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Arbuscular mycorrhizal fungi communities from tropical Africa reveal strong ecological structure

Susana Rodríguez-Echeverría[1], Helena Teixeira[1], Marta Correia[1], Sérgio Timóteo[1,2], Ruben Heleno[1], Maarja Öpik[3] and Mari Moora[3]

[1]Centre for Functional Ecology, Department of Life Sciences, University of Coimbra, Cda Martim de Freitas, 3000-456 Coimbra, Portugal; [2]School of Biological Sciences, University of Bristol, Life Sciences Building, 24 Tyndall Avenue, Bristol, BS8 1TQ, UK; [3]Department of Botany, Institute of Ecology and Earth Sciences, University of Tartu, 40 Lai St, 51005 Tartu, Estonia

Author for correspondence:
Susana Rodríguez-Echeverría
Tel: +351239240767
Email: susanare@ci.uc.pt

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Summary

- Understanding the distribution and diversity of arbuscular mycorrhizal fungi (AMF) and the rules that govern AMF assemblages has been hampered by a lack of data from natural ecosystems. In addition, the current knowledge on AMF diversity is biased towards temperate ecosystems, whereas little is known about other habitats such as dry tropical ecosystems.
- We explored the diversity and structure of AMF communities in grasslands, savannas, dry forests and miombo in a protected area under dry tropical climate (Gorongosa National Park, Mozambique) using 454 pyrosequencing.
- In total, 147 AMF virtual taxa (VT) were detected, including 22 VT new to science. We found a high turnover of AMF with < 12% of VT present in all vegetation types. Forested areas supported more diverse AMF communities than savannas and grassland. Miombo woodlands had the highest AMF richness, number of novel VT, and number of exclusive and indicator taxa.
- Our data reveal a sharp differentiation of AMF communities between forested areas and periodically flooded savannas and grasslands. This marked ecological structure of AMF communities provides the first comprehensive landscape-scale evidence that, at the background of globally low endemism of AMF, local communities are shaped by regional processes including environmental filtering by edaphic properties and natural disturbance.

Introduction

Arbuscular mycorrhizal fungi (AMF, Phylum Glomeromycota) are ubiquitous fungal symbionts that colonize the roots of *c.* 80% of vascular plants, facilitating access to soil mineral nutrients in exchange for carbon (C) compounds (Smith & Read, 2008), and improving plant resistance to drought and pathogens (de la Peña *et al.*, 2006; Egerton-Warburton *et al.*, 2007). However, the outcome of the symbiosis depends on the identity of the partners involved because different AMF may induce different responses in plants (Johnson *et al.*, 1997; van der Heijden *et al.*, 1998). Thus, the composition of AMF communities is fundamental to a determination of the distribution of plant species, and the structure and diversity of plant communities (Moora *et al.*, 2004; Van Der Heijden *et al.*, 2008; Klironomos *et al.*, 2011).

Globally, 93% of known AMF species-level taxa occur in at least two continents and over one third have a cosmopolitan distribution, providing evidence of their low level of endemism (Davison *et al.*, 2015). However, differences in the composition of AMF communities have been detected at regional (Lekberg *et al.*, 2007; van der Gast *et al.*, 2011; Moora *et al.*, 2014) and local scales (Helgason *et al.*, 1999; Davison *et al.*, 2012). It has been suggested that climate and geology shape natural AMF communities at the global scale, whereas soil type, soil pH and ecosystem type are important drivers at regional scales (Öpik *et al.*, 2006, 2013; Dumbrell *et al.*, 2010; Kivlin *et al.*, 2011; Moora *et al.*, 2014). Plant identity, soil properties, AMF dispersal and interactions between fungal taxa could also influence the structure of AMF communities at local scales (Johnson *et al.*, 1992, 2004; Lumini *et al.*, 2010; Davison *et al.*, 2011). However, the rules that govern the distribution and composition of AMF communities are still poorly understood.

Global meta-analyses have shown clear differences in the diversity and composition of AMF communities among major ecosystem types such as forests and grasslands (Öpik *et al.*, 2006, 2010; Kivlin *et al.*, 2011) and declining AMF diversity following land-use intensity (Lumini *et al.*, 2010; Oehl *et al.*, 2010; Moora *et al.*, 2014; Xiang *et al.*, 2014). In spite of the clear differences found in the composition of AMF communities in forests and grasslands in global analyses (Öpik *et al.*, 2006; Moora *et al.*, 2014; Davison *et al.*, 2015), few studies have addressed local variation of AMF in different coexisting habitats. Differences in AMF communities from boreal forests and grasslands have been found at regional (Moora *et al.*, 2014) and local scales (Öpik *et al.*, 2003) in the Northern Hemisphere using molecular methods. Studies based on spore counts in the Southern Hemisphere also

have revealed different AMF community composition among vegetation types (Velázquez *et al.*, 2013), and a higher AMF richness in grasslands than forests (Castillo *et al.*, 2006). Thus, these studies in temperate areas from both hemispheres have revealed similar patterns to those described using global data.

