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Arable agriculture changes soil microbial communities in the South African Grassland Biome

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Many studies, mostly in temperate regions of the northern hemisphere, have demonstrated that agricultural practices affect the composition and diversity of soil microbial communities. However, very little is known about the impact of agriculture on the microbial communities in other regions of the world, most particularly on the African continent. In this study, we used MiSeq amplicon sequencing of bacterial 16S rRNA genes and fungal ITS regions to characterise microbial communities in agricultural and natural grassland soils located in the Mpumalanga Province of South Africa. Nine soil chemical parameters were also measured to evaluate the effects of edaphic factors on microbial community diversity. Bacterial and fungal communities were significantly richer and more diverse in natural grassland than in agricultural soils. Microbial taxonomic composition was also significantly different between the two habitat types. The phylum Acidobacteria was significantly more abundant in natural grassland than in agricultural soils, while Actinobacteria and the family Nectriaceae showed the opposite pattern. Soil pH and phosphorus significantly influenced bacterial communities, whereas phosphorus and calcium influenced fungal communities. These findings may be interpreted as a negative impact of land-use change on soil microbial diversity and composition.

Significance:

- This report is the first of the effect of land-use changes on the diversity of the soil microbial communities in African grassland soils.

- Land-use changes influence the diversity and structure of soil microbial communities in the Grassland Biome of South Africa.

- This study serves as a baseline for future studies on South African soil microbial diversity.

Introduction

Soils represent a reservoir for a wide diversity of microorganisms such as bacteria, fungi and viruses.[1] Bacteria and bacteriophages are typically the most abundant microorganisms present in soils, although their prevalence is highly variable and affected by edaphic factors such as soil mineral content and pH[1,2] and by local plant biodiversity[3]. Soil microbial communities are important drivers of ecosystem functioning and climate change mitigation through the fixation, immobilisation and cycling of greenhouse gases.[4] Plants synthesise organic matter via photosynthetic activities and provide energy to soil microbes through root exudates.[5,6] In return, soil microorganisms provide the plants with critical 'services', including decomposition of organic matter, mineral cycling and biocontrol of soil-borne pathogens.[4,7]

Land use, such as the modification of a natural ecosystem for agriculture purposes, has been proven to have significant effects on soil microbial communities by changing the physical and chemical properties of the soil. Such effects on the microbial community include changes in microbial species abundance, richness and diversity.[8-11] The effect of changing land use on the diversity of soil microbes has also been observed in grassland ecosystems. For example, Acosta-Martinez et al.[12] studied soil bacteria diversity in a single soil type in Texas, USA. They found much higher bacteria diversity in soils under agriculture than in natural grassland soils, providing evidence of the positive influence of land use on soil bacterial diversity. The overall bacterial community diversity and composition in different grassland soils from across South and North America were significantly influenced with soil pH.[13] Similar results were reported in a study of bacterial communities in German grassland and forest soils.[14,15] In a more recent study, both bacterial and fungal communities exhibited contrasting beta diversity among two types of European subalpine/alpine grasslands, and both bacterial and fungal communities were influenced by grassland type.[16] However, most of these studies have been conducted in temperate northern hemisphere grasslands and none is directly relevant to the unique and defined biomes of South Africa.[17] We argue that more geographically diverse comparative analyses are required in order to better understand how microbial communities are altered by land use.

South Africa is one of the world's biodiversity hotspots, and is ranked third in the global list; it is composed of nine biomes which together contain between 250 000 and 1 000 000 species including animals and plants, many of which are endemic to the country.[17] However, while higher eukaryotes have been intensively studied in southern Africa, there has been only very limited focus on the biodiversity of soil microbial communities in any of South Africa's major biomes. Mpumalanga is a province in the northeast of South Africa, much of which is designated as Grassland Biome.[17] The Grassland Biome is the cornerstone of commercial maize cropping, and many grassland areas have been converted to arable production. Sorghum, wheat and sunflowers are also farmed in the region but on a smaller scale.[18] Agriculture is, therefore, one of the important economic sectors in Mpumalanga, with significant contributions toward national economic growth[19], but with major modifications to much of the original natural grassland habitat.

