Deintensification of land use leads to recovery of soil microbial community composition and function after land use change in Ethiopia

Running title: Soil Microbiomes in the Ethiopian Highlands

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Abstract

Ethiopia has undergone significant land use change during the past centuries, particularly deforestation. These changes have resulted in the loss of topsoil as well as the associated soil ecosystem functions. Grazing exclusion and planting of eucalyptus are measures used to recover degraded lands and reduce deforestation, respectively. Using a gradient of the intensity of land use from natural forest to croplands, we investigated whether these measures also result in restoration of the soil microbial community. We identified the soil bacterial and fungal communities using paired-end amplicon sequencing. A total of 12,765 fungal and 12,325 bacterial OTUs were detected in the five land use types, and only ca. 2% and 17% were shared among the land uses, respectively. Total fungal and bacterial OTU richness was not significantly affected by land use change, but the conversion of forest to cropland resulted in the loss of approximately 40% and 11% of the total native fungal and bacterial OTUs, respectively. Soil pH, C, N, and aggregate stability were key factors corresponding to the overall bacterial and fungal community compositions. We also showed relationships between the microbial functional group and enzyme activities. The exclusion of grazing led to an enrichment of soil microbial communities that overlapped with the communities of the natural forest. Our results suggest that remnant native forests act as refugia for microbial communities and that restoration of microbial communities and concomitant recovery of ecosystem function via deintensification of land use is possible.

Keywords: ectomycorrhiza, ericoid mycorrhiza, exclosure, microbial diversity, soil enzymes

1. Introduction

Microbes are the unseen majority in soil and comprise a large portion of life's genetic diversity (Guerra et al., 2019; Van Der Heijden, Bardgett, & Van Straalen, 2008). Several studies have reported the role of the soil microbiome in maintaining the ability of terrestrial ecosystems to regulate the climate by enhancing ecosystem resilience to the changing climate, maintaining soil fertility and health, and supporting land productivity (Bardgett & Van Der Putten, 2014; Dubey et al., 2019; Jiao et al., 2018). Soil microbiomes have a wide amplitude of ecological functions, including fixing atmospheric nitrogen and increasing nutrient uptake, producing various plant growth-promoting substances, and secreting communication signals that trigger plants to develop defense mechanisms against diseases and pests (Kothari, Singh, & Kothari, 2016; Lakshmanan, Selvaraj, & Bais, 2014). Despite their ecological and economic importance, knowledge on the impact of the conversion of natural forest to agricultural land use on belowground microbial diversity is limited, particularly for Africa (Balami, Vašutová, Godbold, Kotas, & Cudlín, 2020).

Many tropical and subtropical forest ecosystems are undergoing uncontrolled land conversion, which is driving environmental change and ecosystem degradation (Cleveland, Townsend, Schmidt, & Constance, 2003; Sun et al., 2020). For example, encroachment into natural forests for agricultural crop production and livestock grazing has been expanding during the last 55 years in northern Ethiopia (Tekle & Hedlund, 2000; Zeleke & Hurni, 2001). The conversion of natural forest to other land uses causes disturbance due to changes in vegetation cover as well as soil disturbance and erosion, all of which can shape the soil microbial community composition (Goss-Souza, Mendes, Borges, Rodrigues, & Tsai, 2019; Kassa, Dondeyne, Poesen, Frankl, & Nyssen, 2017; Szoboszlay, Dohrmann, Poeplau, Don, & Tebbe, 2017). Such conversions have a complex effect on soil ecosystems due to the interwoven interaction between aboveground and belowground processes (Chaparro, Sheflin, Manter, & Vivanco, 2012).

Plant-soil-microbiome interactions exert critical controls on the composition and function of terrestrial ecosystems, yet the fundamental relationships between plant diversity and soil microbial diversity remain elusive (Prober et al., 2015). A study conducted on the long-term impact of forest-to-agriculture land use change in the Amazon, for example, showed that conversion led to microbial community homogenization and loss of soil microbial diversity due to the change in soil properties (Goss-Souza et al., 2019). Similar studies conducted in South

