001	***
	🐂	2.4	0.07	0.002	**	2.5	0.09	0.001	***
Low altitude Grassland	🔥	2.5	0.06	0.002	**	3.5	0.09	0.001	***
	🐂	2.5	0.06	0.002	**	4.2	0.10	0.001	***
	🔥:🐂	2.0	0.05	0.02	*	2.5	0.06	0.002	**

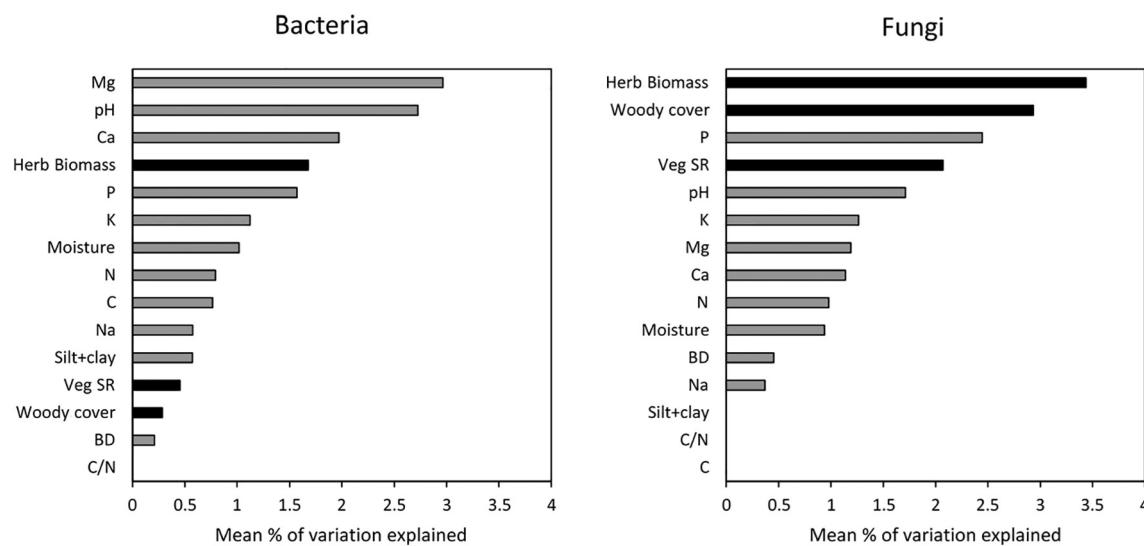


Fig. 4. Average percentage of variation of the fungal and bacterial β -diversity explained by the soil and vegetation parameters (each value is the mean of the constrained ordination models of the six sites). The soil physico-chemical properties are in grey and the vegetation characteristics in black. Abbreviations: bulk density (BD); herbaceous biomass (Herb Biomass); vegetation species richness (Veg SR); available elements (P, Na, Ca, K, Mg); carbon and nitrogen concentration (C, N); soil field moisture (Moisture).

can vary from a disaggregation due to organic matter destruction, to strong aggregation (Mataix-Solera et al., 2011). Mammal grazing has also significantly large, but highly variable effects on grassland SOM (Derner and Schuman, 2007; McSherry and Ritchie, 2013; Pineiro and Paruelo, 2010). The complexity of predicting the ratio between carbon sequestration and release to the atmosphere in response to long-term changes in fire and herbivory regimes represents a challenge in the design of climate-smart land management strategies. Ecosystem-level modelling will be essential if we are to tease apart drivers occurring at various intensities and frequencies, in a diversity of contexts. The long-term effect of fire and herbivory on other soil parameters (pH, available cations, bulk density, moisture) has been the subject of less attention than SOM. Pooling data across all climatic zones, Abdalla et al. (2018) revealed that, on average, increased grazing intensity significantly increased soil bulk density, mainly due to increased animal trampling, but the effect on soil pH was small. Soil pH is increased after fire due to organic acid denaturation, and the consequent release of bound cations including K, Ca, and Mg (Certini, 2005). Long-term fire-induced changes to the cycles of these soil nutrients vary according to the studies and fire recurrence, but are generally ephemeral, i.e. a few months (Alcañiz et al., 2018; Certini, 2005). According to the review of Alcañiz et al. (2018), burn events that did not change pH values were prescribed fires of low intensity and severity applied periodically (every 2 years or more) or single treatments carried out once rather than annually.