In this study, we evaluated the differences in bacterial and fungal communities across a series of paired agricultural and natural grassland soils in the Mpumalanga Province, South Africa. We used an amplicon sequencing based approach to characterise the soil bacterial and fungal communities and to quantify their differences across 15 sites, and related these differences to measured soil chemical parameters. Inventories of the composition and differences in the bacterial and fungal communities in Mpumalanga's agricultural and natural grassland soils will contribute to the body of knowledge, and can provide a description of the core soil microbial communities in the unmodified grassland.

Materials and methods

Sample collection

The study sites are situated on the Highveld escarpment in the Mpumalanga Province, which makes up 6.5% of South Africa's land area, and form part of the Grassland Biome.[20] The climatic conditions of the Grassland Biome of South Africa span a rainfall gradient from about 400 mm to >1200 mm per year, a temperature gradient from frost-free to snow-bound in winter, and an altitude from sea level to >3300 m; a spectrum of soil types occurs in the Grassland Biome, from humic clays to poorly structured sands.[21] In April 2016, 15 paired agricultural and natural sites in the Grassland Biome were localised using a geographic information system (GIS) technology (Supplementary table 1). The distance between sampling sites spanned 18–240 km, and at each sampling site, the sampling points between the two habitat types (agricultural vs natural grassland soil) were approximately 200 m apart. At each GPS-located sampling site, five pseudo-replicate soil samples (50–200 mm depth) were recovered from within a 10 x 10 m quadrat. All pseudo-replicate samples from each habitat type (agricultural vs natural grassland soil) were bulked into a single sample. This process was repeated for all 15 sampling sites, resulting in a total of 30 soil samples (15 agricultural and 15 grassland soils). The samples were transported to the laboratory at 5 °C and stored at -20 °C for subsequent DNA extraction.

Analysis of the soil chemical parameters

Nine soil chemical parameters were analysed to evaluate their influence on the structure and composition of the soil bacterial and fungal communities. Soil pH was measured using a Crison pH meter (Crison Instruments, Barcelona, Spain) from the supernatants of 1:2.5 (g/g) soil/deionised water suspensions, after soil particles had settled after 2 min of vigorous shaking.[22] Total inorganic phosphate was measured from the supernatant of 4 g of soil in 39 mL of the Bray-1 solution after 1 min of vigorous shaking and filtration through Whatman paper.[23,24] Extractable cations (K, Ca, Na, Mg) were measured from the supernatants of 1:10 (g/g) soil/ammonium acetate suspensions.[24] Total carbon content was determined by oxidation with potassium dichromate and sulfuric acid as described previously.[24] Total ammonium (NH_4) and nitrate (NO_3) contents were analysed by Bemlab (Pty) Ltd (Strand, Western Cape, South Africa) using standard protocols.

Genomic DNA extraction, amplification and high-throughput sequencing

Genomic DNA was extracted from 0.25 g soil (dry weight) using the PowerSoil DNA Isolation Kit (Mo Bio Laboratories Inc., Carlsbad, CA, USA) within 48 h of sampling, following the manufacturer's instructions. Soil samples were ground with an electric Powerlyser (Mo Bio Laboratories Inc.) before further processing. Aliquots of 5 μL of genomic DNA were premixed with GelRedTM Nucleic Acid Gel Stain (Biotium, Hayward, CA, USA), separated on 1% agarose gels and visualised under UV light to determine the success of the extraction.

DNA amplification was done at MRDNA (www.mrdnalab.com, Shallowater, TX, USA) sequencing facility in a 30-cycle PCR, using the HotStarTaq Plus Master Mix Kit (Qiagen, Germantown, MD, USA). For bacteria, 16S rRNA gene V4 variable region PCR primers 515/806[25] were used. For fungi, internal transcribed spacer region (ITS) primers ITS1f and ITS4[26] were used. The thermal cycling conditions for amplification of the two gene regions were as follows: 94 °C for 3 min, followed

by 28 cycles of 94 °C for 30 s, 53 °C for 40 s and 72 °C for 1 min, with a final elongation step at 72 °C for 5 min. After amplification, PCR products were checked on 2% agarose gels to determine the success of amplification and the relative intensity of bands. Multiple samples were pooled together (e.g. 30 samples) in equal proportions based on their molecular weight and DNA concentrations. Pooled samples were purified using calibrated Ampure XP beads. Pooled and purified PCR products were used to prepare DNA libraries following the Illumina TruSeq DNA library preparation protocol. High throughput sequencing was performed at MRDNA on a MiSeq platform following the manufacturer's guidelines.