Africa and in the rainforest of Indonesia have also shown that land use change from natural to managed agricultural ecosystems leads to a shift in soil microbial composition and function (Brinkmann et al., 2019; Dube, Valverde, Steyn, Cowan, & van der Waals, 2019). The shift in the overall soil microorganism diversity and community composition has been reported to be a driver of changes in soil biogeochemical processes, thereby regulating soil function (Y. Feng et al., 2019b). In addition to biochemical processes, changes in the soil microbiome of organisms beneficial for plant health may also have positive effects through biotic interactions such as suppression of soil-borne pathogens (Campos et al., 2016; Wei et al., 2015). Biogeochemical processes are mediated by soil enzymes originating from soil microorganisms and plant roots (Ananbeh, Stojanović, Pompeiano, Voběrková, & Trasar-Cepeda, 2019; Wu et al., 2016). These extracellular enzymes degrade complex organic matter, resulting in the release of nutrients for both plants and nutrients and carbon for soil microbes (Ramin & Allison, 2019). The potential enzyme activity in soils is modulated directly both by the community of plants and microorganisms and indirectly by substrate availability (Rosinger, Rousk, & Sandén, 2019). In turn, substrate availability is controlled by biomass inputs from aboveground biomass inputs and belowground inputs from root turnover (C. Feng et al., 2019a).

Recent studies on soil microbiomes using metabarcoding approach based on pair-end amplicon sequencing have demonstrated tremendous potential for the large-scale characterization of the soil microbiomes and for tracking the shift in ecological functions of the soil microbiome in areas with different land uses following native forest conversion (Chang, Haudenshield, Bowen, & Hartman, 2017; Dubey et al., 2019; Kothari et al., 2016). Characterization of the soil microbiome following natural habitat conversion could help to understand the impact of land use change and identify the factors that shape soil microbial communities across space and time to support ecosystem conservation and design better natural resource governance (Guerra et al., 2019; Jiao et al., 2018). Furthermore, this knowledge will have a significant contribution in developing restoration strategies to rehabilitate degraded ecosystems and sustain their ecosystem functions. However, due to the lack of studies using these advanced techniques, generally in African countries, African soils remain the blind spot of the soil microbiome (Guerra et al., 2019; Větrovský et al., 2020). Thus, to better understand and characterize the Ethiopian soil microbiomes, in this study we characterized the soil microbiomes employing paired-end sequencing of fungal and bacterial DNA and investigated their ecological functions in response to land use change for the first time. This study was designed to investigate (i) the effect of natural forest conversion on the taxonomic richness and community

composition of soil microbiomes; (ii) the soil properties corresponding to the microbial OTUs across the different land use types; and (iii) the association between the microbial functional groups and potential soil enzyme activities. Due to the land use change driven by anthropogenic effects on the soil ecosystem, we expected a significant shift in soil microbiomes at the OTU richness, microbial functional group and community composition levels. In accordance with previous studies (Chaparro et al., 2012; Goss-Souza et al., 2019; Szoboszlay et al., 2017), we hypothesized that land use change mediates the change in soil physicochemical parameters, which in turn alter the soil microbial community compositions. The changes in the soil microbiome caused by the conversion of land use types are assumed to be significantly linked with the changes in soil ecological functions (enzyme activities, which are important for C, N and P acquisitions). We also hypothesized that the introduction of exotic tree species (*Eucalyptus camaldulensis*) can introduce or enrich new soil microbiomes into the landscape. Finally, we hypothesized that excluding free-gazing (uncontrolled livestock grazing) activities and human interactions drives the soil microbiome restoration of degraded land to become similar to that of natural forests.

2. Materials and methods

2.1. Site description

The study was conducted near the Village of Ambo Ber in the North Gondar Zone of Northern Ethiopia, located between 12°31′2.87″N and 37°31′24.37″E (Figure S1). The weather is subtropical, with the main rain period between June and September. The mean total annual precipitation is 1,177 mm. The mean monthly temperature varies from 18 °C in August to 22.5 °C in April. Five land use types sharing the same history that are converted from native forest following sequential transition processes were selected for investigation in this study. The studied land uses include natural forest, eucalyptus plantation, grassland, exclosure area, and cropland. The land uses were transformed from native forest at different points in time and dictated by land productivity and population growth with the primary conversion to agricultural land through encroachment followed by other land uses. Eucalyptus plantations were established on degraded communal land by the end of 1970 in response to the urban demand for firewood and construction wood. In 2007, the heavily degraded land in the study site was treated through the

exclusion of human and animal interactions to stimulate natural regeneration. The detailed site description can be found online in the Supplementary Information.