The diversity of AMF communities in natural ecosystems in tropical and subtropical climates is largely unknown because most research has focused on seminatural and anthropogenic systems in Europe and North America (Öpik *et al.*, 2010; Davison *et al.*, 2012). Recent global assessments of AMF communities have provided further data from tropical grasslands, shrublands and rainforests (Öpik *et al.*, 2013; Davison *et al.*, 2015). Overall, the available reports have revealed a higher AMF diversity in rainforests than in temperate forests (Öpik *et al.*, 2006) and a certain specificity between AMF and their hosts (Mangan *et al.*, 2010) that can lead to spatially distinct AMF communities (Lovelock *et al.*, 2003; Uhlmann *et al.*, 2004; Lekberg *et al.*, 2007). In Africa, AMF research has focused mainly on agro-ecosystems and pastoral farmland (Uhlmann *et al.*, 2004, 2006; Lekberg *et al.*, 2007; Tchabi *et al.*, 2008; De Beenhouwer *et al.*, 2015a,b), whereas studies on natural ecosystems have addressed specific host plants from arid and semiarid regions (Jacobson *et al.*, 1993; Yamato *et al.*, 2009), afromontane forests (Wubet *et al.*, 2003, 2006a,b), savannas (Tchabi *et al.*, 2008), natural grasslands, shrublands and rainforests (Öpik *et al.*, 2013; Davison *et al.*, 2015; Gazol *et al.*, 2016). Many of these studies have revealed high ratios of novel taxa in natural tropical ecosystems, particularly in Africa (Wubet *et al.*, 2003, 2006b; Öpik *et al.*, 2013; De Beenhouwer *et al.*, 2015b).

Given the scarcity of data on AMF diversity from dry tropical ecosystems, which are increasingly threatened by growing human population pressure, particularly in Africa (Uhlmann *et al.*, 2006; Tchabi *et al.*, 2008; De Beenhouwer *et al.*, 2015a), and the high proportion of uncultured AMF detected in natural ecosystems (Ohsowski *et al.*, 2014), research in African ecosystems is urgently needed. This study investigated the composition and structure of AMF communities in the main vegetation types of the Gorongosa National Park (GNP), situated in the tropical climatic zone in Mozambique, contributing to fill the knowledge gap on African and tropical AMF communities. The GNP is a highly diverse area in terms of landscapes and vegetation units (Stalmans & Beilfuss, 2008) with a rich wildlife recovering rapidly after a long-lasting war at the end of the 20th Century (Daskin *et al.*, 2016). However, the belowground diversity in GNP remains unstudied. According to the available literature for temperate areas (Öpik *et al.*, 2003; Castillo *et al.*, 2006; Velázquez *et al.*, 2013; Moora *et al.*, 2014), global assessments (Öpik *et al.*, 2006; Kivlin *et al.*, 2011; Davison *et al.*, 2015) and a recent study in a vegetation gradient in tropical Brazil (da Silva *et al.*, 2015), we expect to find different AMF communities in grasslands and forested areas with a higher AMF richness in grasslands. We also expect that the AMF communities from savannas will show a gradual change from grasslands to forests, resembling what is observed for the vegetation aboveground.

Materials and Methods

Study site

This study was conducted at the Gorongosa National Park (GNP), a 4067-km^2 protected area in Mozambique at the southern end of the Great Rift Valley. We sampled the central part of the GNP, characterized by sandy alluvial soils and a tropical savanna climate with monthly mean temperatures of 18°C, mean annual rainfall of 840 mm, and marked wet and dry seasons. Precipitation occurs between November and April resulting in seasonal expansion of Lake Urema and extensive flooding in the lower lands of the GNP (Tinley, 1977; Daskin *et al.*, 2016). A gradient from areas flooded for several months every year to areas permanently above the flood line defines the main vegetation types: floodplain grassland (flooded for 4–5 months yr^{-1}), savannas (flooded for 3–4 months), mixed dry forest (occasionally flooded for 1 month) and miombo woodland (never flooded) (Stalmans & Beilfuss, 2008). Grasslands occupy the areas closer to the lake and are dominated by *Cynodon dactylon* (L.) Pers and *Digitaria swazilandensis* Stent under high grazing pressure, or by *Setaria incrassata* (Hochst.) Hack., *Megathyrsus maximus* (Jacq.) B. K. Simon & S. W. L. Jacobs or *Chrysopogon nigritanus* (Benth.) Veldkamp when grazing pressure is reduced (Stalmans & Beilfuss, 2008). Other common species in the grassland are *Echinochloa stagnina* (Retz.) P. Beauv., *Indigofera spicata* Forssk., *Sporolobus ioclados* (Trin.) Nees and *Urochloa mosambicensis* (Hack.) Dandy. The transition areas between floodplain grasslands and forests are dominated by savannas with similar herbaceous communities and variable tree density dominated either by *Faidherbia albida* (Delile) A. Chev. or *Acacia xanthophloea* Benth. (=*Vachellia xanthophloea* (Benth.) P. J. H. Hurter). Mixed dry forest refers to a diverse and complex mixture of woodlands and dry forest/thickets dominated mainly by *Acacia nigrescens* Oliv., *A. robusta* Burch., *Albizia* spp., *Combretum* spp. and *Milletia stuhlmannii* Taub. Other typical species found in the mixed dry forest are *Borassus aethiopium* Mart., *Dalbergia melanoxylon* Guill. et Perrott., *Dichrostachys cinerea* (L.) Wight & Arn., *Hyphaene coriacea* Gaertn. or *Piliostigma thonningii* (Schumach.) Milne-Redh. (Stalmans & Beilfuss, 2008). Miombo woodlands extend across large areas (2.7 million km^2) in Africa, growing in some of the world's poorest countries (Campbell, 1996; Williams *et al.*, 2008). Miombo refers to seasonally deciduous dry tropical woodlands characterized by the dominance of Caesalpinioideae species, such as *Brachystegia boehmii* Taub, *B. spiciformis* Benth. and *Julbernadia globiflora* (Benth.) Troupin. Other woody species typically found in miombo are *Diplorhynchus condylocarpon* (Müll. Arg.) Pichon, *Pseudarthria hookeri* Wight. & Arn., *Pterocarpus angolensis* DC. and *Sterculia quinqueloba* (Garcke) K. Schum. Both dry forest and miombo can be highly diverse in terms of landscapes and floristic composition. More information on GNP vegetation can be found in Tinley (1977) and Stalmans & Beilfuss (2008). All mentioned species form arbuscular mycorrhizas.