Sequence analysis

Sequences were processed using Qiime V1.9.1.[27] Bacterial and fungal sequences were analysed independently. Firstly, the mapping file was checked to ensure that it is formatted correctly. Barcodes were extracted from the sequence reads. The split libraries command was used to demultiplex the fastq file and assign sequence reads to their respective sample according to their corresponding barcodes, using sample mapping information (Supplementary tables 2 and 3) at the default parameter implemented in Qiime. Chimeric sequences were screened and then removed using the USEARCH software[28] and the Greengenes-derived 16S reference database implemented in Qiime[29] for archaea and bacteria, respectively, and the UNITE-INSD (release 7) derived ITS reference database for fungi. High-quality reads were clustered into operational taxonomic units (OTUs) at 97% sequence similarity and assigned taxonomy using blast search against the Greengenes database (for archaea and bacteria) and the UNITE-INSD database (for fungi), respectively. Sequences identified as singletons were removed before sub-sampling to a depth of 3001 (for bacteria) and 12 358 (for fungi) sequences per sample to ensure equal sampling effort across samples.

Data access

The DNA sequences from this study were deposited in the Sequence Read Archive (SRA) of the National Center for Biotechnology Information (NCBI) database, with accession numbers SRR5341506 and SRR5341505 for bacterial 16S and fungal ITS, respectively.

Statistical analyses

All statistical analyses were conducted in R version 3.2.1.[30] Differences in community composition were visualised using non-metric multidimensional scaling with Hellinger-transformed Bray–Curtis dissimilarities. Abiotic data were standardised (mean=0, SD=1) and the resulting data matrices were used to perform principal component analysis to evaluate differences in soil chemistry between agricultural soils and natural grassland soils. An analysis of similarity (ANOSIM) was performed to determine whether differences between habitats (agricultural vs natural grassland soils) were statistically significant (999 permutations, $\alpha<0.05$). The number of shared OTUs between communities or samples was visualised using Venn diagrams. Differences in means for bacterial and fungal diversities metrics (species richness, Shannon–Wiener index, Simpson index and Pielou's evenness), phyla abundance and abiotic data were compared using paired two-tailed Student's *t*-tests. The compositions of major bacterial and fungal phyla were visualised using a heatmap with unweighted pair group method with arithmetic mean (UPGMA) clustering on Bray–Curtis distances after Hellinger transformation. Distance-based redundancy analysis was used to evaluate the effects of the environment on microbial community composition.[31]

Results and discussion

We evaluated and compared the compositions of the bacterial and fungal communities in agricultural and natural grassland soils from 15 sites in the Mpumalanga Province in the Grassland Biome of South Africa using deep amplicon sequencing. We also evaluated the effect of edaphic factors on the patterns of the bacterial and fungal communities in the two habitats.

After quality filtering, removal of chimeras and singletons and sub-sampling, 90 030 bacterial and 370 740 fungal sequences remained, clustering into 42 866 bacterial and 9730 fungal OTUs (Figure 1, Table 1), respectively. Although the relative numbers of bacterial versus fungal taxa cannot be

quantified from our data, our results suggest that natural grassland soils are richer in bacterial taxa than in fungal taxa, which is in agreement with previous studies showing that soil microbial biomass in natural grasslands is usually strongly dominated by bacteria.[32,33]