2.2. Sample collection

The soil samples were taken from the biologically active layer (0-10). Seven replicates of soil samples were analyzed for each land use type (5 land use types × 7 replicates), with each replicate consisting of 5 pooled samples. Visible plant roots, stones, litter, and debris were removed from the composite soil samples using a 2-mm mesh size sieve. The soil preparation was undertaken carefully to avoid cross contamination between soil samples. The soil sample was split into two subsamples, where one subsample was stored at − 20 °C for DNA-based molecular analysis, and the second subsample was stored at + 4 °C for analysis of the soil biological activities (enzymes), physicochemical properties [soil pH, total soil organic C, N and P, and soil aggregate stability (SAS)]. The soil sampling and sample preparation were performed as described previously (Delelegn et al., 2017).

2.3. Soil physicochemical and soil enzyme analysis

The soil chemical properties were analyzed as described in a previously published report (Delelegn et al., 2018). Briefly, the soil pH (pH) was determined in 1:2.5 soil suspensions in deionized water using a potentiometric pH meter. The soil organic C (C) and total soil N (N) were analyzed with a LECO CN analyzer (TruSpec® CN, LECO Inc.). Labile and moderately labile P were determined for all samples using sodium bicarbonate and sodium hydroxide extraction methods as described previously (Cherubin et al., 2016; Hedley & Stewart, 1982). Briefly, P was sequentially extracted from 1 g of the dry soil samples, where labile P was extracted with 30 ml of 0.5 M $NaHCO_3$ followed by extraction of moderately labile P with 30 ml of 0.1 M NaOH. The bicarbonate and hydroxide extracts were neutralized using HCl and H_2SO_4, respectively. The extracted labile P and moderately labile P concentrations were determined colorimetrically with a phosphomolybdate method (Murphy & Riley, 1962). The SAS was estimated based on the methods and apparatus of ÖNORM L 1072, 2014-04-01 (Austrian Standard) following the previously described method (Delelegn et al., 2017). Briefly, the SAS was determined from 4 g of air-dried sample using a wet-sieving apparatus. The estimation of the soil aggregate stability following the wet-sieving procedures was calculated as the mass of the aggregated soil remaining after wet sieving as a percent of the total

mass of the soil without sand. The analysis of potential extracellular enzymatic activities, including β-glucosidase, which mobilizes C; chitinase (N-acetyl-glucosaminidase), which mobilizes N and C from chitin; acid phosphatase, which mobilizes P; and protease (leucine aminopeptidase), which mobilizes N, were analyzed as described in a previous study (Delelegn et al., 2017). The method used by (Marx, Wood, & Jarvis, 2001) was applied to measure the potential enzymatic activity based on fluorescently labeled substrates, including 7-amino-4-methylcoumarin (AMC) for protease and 4-methylumbelliferone (MUB) for the other three enzymatic activities. A fluorescence spectrophotometer (Perkin Elmer Enspire Plate Reader) was used to measure the enzymatic activities. The potential enzyme activities were calculated in units of nmol h^{-1} g^{-1} dry mass as described in a previous work (Delelegn et al., 2017)

2.4. DNA extraction and Illumina sequencing

Total soil microbiome DNA was isolated from 0.25 g of each homogenized soil sample using the ZR Soil Microbe DNA MiniPrep Kit (Zymo Research, Irvine, CA, USA) following the manufacturer's protocols. The quality and quantity of the DNA samples were evaluated through gel electrophoresis of 5 µl subsamples on a 1.5% agarose gel, and the DNA concentrations were quantified using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Dreieich, Germany) as recommended by the manufacturer. Bacterial and fungal amplicon libraries were obtained separately for Illumina sequencing using the primer combination 341F (5'CCTACGGGNGGCWGCAG3') and 785R (5'GACTACHVGGGTATCTAAKCC3'), which target the V3–V4 region of the 16S rRNA gene, and fITS7 (5'GTGARTCATCGAATCTTTG3') (Ihrmark et al., 2012) and ITS4 (5'TCCTCCGCTTATTGATATGC3') (White, Bruns, Lee, & Taylor, 1990), which target the fungal ITS2 region. The PCR mix included 5 ng of DNA extract as template, 15 pmol of each forward primer (341F for bacteria and fITS7 for fungi) and reverse primer (785R for bacteria and ITS4 for fungi) in a 20 µL volume of MyTaq buffer containing 1.5 units MyTaq DNA polymerase (high-performance polymerase, Bioline) and 2 ml of Bio-StabII PCR Enhancer (Sigma, USA). For each sample, the forward and reverse primers had the same 8-nt barcode sequence. PCR was performed for 30 cycles using the following parameters: 2 min 96 °C predenaturation; 96 °C for 15 s, 50 °C for 30 s, 72 °C for 60 s and a final extension at 72 °C for 5 min. The concentration of amplicons was determined by gel electrophoresis. Approximately 20 ng amplicons of each sample were pooled for up to 48 samples carrying different barcodes. The