Sampling

Samples of soil were collected in June 2014 from three different sites in each of the five vegetation types (15 sites in total): grassland, *F. albida* (hereafter Faidherbia) savanna, *A. xanthophloea* (hereafter Acacia) savanna, mixed dry forest (hereafter forest) and miombo (Supporting Information Table S1). Sampled sites were at least 1 km apart from each other. Nine samples were collected from a regularly spaced grid (10 × 10 m) in each site for grassland, forest and miombo. In Acacia and Faidherbia savannas sampling did not follow a regular grid because we wanted to characterize the communities closely associated with these dominant tree species, so samples were collected close to the roots of five individual trees in each site. A total of 111 samples were collected (81 samples from grassland, forest and miombo (3 × 3 × 9) plus 30 samples for the two savannas (2 × 3 × 5)). Each sample consisted of 8 g of soil collected from the 2–10 cm topsoil with disposable equipment to avoid cross-contamination. Samples were dried with silica gel, and stored airtight at room temperature before further analyses. In addition, five samples were collected for chemical analysis in each site from 2 to 10 cm topsoil. Samples were pooled by site, rendering a total of 15 soil samples, air dried and stored at room temperature until processing.

Soil chemical analysis

Soil analyses for pH, organic carbon, total nitrogen (N), N-NH_4^+, N-NO_3^-, available phosphorus (P) and potassium (K) were performed following standard protocols after soils were air-dried, and sieved through a 2-mm sieve. Soil pH was measured in soil suspensions in distilled water. Soil organic carbon was estimated after combustion of samples at 550°C (Rossell *et al.*, 2001). Total N was estimated following the Kjeldahl method (Bremner & Mulvaney, 1982). N-NH_4^+ and N-NO_3^- were extracted using $CaCl_2$, and measured using a molecular absorption spectrophotometer following a modified protocol of Keeney & Nelson (1982). Available potassium and phosphorus were extracted using ammonium lactate and acetic acid, and measured using an atomic absorption spectrophotometer for potassium and a colorimetric method for phosphorus (Watanabe & Olsen, 1965). Differences in all soil properties between vegetation types were tested using ANOVA and Bonferroni comparisons.

Molecular analysis of AMF

DNA was extracted from 250 mg of dried soil using the PowerSoil DNA Isolation Kit (Mo Bio Laboratories Inc., Carlsbad, CA, USA). Glomeromycota sequences were amplified from soil DNA extracts using the small subunit (SSU) rRNA gene primers NS31 and AML2 (Simon *et al.*, 1992; Lee *et al.*, 2008) linked to 454-sequencing adaptors A and B, respectively, and following the 454-sequencing approach of Öpik *et al.* (2013). PCR was carried out in a two-step procedure of which the first reaction used the specific primers with multiplex identifying barcodes and partial adapters for sequencing, and the second PCR was used to complete the sequencing adapters. In between sequential reactions, amplicons were diluted 10-fold. Total reaction volume of PCRs was 30 µl and contained primers with 0.2 µM final concentrations and Smart-Taq Hot Red 2× PCR Mix (Naxo OU, Estonia). Conditions for both PCR were 95°C 15 min, five cycles of 92°C 45 s +42°C 30 s +72°C 90 s, 20 cycles of 92°C 45 s +65°C 30 c +72°C 90 s and a final extension step of 10 min at 72°C.

PCR products were separated by electrophoresis in 1.5% agarose gel and 0.5× TBE and purified from the gel using Agencourt AMPureXP (Beckman Coulter Inc., Pasadena, CA, USA). DNA concentration of purified amplicons was measured using Appliskan fluorescence-based microplate reader (Thermo Scientific, Waltham, MA, USA) and PicoGreen dsDNA Quantitation Reagent (Quant-iT ds DNABroad Range Assay Kit, Invitrogen). Afterwards, amplicons were sequenced using a Roche 454 FLX sequencing platform at Microsynth. Preparatory procedures for 454-sequencing were performed by BiotaP Ltd (Tallinn, Estonia).

Bioinformatical analyses

Operational taxonomic unit (OTU) delimitation and taxa assignment were done using the Maarj*AM* database (Öpik *et al.*, 2010) as reference. The Maarj*AM* database contains representative SSU rRNA gene sequences covering the NS31/AML2 amplicon from published environmental Glomeromycota sequence groups and known taxa. As of February 2015, it contained a total of 6064 SSU rRNA gene sequence records that had been classified on the basis of phylogenetic analysis into manually curated OTUs with sequence similarity threshold ≥ 97% or virtual taxa (VT cf. Öpik *et al.*, 2009, 2014). VT nomenclature allows easy comparison of data from individual datasets and consistent taxon naming. The taxonomic nomenclature used in Maarj*AM* follows recent changes in Glomeromycota taxonomy where possible (discussed in Öpik *et al.*, 2014).

Sequence reads were included in subsequent analyses only if they carried the correct barcode and forward primer sequences and were at least 170 bp long (excluding the barcode and primer sequence). Quality score (Q score) threshold was set at 25. Potential chimeras were detected using UCHIME (Edgar *et al.*, 2011) with the default settings in reference database mode (Maarj*AM*; Öpik *et al.*, 2010) and removed from the analyses (4499 chimeras). After stripping the barcode and primer sequences and removing chimeras, we used an open-reference OTU picking approach to match obtained reads against taxa in the Maarj*AM* database (accessed 4 February 2015). 454-reads were assigned to VT by conducting a BLAST search against the reference database with the following criteria required for a match: sequence similarity ≥ 97%; alignment length not differing from the length of the shorter of the query (454-read) and subject (reference database sequence) sequences by > 5%; and a BLAST *e*-value < 1e-50.