Bacterial alpha diversity metrics (Shannon and Simpson diversity indices and Pielou's evenness) revealed no significant difference (paired t-test, $p > 0.05$) between agricultural and natural grassland soils (Table 1). However, natural grassland soils were significantly richer (paired t-test, $p < 0.05$) in bacterial OTUs than were agricultural soils (Table 1), confirming the effects of land-use perturbation in decreasing soil microbial diversity.[34,35] However, this finding contradicts previous studies on grassland soils, in which higher bacterial diversity was associated with land-use changes.[11,36,37] The high level of bacterial genotypic richness detected in natural grassland may be explained by the influence of the high diversity of plant species in the grassland ecosystem, compared to the monoculture of agricultural crops.[8,38] Plant species differ in the quantity and variety of their root exudates, as well as in the range of organic compounds in plant litter detritus: both factors are potential drivers of the higher microbial diversity often associated with more diverse plant communities.[34,39-41] Our results are therefore consistent with the view that plant diversity promotes soil microbial diversity in grassland ecosystems.[3]

Fungal alpha diversity metrics (species richness, Shannon and Simpson diversity indices, and Pielou's evenness) were significantly higher (paired t-test, $p < 0.05$) in natural grassland soils than in agricultural soils (Figure 1, Table 1), which is also consistent with previous studies on grasslands.[11,36,37] The relatively low fungal diversity detected in agricultural soils is probably as a result of land-use management processes such as tillage. Tillage has been shown to damage fungal mycelia, leading to a reduction in fungal abundance and diversity in the soil.[42,43] However, these results should be treated with caution as rarefaction curves obtained for both bacteria and fungi (Supplementary figures 1 and 2) did not plateau, indicating that the diversity of bacteria and fungi in both agricultural and natural grassland soils had not been fully sampled.

Differences in microbial diversity in managed agricultural soils, as compared to natural grassland soils, have been disputed in several studies. Some studies reported high bacterial diversity in conventionally managed arable soils as well as grassland soils.[32,33,36,37] Others have shown that extensively managed grassland soils are richer in fungi than bacteria.[37,44]
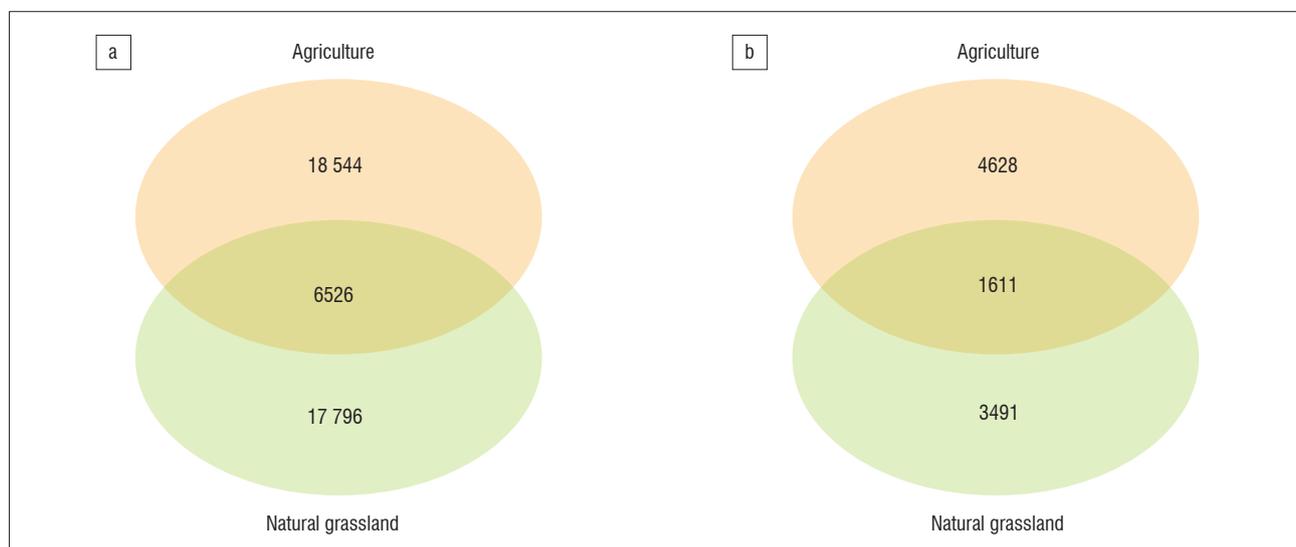
Figure 1: Venn diagrams of shared and unique (a) bacterial and (b) fungal operational taxonomic units between agricultural and natural grassland soil communities.