The above-mentioned reviews and meta-analyses suggest that the lack of consensus is due to the diversity of fire and herbivory regimes, plant communities and ecosystem properties (climate, topography, soil type). Fire severity differs among biomes and fire types. Low intensity (energy) fires such as prescribed fires are commonly also of low severity or ecosystem impact as they are intended to manage and reduce fuel accumulation, and changes to the soil produced by this type of fire are in most cases only transient (Mataix-Solera et al., 2011). However, high intensity fires that severely affect biota, such as summer wildfires, generally have similarly severe effects on soil (Certini, 2005). Weather conditions (temperature, humidity, wind, and rainfall) and consequent fuel characteristics (amount, moisture and structural and chemical composition) affects both fire intensity and herbivory. Soil fertility determines herbivore diversity and the magnitude of herbivores effect on soil and soil biota. As reviewed in Bardgett and Wardle (2003), in productive ecosystems, which are dominated by palatable, nutrient rich plants and support a greater diversity and level of herbivory, labile

nutrients are returned to the soil, leading to increased microbial activity (mineralization) and hence plant productivity. In contrast, in unproductive ecosystems, with low herbivory rates, selective grazing leads to changes in the functional composition of the vegetation and especially the dominance of plant species that produce litter of poor nutritional quality to decomposers. In addition to the difficulty of predicting the impact of fire and herbivory individually, the fact that these two consumers of aboveground biomass interact and can either promote or exclude each other represents another challenge. Persistent heavy grazing can prevent the spread of fire by breaking up the grass layer; in contrast, frequent burning might inhibit the persistence of grazed patches by attracting grazers to the post-burn green flush (Archibald and Hempson, 2016). In the present study, the different sites supported different types of grazing and browsing ungulates (antelopes, elephants, rhinoceroses, giraffe and buffalo). Grasslands, particularly at high altitude, support large grazing ungulates such as eland and medium sized antelopes such as oribi and red hartebeest. While fire frequencies were known, fire intensity was also likely different between the sites and between years within sites due to differences in available moisture and fuel. As with many other studies we were constrained to the available fire and herbivore/mowing regimes inherent in the LTers. It is likely that a larger range of fire frequencies or intensities, and herbivore abundances and densities, as well as finer characterization of herbivore types (elephants versus antelope) could improve our ability to explain the variance in soil responses to disturbance.

Despite variability in site responses, we distinguished distinct mechanisms by which fire and herbivory influenced bacterial and fungal communities. Edaphic variables, i.e. Mg > pH > Ca, were the main drivers of bacterial communities. This is in line with global trends that reveal that edaphic variables, particularly pH, control bacterial community composition (Bahram et al., 2018; Fierer and Jackson, 2006; Kaiser et al., 2016; Lauber et al., 2009). The inclusion of exchangeable cations distinguishes our study from these global analysis (for example Fierer and Jackson, 2006, Kaiser et al., 2016 and Lauber et al., 2009). Likewise, most studies on the impact of fire and herbivory on microbes focus on pH and SOM, while the available cations and physical soil parameters are less often considered. Importantly, we found that fire and herbivore-induced changes in the available essential nutrient cations were the main correlates of bacterial diversity, after pH. Given our results, and the fact that these cations are known to play an important role in bacterial metabolism (Paul, 2014), this study indicates that these cations should be considered in future studies to understand the

impact of fire and herbivory on soil microorganisms. In contrast to bacteria, vegetation variables were the main drivers of fungal communities, i.e. herbaceous biomass > woody cover > P. At the global scale, fungi are known to follow similar biogeographic patterns as plants, and are more affected by climatic factors than bacteria (Bahram et al., 2018; Tedersoo et al., 2014). Phosphorus is also known to play a major role for fungal ecology (Coleman et al., 2017; Paul, 2014; Tedersoo et al., 2014). As fungi are heterotrophs, they rely heavily on plants for their substrate, i.e. plant litter for saprotrophs, and host metabolites for symbiotrophs and pathotrophs (mycorrhizal and parasitic fungi). The effect of fire and herbivory on vegetation was relatively consistent across our sites. Exclusion of fire and herbivory led to dramatic accumulations of woody biomass in both grasslands and savannas, though this varied in extent depending on site-specific conditions. Very little woody encroachment occurred in the high-altitude grassland site when disturbance was removed, probably due to low temperatures, associated frost events and nutrient limitations which act as bottlenecks to woody seedling establishment (Bond, 2008). However, removal of disturbance from the low altitude grassland site, where there has been neither burning nor mowing since 1950, resulted in dense encroachment by a range of exotic e.g. *Acacia mearnsii*, and indigenous tree species, e.g. *Vachellia nilotica* (Fig. 2). In this site, where disturbance exclusion had the biggest impact on woody cover, a dramatic increase of symbiotroph fungi was observed, from a relative abundance of less than 10% in treatments with disturbance to ca. 30% when disturbances were excluded. The MAP of the low altitude grassland (820 mm) is above the threshold defined by Sankaran et al. (2008), and can be considered an 'unstable' savanna in which MAP is sufficient for woody canopy closure, and disturbances (fire, herbivory) are required for the coexistence of trees and grass. The savanna sites were under this threshold, with MAPs of 560 mm for the moist site, and 400 mm for the dry site. In these sites, maximum woody cover is constrained by MAP, and can be considered as 'stable' savanna while fire and herbivory interact to reduce woody cover below the MAP-controlled upper bound. However, the presence of the river induced a higher nutrient and water availability locally, and the fire and herbivory exclusion resulted in a complete switch, from savanna to closed forest, especially in the moist savanna riparian site.