Sequences not receiving a match against Maarj*AM* database (59% of quality-checked reads) were compared against the INSD database using a similarity threshold of 90% and an alignment length differing by < 10 nucleotides between the query and the

subject as in previous studies (e.g. Öpik *et al.*, 2009). Putatively Glomeromycotan sequences longer than 450 nucleotides were clustered at 100% similarity level and the four longest sequences for each cluster retained for subsequent phylogenetic analysis. Clusters with less than two sequences longer than 450 nucleotides were removed. The new sequences were aligned with VT type sequences available in the Maarj*AM* database (status 4 February 2015) using the MAFFT multiple sequence alignment web service v.7.245 (Katoh & Standley, 2013) and subjected to a neighbor-joining analysis (F84 model with gamma substitution rates) in TOPALI v.2.5 (Milne *et al.*, 2009). A representative sequence from groups that were both well resolved in the tree and with high bootstrap values were considered as novel VT sequences and added to the reference dataset. Then, a second BLAST was performed against this updated reference sequence set using the same parameters as described earlier. After this new taxonomic assignment, 13 singletons and two samples with < 10 reads were omitted from further analyses (following practices of Öpik *et al.*, 2009; Hiiesalu *et al.*, 2014).

Representative sequences of each VT found in this study have been deposited at ENA under accession numbers LT223170–LT223556.

Statistical analysis

Sampling efficacy was assessed using the Coleman method in the SPECACCUM() function from R package VEGAN (Oksanen *et al.*, 2015). A general linear model (GLM) was used to test for significant differences in the number of different VT per site using vegetation as fixed factor. AMF diversity was estimated as the effective number of species using the exponential of Shannon diversity index (Jost, 2006). Samples were rarified to the median number of sequence reads (de Cárcer *et al.*, 2011) using the R package GUNIFRAC (Chen, 2012). General linear mixed models (GLMM) with vegetation type as fixed factor and site as random factor were used to test for differences in AMF diversity, and observed and rarefied richness to detect potential differences in sequencing depth. Both the proportion of reads and VT were used as dependent variables to analyze the relative abundance of Glomeromycotan families. These analyses were done using the R package LME4 (Bates *et al.*, 2014) and multiple *post hoc* pairwise comparisons were fitted using the function GLHT() from R package MULTCOMP (Hothorn *et al.*, 2008).

The diversity and overlap of AMF virtual taxa across the five habitats was visualized by assembling a bipartite network with R package BIPARTITE (Dormann *et al.*, 2008). To identify the AMF taxa associated with particular habitats we used indicator species analysis (Dufrene & Legendre, 1997) as implemented by functions MULTIPATT(), and SIGNASSOC() to obtain *P*-values corrected for multiple comparisons, from the R package INDICSPECIES (de Cáceres & Legendre, 2009), using 9999 permutations.

The structure of AMF communities was compared using the proportion of different VT reads as a proxy for the relative abundance of AMF taxa per sample (Öpik *et al.*, 2009). Turnover of species composition across communities was evaluated using the Bray–Curtis dissimilarity index. Variation in AMF community composition was visualized using nonmetric multidimensional scaling (NMDS) with 1000 iterations, using function METAMDS () from R package VEGAN (Oksanen *et al.*, 2015). Environmental variables (soil chemical properties and vegetation types) were fitted to the NMDS ordination using ENVFIT(). The effect of habitat on the composition of the AMF communities subsequently was analyzed by permutational multivariate analysis of variance (PerMANOVA; Anderson, 2001), based on Bray–Curtis distances and using 9999 permutations, constrained by site with the function ADONIS() from R package VEGAN (Oksanen *et al.*, 2015).

Results

A total of 715 665 reads (average length 531 bp excluding primers) were obtained, and 567 887 sequences were kept for subsequent analysis after quality check and chimera detection. From those sequences, 213 677 (38% of reads) matched Glomeromycota SSU rRNA gene sequences from the Maarj*AM* database (similarity ≥ 97%) corresponding to 147 VT, of which 22 VT (15%) were novel and 18 VT corresponded to currently described AMF morphospecies (Table S2). The 22 novel VT detected in this study belonged to the genera *Glomus* (eight), *Paraglomus* (six), *Claroideoglomus* (three), *Archaeospora* (two), *Diversispora* (two) and *Scutellospora* (one) (Table S2).

The sampling strategy was effective to detect the majority of AMF taxa present in each vegetation type (Fig. S1). Only for Acacia sites adding more samples would have significantly increased the number of VTs. In total, 29 different VT were detected in Acacia (mean and SE per site: 18.5 ± 0.4), 42 in Faidherbia (21 ± 2.5), 56 in grasslands (32.67 ± 3.9), 101 in forests (65 ± 18.4) and 121 in miombo (80.67 ± 10.3) (Table S3; Fig. 1). Significant differences in the mean number of VT per site were found between all vegetation types ($F_{4,11} = 48.13$, $P < 0.001$) except for forest and miombo, and for Acacia and Faidherbia savannas (Fig. 1). Significant differences in the mean effective number of species per site were found between open (grassland and savannas) and forested (forest and miombo) areas ($F_{4,11} = 10.44$, $P < 0.01$) (Fig. 1). Consistent significant differences between forested and open areas were also found for both observed ($\chi^2 = 16.13$, $P < 0.01$) and rarified number of VT per sample ($\chi^2 = 13.77$, $P < 0.01$) (Fig. S2). The relative abundance and overlap of VT across the five habitat types was visualized in the form of a quantitative bipartite network using the number of reads of each VT per habitat as a proxy for interaction frequency (Fig. 2).