Table 1: Characteristics of sequences, diversity and richness between agricultural soils and natural grassland soils

	Bacteria		Fungi	
	Agriculture	**Grassland**	**Agriculture**	**Grassland**
Raw sequences	1 910 115	1 537 765	674 574	502 509
Post pre-processing	80 112	82 105	605 544	453 965
Post sub-sample and filter[†]	45 015	45 015	185 370	185 370
Number of operational taxonomic units	25 070	24 322	5102	6239
Unclassified	5663 (22.6%)	5015 (20.6%)	104 (2.04%)	116 (1.9%)
Richness	2116[a] ±242.1	2250[b] ±105.4	556.4[a] ±176.3	748.5[b] ±137.8
Shannon index	7.38[a] ±0.37	7.54[a] ±0.11	3.48[a] ±0.8	4.09[b] ±0.6
Simpson index	1.00[a] ±0.005	1.00[a] ±0.0004	0.88[a] ±0.12	0.93[a] ±0.006
Pielou's evenness	0.96[a] ±0.04	0.98[a] ±0.009	0.55[a] ±0.11	0.62[a] ±0.009

[†]*Sub-sample depth was at 3001 (for bacteria) and 12 358 (for fungi) sequences per sample. Values are the mean ± standard deviation.*

Diversity indices followed by different superscript letters are significantly different (agricultural vs grassland soils) according to paired Student's t-tests (p < 0.05).

The contrasting pattern of bacterial and fungal diversity in agricultural compared to natural grassland soils has been explained by the difference in nutrient availability in the two ecosystems.[45,46] In extensively managed agricultural soils, high fertility and nutrient availability derived from the input of fertiliser favour bacterial communities, while the low soil fertility characteristics of natural grassland soils favour the fungal communities.[45,46] In comparison, the factors that shape the differences in bacterial and fungal diversity in managed soils are still largely unknown. The bacterial and fungal taxa unique to agricultural soils and natural grassland soils accounted for the majority of the raw sequences (Figure 1, Table 1). In addition, more than 20% of the bacterial sequences and about 2% of the fungal sequences could not be classified (Table 1), suggesting that these soils remain understudied.

A total of 13 prokaryotic phyla (12 bacterial phyla and 1 archaeal phylum) were detected in the two habitats (Figure 2), of which Actinobacteria, Proteobacteria and Acidobacteria were the most abundant (Supplementary table 4). These three bacterial phyla are the most dominant taxa found in soils worldwide and make up the large proportion of bacterial 16S rRNA sequences available in public databases.[47,48] Seven fungal phyla were detected in the two habitats (Figure 3), of which Ascomycota accounted for the majority of the sequences (71.6% and 63.4%) in agricultural and natural grassland soil samples, respectively (Supplementary table 4). Both the bacterial and fungal phyla identified are typically ubiquitous in soils and are thought to be important role players in soil geochemical cycling processes.[3,14]

Species of the phylum Acidobacteria were significantly more abundant (paired *t*-test, $p < 0.05$) in natural grassland soils than in agricultural soils (Figure 2, Supplementary table 4). Acidobacteria abundance is commonly reduced in soils under conventional tillage[11,49], which

may explain this pattern. This phylum includes many environmentally important species, which provide a wide range of functions including the biodegradation of cellulose, hemicelluloses and chitin, nitrate and nitrite reduction, and the production of antimicrobial compounds.[50]

The most abundant bacterial and fungal taxa accounted for at least 10% of the total number of sequences in both agricultural and natural grassland soil samples. Analysis of these sequences at class, order, family and genus levels is shown in Supplementary table 4. At the class and order levels, Actinobacteria were significantly more abundant (paired *t*-test, $p < 0.05$) in agricultural soils than in natural grassland soils (Supplementary table 4). These results suggest that land-use changes favour Actinobacteria abundance, which contradicts the results of previous related studies on non-African grassland soils, which suggested that Actinobacteria are more abundant in non-disturbed grassland soils than in agricultural managed soils.[11,12] Actinobacteria are major producers of extracellular enzymes and secondary metabolites, and are thought to play significant roles in carbon cycling, plant disease suppression and enhancement of plant growth.[51,52]