amplicon pools were purified with one volume of AMPure XP beads (Agencourt) to remove primer dimers and other short mispriming products, followed by additional purification on MinElute columns (Qiagen). Approximately 100 ng of each purified amplicon pool was used to construct Illumina libraries using the Ovation Rapid DR Multiplex System 1 - 96 (NuGEN). Illumina libraries were pooled, and size was selected by preparative gel electrophoresis. Sequencing was performed on an Illumina MiSeq using V3 Chemistry (Illumina) at LGC Genomics, Berlin, Germany.

2.5. Bioinformatics

A bioinformatic analysis was performed to filter out high-quality reads from the paired-end sequences generated by the Illumina MiSeq sequencing platform using MOTHUR (Schloss et al., 2009) and OBI Tools (Boyer et al., 2016) software suits. Read pairs were extracted from raw libraries when the two reads held the expected primer (forward primer for forward library, reverse primer for reverse library) at its 5' end. Forward and reverse raw reads from the same sample were assembled using the simple Bayesian algorithm with a threshold of 0.6 and a minimum overlap of 15 nucleotides as implemented in PANDAseq (Masella, Bartram, Truszkowski, Brown, & Neufeld, 2012). Reads fulfilling the following criteria were retained for further analyses: a minimum length of 250 (bacteria) and 200 (fungi) nt; a minimum average quality of 25 Phred score; containing homopolymers with a maximum length of 10 nt; and without ambiguous nucleotides. We then preclustered the reads using CD-HIT-EST with a maximum of 1% dissimilarity and only one base allowed per indel (Niu, Fu, Sun, & Li, 2010) to merge those reads likely arising from sequencing errors (Huse, Welch, Morrison, & Sogin, 2010). We detected chimeric sequences using the UCHIME algorithm (Edgar, Haas, Clemente, Quince, & Knight, 2011) as implemented in MOTHUR and removed them from the datasets. The obtained reads were then clustered into operational taxonomic units (OTUs) using the CD-HIT-EST algorithm (Fu, Niu, Zhu, Wu, & Li, 2012) at a threshold of 97% sequence similarity. The OTU representative sequences (defined as the most abundant sequence in each OTU) were taxonomically assigned against the reference sequences from the SILVA database v132 for prokaryote 16S and the UNITE database (version Unite. v7) (Kõljalg et al., 2013) using the naive Bayesian classifier (Wang, Garrity, Tiedje, & Cole, 2007) as implemented in MOTHUR using the default parameters. To improve their taxonomic annotation, the sequences identified as fungi were further classified against the full version of the UNITE (v7.0) database. Finally, all of the sequences identified as fungi were again classified against fungal sequences of the UNITE database augmented with nonfungal eukaryotic sequences

from NCBI (version 211) (Benson et al., 2013) to detect sequences from nontarget organisms. The read counts were normalized to the smallest read number per sample (10,205 and 9,273 reads for bacteria and fungi, respectively). Therefore, the final normalized datasets were used for further statistical analysis unless otherwise stated. Specific ecological functions were determined for each OTU using FAPROTAX for bacteria (Louca, Parfrey, & Doebeli, 2016) and FUNGuild (Nguyen et al., 2016) for fungi. The bacterial 16S and fungal ITS2 raw read sequence datasets are deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) under bioproject number PRJNA667338.