Only 11 VT – 10 *Glomus* and one *Paraglomus* VT – were detected in all five vegetation types (Table S2; Fig. 2). This value represents 11.8% of the number of VT detected in at least five samples. In pairwise comparisons, forest and miombo shared 90 VT (68% of total number of different taxa for both vegetation types), whereas fewer taxa were shared between grassland and these two forest types: 43 VT (38%) for grassland and forest, and 42 VT (31%) for grassland and miombo (Table S3). Acacia showed the lowest values for shared VTs ranging between 16% with miombo and 25% with grassland (Table S3).

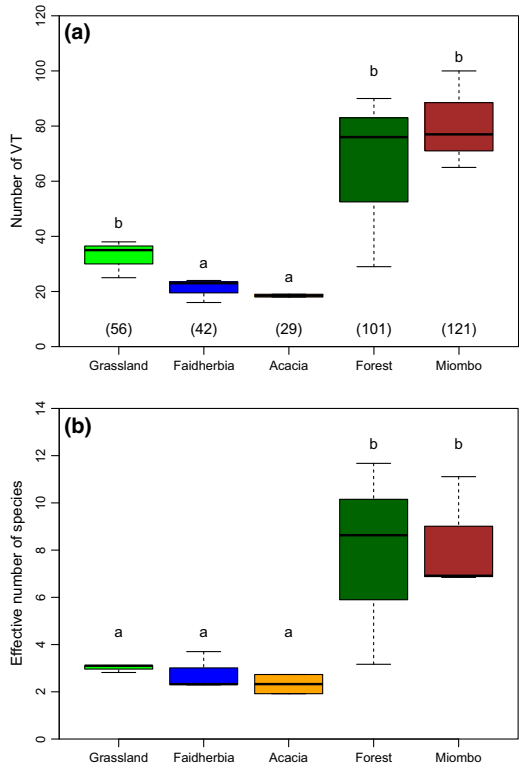

Fig. 1 Diversity of arbuscular mycorrhizal fungi (AMF) in each vegetation type. (a) Number of AMF taxa per site (mean ± SE) found in each vegetation type and total number of different virtual taxa (VT) found in each habitat (in brackets). (b) Effective number of species per site, calculated as the exponential of Shannon–Weiver diversity index. Solid lines indicate medians; boxes and whiskers indicate quartiles and ranges, respectively. Different letters represent significant differences after general linear model (GLM) and Tukey contrast for multiple comparisons ($P < 0.05$).

Indicator species analysis detected 25 VT significantly associated with a single vegetation type after correction for multiple testing (Table S4). Miombo had the highest number of indicator VT (15 VT from five genera; Table S4) and the highest number of exclusive VT (28 VT from six genera, Table S2).

Most of the sequences and detected VT belonged to the Glomeraceae, genus *Glomus* (73% of the total number of Glomeromycota sequences and 67% of the number of VT), whereas the contribution of other families was lower: Paraglomeraceae (16% and 7%), Claroideoglomeraceae (7% and 5%), Gigasporaceae (2% and 6%), Diversisporaceae (1.3% and 9.5%), Archaeosporaceae (0.15% and 3.4%) and Acaulosporaceae (0.08% and 1.36%) (Fig. S3). Glomeraceae was the dominant family in all vegetation types except grassland, which was dominated by Paraglomeraceae (Fig. 3). Glomeraceae and Paraglomeraceae were the only families found in Acacia savannas. The abundance of Claroideoglomeraceae was significantly higher in grassland and savannas (VT: $\chi^2 = 18.91$, $P < 0.001$; reads: $\chi^2 = 19.17$, $P < 0.001$) whereas Diversisporaceae (VT: $\chi^2 = 20.19$, $P < 0.001$; reads: $\chi^2 = 12.39$, $P = 0.014$) and Gigasporaceae (VT: $\chi^2 = 17.98$, $P = 0.001$) were more abundant in the miombo than in other vegetation types (Fig. 3).

Soils were acidic with significant differences between Acacia savannas (pH 5.8) and all other habitats (pH 4.1–4.5) (Table 1). Acacia savannas also had the lowest values of soil organic C, total N and NH_4 contents, whereas the highest values were found in grassland and Faidherbia savannas. There were quite large differences in NO_3, P and K contents among the different habitats (Table 1). Soil NO_3 content was very low in all vegetation types except in Faidherbia savannas where it reached 29 mg g^{-1}. P content was significantly lower in Acacia savannas and miombo, whereas K content was significantly higher in forest and miombo (Table 1). Collinearity ($R^2 > 0.77$) was detected between total N, soil organic C and pH.

NMDS ordination and PerMANOVA revealed a significant effect of vegetation type on the taxonomic composition of AMF communities (Pseudo-$F = 8.04$, $R^2 = 0.23$, $P = 0.001$). There was a clear separation between the AMF communities in grassland and Faidherbia savannas from those in forest, miombo and Acacia savannas along axis 1 (Figs 4, S4). Vegetation type and all soil chemical variables, except K, were significantly correlated with the ordination obtained (Table S5). Total N, total soil organic C and pH had the highest correlation values with axis 1, thus, being the most important soil properties affecting AMF communities (Table S5; Fig. 4).