At family level, Nectriaceae (Hypocreales) was the most abundant fungal family with 17.4% and 8.7% of all the fungal raw sequences in agricultural soils and natural grassland soils, respectively (Supplementary table 4). However, species of the family were significantly more abundant (paired *t*-test, $p < 0.05$) in agricultural soils than in natural grassland soils (Supplementary table 4). The family Nectriaceae includes species of both economic and health importance. Species of the genus *Fusarium* produce mycotoxin and are both crop and opportunistic human pathogens.[53,54]
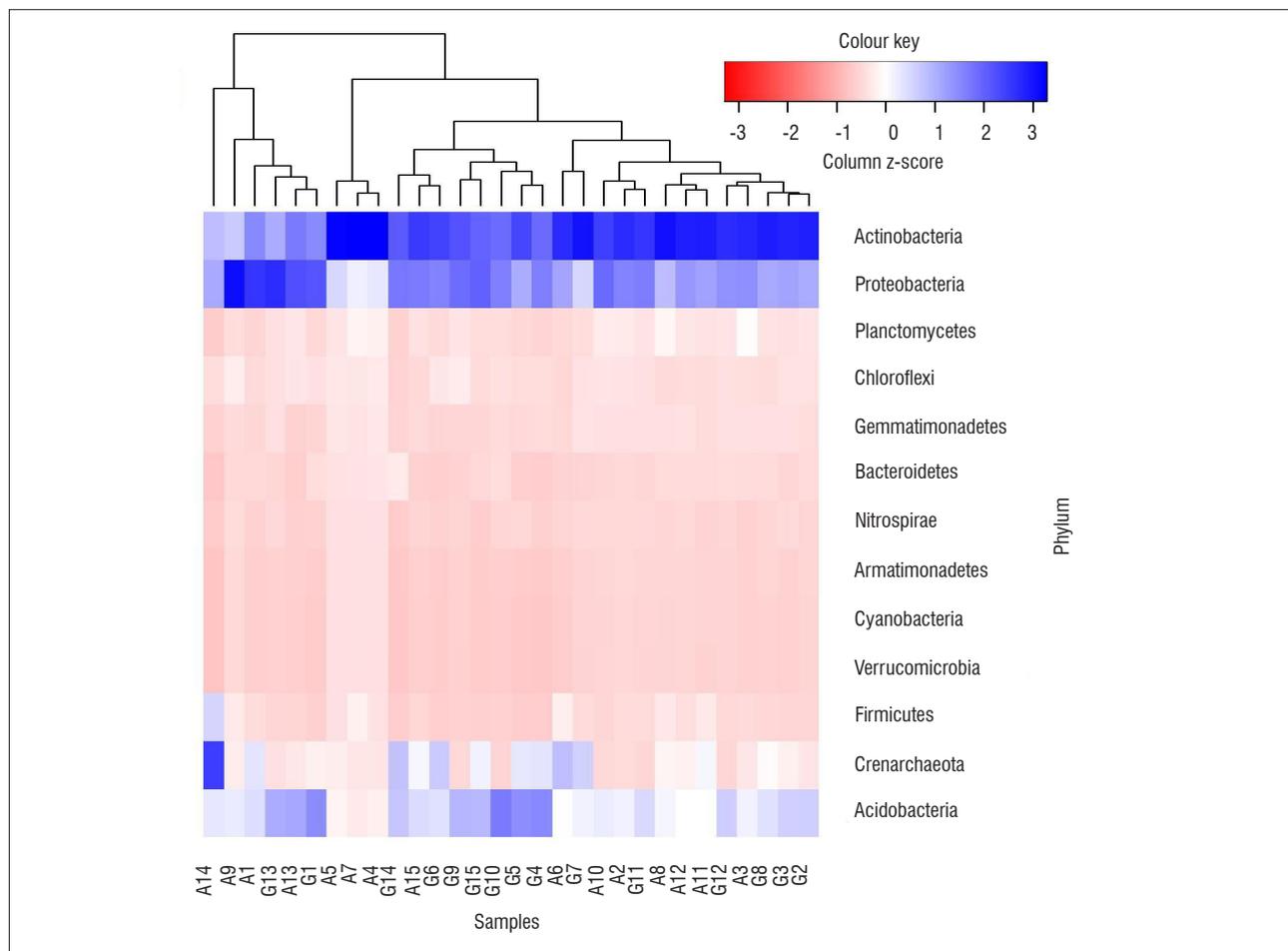