Another important generalization from our study was that disturbances and associated edaphic and vegetation properties explained a significant but small proportion of the total diversity of bacterial and fungal communities (Table 3, Fig. 4). Fungal communities were more impacted by disturbances than were bacterial communities. In several recent reviews and meta-analyses, fire was revealed to have a greater effect on fungi than on bacteria (Dooley and Treseder, 2012; Dove and Hart, 2017; Pressler et al., 2019). Previous evidence suggests that this is due to both the lower thermal tolerance of fungi, their greater abundance in the superficial soil horizons (Baldrian et al., 2012) and, for mycorrhizal fungi, the mortality of plant hosts during fire (Neary et al., 1999). Pyric-herbivory or the temporal and spatial variation of burnt and grazed areas in contiguous patches is known to create heterogeneity and diverse habitats (Fuhlendorf et al., 2009; Fuhlendorf and Engle, 2004) and might explain part of the unexplained microbial communities variation in our study. In addition, it is possible that soil properties modifications due to fire and herbivory, or the exclusion thereof, may still emerge in the coming decades. LTERs are valuable long-term field experiments established between 18 and 70 years ago. However, a change in soil forming factors, including fire and grazing or browsing regimes, often only results in changes in soil characteristics over decadal or longer time scales. Changes in microbial communities might follow belowground (pedogenic) timescales and pedo-diversity more than aboveground-timescales and -biodiversity. Illustrating such timescales, in a podzolic chronosequence, a distinct shift in microbial communities was observed after 330 years (Vermeire et al., 2018). Several mechanisms might explain the stability of microbial communities (comprised of resistance and resilience) (see Allison and Martiny, 2008 and Shade et al., 2012 for reviews): (1) individual properties, like physiological plasticity and stress tolerance (gene expression) or

dormancy; (2) population properties, like adaptation (evolution of population traits), growth rate, stochastic gene expression and dispersal; and (3) community properties, like diversity, compositional turnover and emergent properties. Whatever the mechanisms, the resistance to change of microbial communities in disturbance-driven grassy ecosystems may be linked to an evolution along with those very disturbances.

5. Conclusion

Given the roles of soil microbial communities in regulating the biogeochemical cycles, their effective management is among our most powerful weapons in the fight against the global threats of biodiversity loss and climate change (Crowther et al., 2019). Our study highlights the complexity of an effective management of fire and herbivory to promote carbon sequestration in soil and vegetation. On one hand, the removal of disturbances led to an increase of vegetation biomass in the sites where ecological context allowed tree establishment. On the other hand, the consequences of disturbances on soil C and microbial biomass varied depending on the site, and the stock of soil OM was not correlated with the vegetation biomass. Considering the highly site-specific nature of the responses of the microbial communities to manipulation of fire and herbivory, future efforts need to address the large variety of interactive environmental and biotic factors in order to disentangle ecological thresholds. In particular, fire and herbivory are mostly considered separately. However, our results highlight the intricate relationship between these two disturbances, resulting in varying and sometimes opposing effects on soil parameters. Their interaction in both time and space should be considered (Fuhlendorf et al., 2009). In addition, more analyses should consider the global south including fire-driven African landscapes, as most studies in the field focus on European and North American ecosystems. Finally, since the changes in microbial communities we observed in response to a modification of fire and herbivory regimes were muted, even with treatments over decades, longer (pedogenic) timescales should be investigated. This is an important goal for LTERs and ecosystem modelling alike.

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Data availability

This study is part of a project that adheres to FAIR data. Soil microbial DNA sequences will be made available on the International Nucleotide Sequence Database Collaboration (NCBI, <https://www.ncbi.nlm.nih.gov/genbank/collab/>) upon manuscript publication. Edaphic and vegetation data will likewise be made available on the University of Cape Town's data repository, ZivaHub (<https://zivahub.uct.ac.za/>).

CRediT authorship contribution statement

HJH, MC, JK, JT and MLV conceived the ideas and designed methodology; MLV, JT, GW, HJH and ZV collected field data; KK, PG, FS, AS and MT contributed data; JT and MLV processed samples, KL, SV and CB helped with the processing of the metabarcoding data; MLV, JT and HJH analysed the data; MLV led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Declaration of competing interest

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

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