Discussion

To our knowledge, this is the first comprehensive assessment of arbuscular mycorrhizal fungi (AMF) communities in multiple tropical natural ecosystems at the landscape scale. Because the majority of studies have targeted agro-ecosystems, temperate grasslands and annual plant species (Ohsowski *et al.*, 2014) our

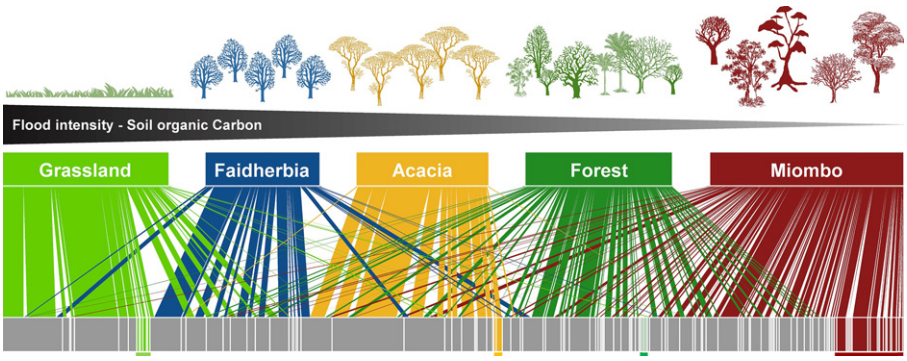

Fig. 2 Bipartite network representing the diversity and abundance of arbuscular mycorrhizal fungi (AMF) across the five studied vegetation types in the Gorongosa National Park, Mozambique. Each box in the bottom level represents an AMF virtual taxa (VT) which is linked to the habitat(s) were it was found (boxes on the top level). The width of each AMF and habitat box is proportional to the number of reads for each VT and for each habitat, respectively. AMF exclusive to a single habitat are colored with the color of that habitat.

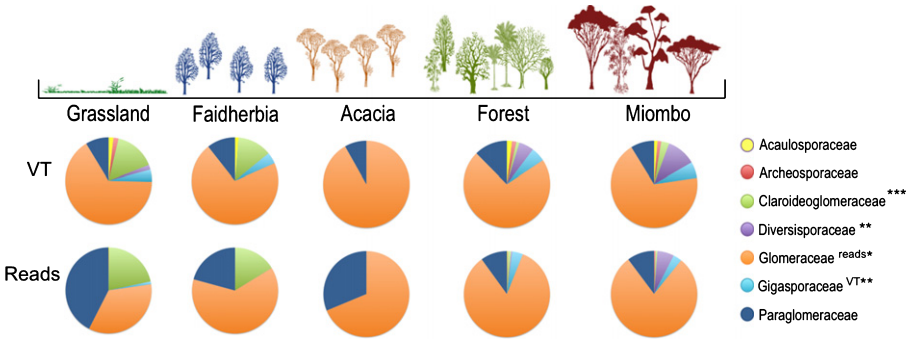

Fig. 3 Relative abundance of Glomeromycota families in each vegetation type, both in terms of the number of virtual taxa (VT) and number of reads per family. Asterisks mean that significant differences on the abundance of that family were found between the different habitats either for VT, reads or both after linear mixed models (LMM). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Table 1 Soil chemical properties (mean ± SE) for each of the vegetation types sampled

	pH	Organic C (%)	Total N (%)	NH_4 (mg g^{-1})	NO_3 (mg g^{-1})	P (mg g^{-1})	K (mg g^{-1})
Grassland	4.15 ± 0.03a	10.94 ± 0.72b	0.27 ± 0.02c	128.94 ± 23.01b	1.34 ± 0.65a	229.48 ± 45.79ab	291.61 ± 18.94b
Faidherbia	4.24 ± 0.03a	9.72 ± 1.18b	0.25 ± 0.02bc	17.26 ± 5.1a	28.94 ± 5.62b	134.73 ± 11.03ab	187.3 ± 18.99ab
Acacia	5.81 ± 0.23b	5.16 ± 0.22a	0.09 ± 0.01a	9.03 ± 1.82a	0.92 ± 0.36a	89.73 ± 8.03a	134.46 ± 7.47a
Forest	4.55 ± 0.05a	7.54 ± 0.26a	0.20 ± 0.01bc	98.00 ± 7.02b	2.13 ± 0.82a	265.37 ± 36.64b	449.59 ± 38.36c
Miombo	4.56 ± 0.11a	7.40 ± 0.21a	0.18 ± 0.02b	83.03 ± 5.35b	0.32 ± 0.16a	80.98 ± 18.82a	240.98 ± 8.85ab
F	42.791	9.347	13.023	17.157	20.820	7.425	25.888
P	< 0.0001	0.0029	0.0009	0.0003	0.0001	0.0063	< 0.0001

Phosphorus (P) and potassium (K) are bioavailable values for both nutrients, measured as ammonium-lactate and acetate extractable, respectively. Different letters mean significant differences between habitats for each measurement after ANOVA and Bonferroni comparisons. *F* and *P*-values obtained in ANOVAs are shown at the bottom of the table. C, carbon; N, nitrogen.