Figure 2: Heatmap displaying the bacteria and archaea phyla detected. Paired-site samples are clustered based on the per cent relative abundance. Each row was scaled so that the mean of each taxonomic group across samples was calculated and coloured by the corresponding z-score of each cell. Sample names starting with A were obtained from agricultural soils while those starting with G were from grassland soils.
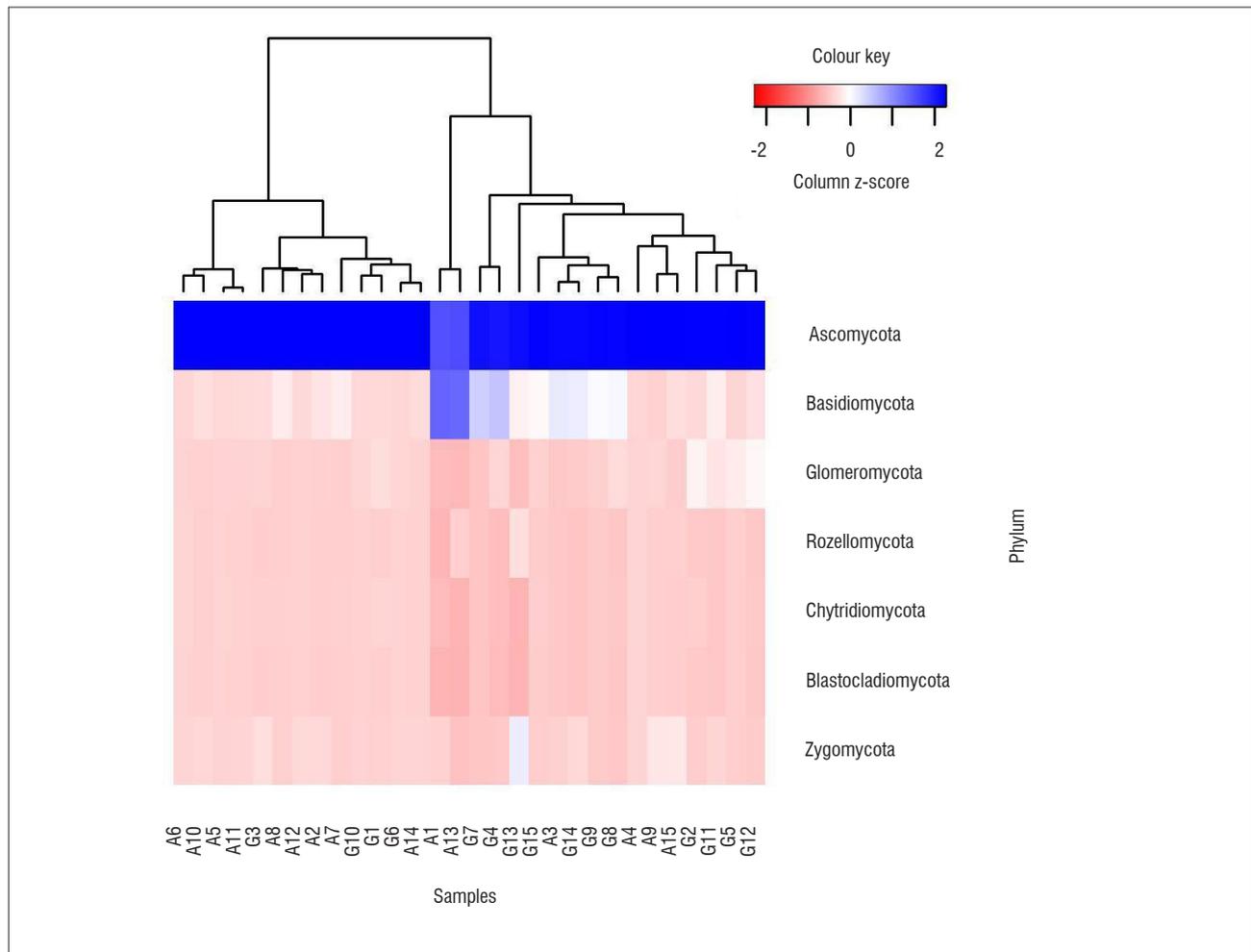
Figure 3: Heatmap displaying the fungal phyla detected. Paired-site samples are clustered based on the per cent relative abundance. Each row was scaled so that the mean of each taxonomic group across samples was calculated and coloured by the corresponding z-score of each cell. Sample names starting with A were obtained from agricultural soils while those starting with G were from grassland soils.