2.6. Statistical analysis

Total and specific functional group community richness, which refers to the number of fungal and bacterial OTUs per sample, was used to measure the soil microbial diversity. The effects of the land use types on the total and specific functional group community richness were analyzed using one-way ANOVA incorporated with Tukey's *post hoc* test using PAST. The richness datasets were tested for normality and equality of group variance using the Jarque–Bera test and Levene test, respectively. The composition of microbial communities (total and specific functional groups) and the corresponding factors were visualized using nonmetric multidimensional scaling (NMDS) based on presence/absence data and Jaccard distance measurements using the PAST program (Hammer, Harper, & Ryan, 2001). When the stress values from NMDS ordinations were higher than 0.20, a principal coordinate analysis (PCoA) based on presence/absence data and the Jaccard distance measure were employed. The first and second axes of ordinations (NMDS and PCoA) were used to capture the information on community composition. Relationships between microbial community composition and the key soil parameters were analyzed using Spearman's rank correlation. The effects of the land use types on the total and specific functional group community compositions were analyzed using one-way analysis of similarity (ANOSIM) based on presence/absence data and the Jaccard distance measure. Accordingly, an overall similarity and "pairwise tests" of the microbial functional group richness were analyzed. The statistical significance of the differences in both fungal and bacterial community compositions was determined based on 999 permutations. Bonferroni-corrected *P* values were applied because more than two groups were compared. ANOSIM produces a sample statistic (*R*), which is supposed to vary between the test groups ranging from -1 to 1 ($R = 0$, no separation; $R = 0.30 – 0.75$, there is separation but with some degree of overlapping;

244 R = 1, complete separation (Purahong et al., 2014). Correlations between microbial richness, community composition
245 and enzyme activities were analyzed using Spearman's rank correlation. All statistical tests were performed using the
246 PAST program (Hammer et al., 2001). P values < 0.05 were considered significant.
247
248 **3. Results**
249
250 ***3.1. General overview of the soil microbiome: gains and losses***
251 A total of 12,765 fungal OTUs were detected in the five land use types, and approximately 2% (282 OTUs) of the
252 total fungal OTUs were shared among the land uses. The conversion of natural forest led to a significant loss of
253 native fungal OTUs, which accounted for approximately 40% (1,689 OTUs; 13% of the total detected fungal OTUs).
254 On the other hand, the conversion of the natural forest resulted in approximately 67% (8,586 OTUs) of OTUs that
255 were not detected in the natural forest (Table 1). The total fungal net change was less than 5%, but we estimated the
256 highest gain of ectomycorrhizal and ericoid mycorrhizal functional groups in eucalyptus plantations (51% and 99%,
257 respectively). The ectomycorrhizal-forming fungal species that were abundant in the eucalyptus compared with the
258 natural forest were *Inocybe* sp., *Laccaria* sp., *Ruhlandiella peregrine* and *Descomyces* sp. Among them, *Descomyces*
259 sp. was unique to the eucalyptus land use (Table S17A). The ericoid mycorrhizal fungi detected in the eucalyptus
260 plantation were mainly from the *Myxotrichaceae* family and included *Oidiodendron maius* (481 OTUs), *O. griseum*
261 (125 OTUs), and *O. echinulatum* (1 OTU), which were not detected in any of the other land uses (Table S17B).
262 Comparatively, the highest losses in arbuscular mycorrhizae, plant pathogens, and saprotrophic fungal functional
263 groups were recorded in the eucalyptus land use (30%, 31%, and 22%, respectively). The grassland and cropland
264 showed a net gain of the plant pathogen fungal functional groups (11% and 3%, respectively), while other land uses
265 exhibited a net loss in the plant pathogen fungal OTUs (Table 1 and Figure S2A; for details, see Table S1). Some of
266 the dominant plant pathogenic fungi found in cropland were *Gibberella intricans, Edenia gomezpompae*, and *Lectera*
267 *longa*; meanwhile, in grassland, they were *Gibberella intricans, Ascochyta rabiei,* and *Macrophomina phaseolina*. A
268 newly detected plant pathogenic fungal species in croplands and grasslands was *Macrophomina phaseolina* (Table
269 S17).

A total of 12,325 bacterial OTUs were detected in the five land use types, and approximately 17% (2,088 OTUs) of the total bacterial OTUs were shared among the land uses. The conversion of the natural forest led to approximately 11% (683 OTUs; 6% of total detected bacterial OTUs) loss of native bacterial OTUs. On the other hand, the conversion of the natural forest led to new bacterial OTUs, which accounted for approximately 48% (5,956 OTUs) of the total OTUs that did not exist originally (Table 1). The total bacterial net change was less than 5%, but all land use types showed a net loss of aromatic compound-degrading and sulfur cycling bacteria except for exclosure areas, where they did not decrease. In contrast, a net gain of soil carbon cycling bacterial communities was recorded by all land uses (Table 1 and Figure S2B; for details, see Table S2).