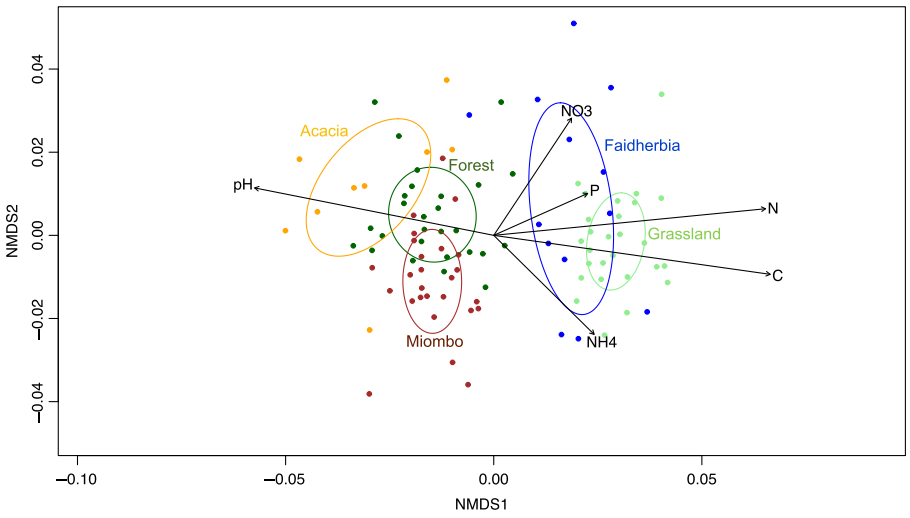

Fig. 4 Nonmetric multidimensional scaling (NMDS) ordination plot of the community composition of arbuscular mycorrhizal fungi (AMF) in the different habitats of Gorongosa National Park based on Bray–Curtis dissimilarity between samples (stress = 0.21). Ellipses represent confidence regions based on SD from the centroid for each ecological unit. Environmental variables with significant correlation with the ordination are shown. The length and orientation of each arrow are proportional to the strength and direction of the correlation between the ordination and the variables.

data contribute towards a more complete understanding of the diversity and ecology of AMF species. The present work shows that dry tropical ecosystems can sustain highly diverse AMF communities and that the structure of these communities is strongly related to vegetation type.

We detected a total of 147 AMF taxa in five habitats, which reveals a high AMF richness compared with other studies using SSU 454-pyrosequencing at the landscape scale at higher latitudes (e.g. 20–80 operational taxonomic units (OTUs) in five Sardinian ecosystems following a gradient of land use (Lumini *et al.*, 2010); 90 OTUs in a farmland–grassland ecotone (Xiang *et al.*, 2014); 113 OTUs from ten sites through a 120 000-yr chronosequence (Martínez-García *et al.*, 2015)). However, direct comparisons between different studies should be taken cautiously because virtual taxa (VT) richness is highly dependent on the pipeline and thresholds used. Interestingly, there were many new novel VTs discovered in this study, which constituted 15% of all detected taxa. Similar values were found in dry afromontane forests in Ethiopia where up to 18% of OTUs were new to science (Wubet *et al.*, 2003). The diversity of AMF in natural woodlands and forests is probably underestimated not only in tropical areas, but also in other biomes because even higher rates

of novel taxa (up to 30%) have been recently found in Mediterranean shrublands using 454-pyrosequencing (Varela-Cervero *et al.*, 2015).

Most VT detected in this study belonged to the Glomeraceae, which is the most widespread family in natural and managed ecosystems (Lumini *et al.*, 2010; Oehl *et al.*, 2010; Brearley *et al.*, 2016). Surprisingly, Paraglomeraceae, a family rarely detected in high numbers even in other tropical and temperate grasslands (Uhlmann *et al.*, 2006; Moora *et al.*, 2014; Xiang *et al.*, 2014) was the most abundant family in Gorongosa National Park (GNP) grasslands. We also found evidence of ecosystem preference for different Glomeromycotan families with a higher abundance and richness of Paraglomeraceae and Claroideoglomeraceae in open areas, whereas Diversisporaceae and Gigasporaceae increased in forested areas. Although other studies have found that the distribution of AMF taxa might differ between ecosystems (Öpik *et al.*, 2006; Kivlin *et al.*, 2011; Moora *et al.*, 2014) to our knowledge this is the first time that differences between neighboring ecosystems have been detected at the family level, reinforcing the existence of ecological specificity for AMF.

Differences among habitats were also found at finer taxonomic levels. Grasslands, savannas, dry forests and miombo shared < 12% of detected AMF and presented distinct AMF communities with specifically associated taxa. We observed a clear differentiation in AMF communities between grasslands and forested areas as reported before for temperate ecosystems (Öpik *et al.*, 2006; Kivlin *et al.*, 2011; Moora *et al.*, 2014). Both dry mixed forest and miombo had the highest values of AMF richness and diversity. Miombo had particularly rich and distinctive AMF communities, holding the highest richness, the highest number of novel AMF taxa, and the highest number and diversity of indicator AMF taxa. However, this ecosystem is under increasingly high destruction rates due to increasing subsistence slash-and-burn farming and demand for firewood by the growing population in Africa (Abbot & Homewood, 1999), which poses an important risk for the conservation of AMF diversity in dry tropical areas.

We expected to detect a gradual transition in AMF diversity values and community structure from grasslands to forested areas, but our results did not support this hypothesis. Instead we found that Acacia and Faidherbia savannas occupy soils with different chemical properties, and it might be the soil properties rather than vegetation composition that primarily drive differences in AMF communities among these habitats. Distinct soil properties may also explain why these trees do not grow intermingled in spite of both being tolerant to waterlogged soils and, thus, being able to grow as pioneer savanna woody species (Dharani *et al.*, 2006; Gope *et al.*, 2015). Savannas displayed AMF diversity values similar to those found in grasslands. This might suggest that the presence of sparse trees is not enough to increase AMF diversity towards the high values observed in forests of this study. However, we need to take into account that the reported low AMF diversity values could be affected by the different sampling design used in savannas.