Non-metric multidimensional scaling analysis showed that bacterial and fungal communities in agricultural soils were distinct from those of the natural grassland soils (Supplementary figures 3 and 4). These results were confirmed by an analysis of similarity (ANOSIM: $R_{bacteria}=0.46$, $p=0.001$; $R_{fungi}=0.16$, $p=0.001$). A principal component analysis showed a clear discrimination of soil chemistry between agricultural soils and natural grassland soils. Soil pH and Ca concentrations tended to be higher in natural grassland soils, while NH_4, NO_3, K, C, P and Na concentrations were higher in agricultural soils (Supplementary figure 5). However, only three of the chemical parameters evaluated (Ca, P, pH) were significantly different (paired *t*-test, $p<0.05$) between the two habitats (Supplementary table 5). The high concentration of phosphorus observed in agricultural soils may be explained by mineral fertilisation commonly used in agriculture practices to improve plant performance.[55,56] Members of the soil microbial community, particularly bacteria, are capable of solubilising soil phosphate minerals into a usable form for plant uptake, which may influence the mineral concentration in the soil.[57] The differences observed in the composition and pattern of the bacterial and fungal communities (according to ANOSIM analyses) in agricultural soils compared with those in natural grassland soils, can potentially be explained by the differences in the soil chemistry of the two habitats. In previous studies on grassland soils, soil fertilisation and land-use intensification have both been linked to shifts in richness and diversity of soil microbial communities.[55,56]

Using distance-based redundancy analysis, we found that the composition and pattern of the bacterial communities in agricultural soils compared to natural grassland soils were influenced significantly by both soil pH (R=0.008, $p=0.002$) and phosphorus concentrations (R=0.013, $p=0.002$) (Supplementary figure 6). Our results are in agreement with previous studies which have shown that soil pH and nutrient availability shaped soil bacterial diversity.[2,9,13-15] In contrast, the differences found in the composition and pattern of the fungal communities between the two habitats were better explained by calcium (R=0.019, $p=0.01$) and phosphorus (R=0.01, $p=0.004$) concentrations (Supplementary figure 7), and corroborated previous studies.[9,58]

In summary, our results have shown that bacterial and fungal communities were significantly influenced by land-use changes, with agricultural soils containing distinct bacterial and fungal communities compared with natural grassland soils. Natural grassland soils consistently exhibited higher numbers of OTUs than did agricultural soils. The differences in microbial communities between the two habitat types were influenced by differences in soil chemical properties, supporting the concept that both soil chemical properties and microbial community compositions and diversities are altered after a conversion from a natural to an agricultural ecosystem.

This study was focused on a single economically important biome and represents the first demonstration of the effect of land-use changes on the diversity and structure of the soil microbial communities in African grassland soils. The study therefore serves as the benchmark for future studies on South African soil microbial diversity, and for monitoring future changes in soil microbial communities resulting from changing land use and climate.

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