3.2. Fungal and bacterial taxonomic richness across different land use types: total and specific functional groups

The total fungal and bacterial OTU-based richness showed no significant differences across different land use types ($P > 0.05$) (Figures 1A and 2A); however, differences in specific fungal and bacterial richness among the land uses were detected (Figures 1 and 2). The deeper analysis of specific fungal richness showed that the conversion of the natural forest to eucalyptus promoted a significant reduction in arbuscular mycorrhizae, plant pathogens and saprotrophs (Figure 1B, 1E and 1F). However, the conversion to eucalyptus promoted a significant increase in ectomycorrhizal and ericoid mycorrhizal richness (Figure 1C and 1D). Comparatively higher plant pathogenic fungal richness was detected in grassland and cropland, as shown in the natural forest (Figure 1E).

The specific bacterial OTU-based richness analysis confirmed significant variation only in carbon and nitrogen cycling bacterial richness following land use change (Figure 2C and 2E). The results indicated no differences in aromatic compound degradation, sulfur cycling, and predatory/exoparasitic bacterial richness due to land use changes (Figure 2B, 2D, and 2F). The carbon-cycling bacteria richness in eucalyptus plantations was significantly higher than that in natural forest and exclosure areas ($P = 0.02$ and $P = 0.03$, respectively) (Figure 2C).

3.3. Effects of land use types on soil microbial community compositions

Overall, the total and specific fungal and bacterial OTUs were significantly impacted by the land use types. The transition of natural forest to other land uses shifted the total fungal and bacterial OTUs ($R_{ANOSIM} = 0.47 – 0.94$, $P < 0.05$; $R_{ANOSIM} = 0.52 – 0.77$, $P < 0.05$, respectively). The exclosure showed a significant overlap in both the fungal

297 and bacterial communities with the natural forest (R_{ANOSIM} = 0.41, P = 0.07; R_{ANOSIM} = 0.23, P =0.32) (Table 2; for
298 details see Tables S3 and S9).

299 The land use types with woody vegetation showed significant separation from other land uses. The results
300 revealed that the fungal community compositions in the natural forest and eucalyptus plantations were strongly
301 separated from those in the grassland and cropland (R_{ANOSIM} > 0.75, P < 0.05). However, we observed only a
302 moderate separation of the fungal community compositions of exclosure areas from grassland and cropland (R_{ANOSIM}
303 = 0.58, P = 0.02; R_{ANOSIM} = 0.66, P = 0.01, respectively) (Table 2; for details see Table S3).

304 The pairwise ANOSIM for arbuscular mycorrhizal fungi OTUs showed a strong similarity between the
305 forested land use types (natural forest, eucalyptus plantations and exclosure areas) (R_{ANOSIM} = 0.18 – 0.28, P > 0.05;
306 Table 2; for details see Table S4). The eucalyptus plantations exhibited a significantly different composition of
307 ectomycorrhizal and ericoid mycorrhizal communities than other land uses (R_{ANOSIM} = 0.31 – 0.50, P < 0.05; R_{ANOSIM}
308 = 0.46 – 0.52, P < 0.05; Tables 2, S5 and S6). The plant pathogen fungal compositions in the eucalyptus plantations
309 were significantly separated from cropland, grassland, and natural forest, but a strong separation from cropland and
310 grassland was observed (R_{ANOSIM} = 0. 80, P = 0.01; R_{ANOSIM} = 0. 85, P = 0.02; Tables 2 and S7). The saprotrophic
311 fungal compositions in the exclosure exhibited similarities with those in the natural forest (R_{ANOSIM} = 0. 42, P = 0.06).
312 Notably, the saprotrophic fungal compositions in the eucalyptus land use were strongly separated from those in the
313 cropland and grassland (R_{ANOSIM} = 0. 85, P = 0.01; R_{ANOSIM} = 0. 91, P = 0.02; Tables 2 and S8).

314 The aromatic compound-degrading bacterial compositions in the three forested land uses were significantly
315 separated from grassland and cropland, except for the high level of similarity observed between the exclosure areas
316 and cropland (Tables 2 and S10). The carbon-cycling bacterial community composition in the eucalyptus was
317 significantly separated from that in the natural forest and exclosure grassland (Tables 2 and S11). Significant
318 similarities were observed in chemoheterotrophic and nitrogen cycling bacterial compositions between natural forest
319 and exclosure areas (Tables 2, S12 and S13). The predatory/exoparasitic bacterial community composition in natural
320 forest did not show significant differences from exclosure and grassland (Tables 2 and S14). The sulfur-cycling
321 bacterial community compositions in natural forest showed a significant difference from that of eucalyptus
322 plantations, grassland and cropland, and the composition in eucalyptus plantations was significantly distinct from
323 that of cropland (Tables 2 and S15).