Vegetation type was the main driver of AMF community structure, although a significant role of soil pH, soil organic carbon (C) and soil nitrogen (N) content was also apparent. The relative effect of each factor on AMF communities could be defined by fungal sensitivity to pH (Dumbrell *et al.*, 2010; Jansa *et al.*, 2014; Lekberg *et al.*, 2011; Davison *et al.*, 2015), ability to enhance organic matter decomposition (Hodge & Fitter, 2010; Herman *et al.*, 2012; Hodge & Storer, 2015) and uptake of N (Jumpponen *et al.*, 2005; van Diepen *et al.*, 2011). However, at the landscape scale these factors are likely to co-vary, and in this study, soil pH and contents of organic C and N were significantly negatively correlated, with increasing N and C content at lower pH values. Furthermore, vegetation type was also correlated with soil properties following a gradient defined by soil pH, organic C and N. Thus, the composition of AMF communities will be affected by the combination of biotic (plant communities) and abiotic characteristics (soil pH, N, C) that define each ecosystem and that are likely driven by seasonal flooding.

Grasslands of our study occupy the floodplains around Lake Urema with seasonal inundation that imposes an abiotic filter for both plants (Jackson & Colmer, 2005) and AMF species (Wang *et al.*, 2011). Flooding affects soil fertility and imposes major constraints for germination and growth of flood-intolerant species, thus influencing the composition of plant communities (Jackson & Colmer, 2005). Intense floods may also have a significant detrimental effect on AMF richness and root colonization levels (Miller & Jastrow, 2000; Wang *et al.*, 2011), although some AMF can grow in aquatic habitats such as freshwater lakes (Nielsen *et al.*, 2004; Sudova *et al.*, 2015; Moora *et al.*, 2016), mangroves (Wang *et al.*, 2011) and periodically flooded fields (Watanarojanaporn *et al.*, 2013). However, the effect of periodic flooding in AMF diversity is little understood and could act as an environmental filter for AMF communities in the studied floodplains.

Whether tropical and temperate ecosystems hold different values of AMF richness is still under debate. Although some studies have found similar numbers of AMF taxa in tropical and temperate ecosystems (Brearley *et al.*, 2016), the diversity of AMF colonizing the roots of woody species from tropical forests has been reported to be higher than in temperate grasslands, forests or woodlands (Helgason *et al.*, 1999; Husband *et al.*, 2002; Wubet *et al.*, 2003). Making good comparisons between studies is difficult due to the scarcity of studies in natural ecosystems, particularly from tropical regions, and the different methodologies used (spore identification, PCR-cloning, Terminal Restriction Fragment Length Polymorphism (T-RFLP), pyrosequencing) which hinder many comparisons (but see global studies by Öpik *et al.*, 2013; Davison *et al.*, 2015). By comparing with reports that also used 454-pyrosequencing targeting the SSU region, our data show that dry tropical forests have a higher AMF diversity than temperate forests whereas the contrary is observed for grasslands and open areas (Hiiesalu *et al.*, 2014; Moora *et al.*, 2014; Saks *et al.*, 2014).

Contrary to predictions based on global analyses (Davison *et al.*, 2015) and temperate areas (Öpik *et al.*, 2006; Moora *et al.*, 2014), AMF communities were more diverse in forests than in grasslands in the dry tropical ecosystems studied. This difference could be explained by regular disturbance of GNP grasslands by

flooding which could impose a strong environmental filter on AMF communities, as discussed earlier; and by the highest plant diversity and, more particularly, the large number of woody plant species forming AMF associations in tropical forests (e.g. Hogberg, 1982; Bakarr & Janos, 1996). Although we do not have information on the AMF diversity associated with different plant species in the studied habitats, a higher richness of host plants should result in more diverse AMF communities because a certain host-AMF specificity has been found in different tropical ecosystems (Uhlmann *et al.*, 2004; Wubet *et al.*, 2009; Mangan *et al.*, 2010).

In conclusion, this study provides evidence of structured AMF communities with < 12% of the taxa shared across five main habitats of a natural park in tropical Africa. Clear differences in the diversity and composition of AMF communities were found between open and forested areas. Forested ecosystems presented an unexpected high AMF richness and diversity, thus, revealing the importance of dry tropical woodlands and forests as reservoirs of mycorrhizal diversity. Because the diversity of AMF in African ecosystems can be drastically reduced by agricultural practices (Tchabi *et al.*, 2008; De Beenhouwer *et al.*, 2015a), long-term conservation strategies such as those now implemented in the GNP are crucial to preserve this unknown belowground biodiversity along with the most iconic African landscapes and species. The strong ecological structure of AMF communities shows a clear belowground–aboveground interdependence at the landscape scale in dry tropical natural ecosystems. Further research is needed to clarify how natural disturbance, soil properties and host–AMF specificity shape plant–AMF assemblages.

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Author contributions

S.R-E., R.H., M.M. and M.O. planned and designed the research; S.R-E., H.T., M.C., S.T. and R.H. performed field and laboratory work; S.R-E. and R.H. analyzed data with helpful insights from M.M. and M.O.; S.R-E. wrote the manuscript. All authors contributed to the discussion and final version of the manuscript.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information tab for this article:

Fig. S1 Coleman accumulation curves of arbuscular mycorrhizal fungal virtual taxa for each vegetation type sampled in this study.

Fig. S2 Observed and rarefied arbuscular mycorrhizal fungal taxon richness per soil sample in different habitats.

Fig. S3 Relative abundance of Glomeromycota families in Gorongosa National Park, either for number of sequences and for VT for each family.

Fig. S4 Shepard plot for the NMDS of AMF community composition in Gorongosa National Park based on Bray–Curtis dissimilarity between samples.

Table S1 Geographical coordinates and altitude of sampled sites.

Table S2 Abundance of VT in each vegetation type in Gorongosa National Park.

Table S3 VTs detected in each vegetation type and shared between pairs of vegetation types.

Table S4 Indicator AMF taxa for each vegetation type.

Table S5 Correlation coefficients and significance values for soil chemical properties and NMDS axes.

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