3.4. Soil properties shaping the distribution of soil microbial functional groups

The difference between land use types in fungal and bacterial communities corresponded to soil properties. Overall, the most important soil properties consistently corresponding to fungal and bacterial community composition (both total and specific functional groups) were soil pH, N, and C and soil aggregate stability (SAS). The total and specific fungal and bacterial correspondence with the soil properties are presented in Figure 3A and Table S16. The total fungal, plant pathogenic, and saprotrophic fungal communities that clustered in the natural forest and exclosure were strongly associated with soil pH, C, and N (Figure 3A, 3C, and 3D). On the other hand, the arbuscular mycorrhizal fungi that separated the natural forest, eucalyptus plantations and exclosure areas from other land uses were correlated with soil C, N, easily available P (BP) and SAS (Figure 3B). Our results showed that the ectomycorrhizal and ericoid mycorrhizal OTUs found mainly in the eucalyptus were significantly correlated only with SAS and soil pH (Table S16A). The total bacterial and chemoheterotrophic bacterial community compositions clearly clustered the land use types into three groups: cropland and grassland, natural forest and exclosure areas, and eucalyptus plantations (Figure 3E and 3F). The total bacterial and chemoheterotrophic bacterial community compositions in the natural forest and exclosure areas were significantly correlated with soil pH, C and N, while the communities in the eucalyptus plantations were correlated with HP and SAS (Figure 3E and 3F).

3.5. Relationship between soil microbial functional groups and potential enzyme activities

The four tested potential enzyme activities ß-glucosidase, chitinase, phosphatase and protease showed a significant correspondence with the total fungal ($\rho = 0.48$, $P < 0.003$; $\rho = 0.50$, $P < 0.002$; $\rho = 0.39$, $P < 0.002$; and $\rho = -0.57$, $P < 0.0004$, respectively) and total bacterial ($\rho = 0.61$, $P < 0.0001$; $\rho = 0.41$, $P < 0.01$; $\rho = 0.46$, $P < 0.005$; and $\rho = -0.42$, $P < 0.015$;, respectively) community compositions. However, the specific microbial functional groups were related differently to the potential enzyme activities (Figure S3A and S3B). The arbuscular mycorrhizal fungi were associated with all potential enzyme activities except phosphatase, while the ectomycorrhizal community showed a significant correspondence with chitinase and protease enzyme activities. The ericoid mycorrhizal community showed a significant correspondence with chitinase, protease and phosphatase. The plant pathogen community was significantly correlated only with protease activity, whereas the saprotrophic community corresponded with

glucosidase and protease (Figure S3A). For the bacterial functional groups, the aromatic compound degradation and chemoheterotrophic bacterial community showed a significant correlation with phosphatase and protease (Figure S3B). The carbon cycling bacterial community was associated with only chitinase, while the nitrogen cycling bacterial community showed a significant correspondence with ß glucosidase, chitinase and protease. The predator/exoparasitic community was significantly correlated with chitinase and protease.

Focusing on richness, we found that total fungal community richness was significantly correlated with ß-glucosidase, chitinase, and protease but not with phosphatase (Figure S4A and S4B). Arbuscular mycorrhizal fungal community richness showed a significant correlation with ß-glucosidase and chitinase, whereas ectomycorrhizal fungal community richness was correlated with chitinase and protease. Ericoid mycorrhizae and saprotrophic mycorrhizae exhibited a significant correlation only with chitinase. Plant pathogenic fungal community richness did not show any correspondence with the potential enzymatic activities. Overall, neither fungal nor bacterial community richness were correlated with phosphatase.

4. Discussion

4.1. Land use change altered the total fungal and bacterial community compositions but not richness

This study demonstrated distinct differences in the fungal and bacterial community composition among land use types that could be connected to changes in soil properties and the quality of soil substrates in response to land use change. Although the total fungal and bacterial OTU richness was not significantly affected, we showed that conversion of forest to cropland resulted in a loss of approximately 40% and 11% of the total native fungal and bacterial OTUs, respectively. The higher fungal OTU loss compared with the bacterial OTUs in our study may suggest that fungal communities are more sensitive to disturbance than bacteria (Kumar & Ghoshal, 2017), which could partly be because a large part of the fungal species live in closer and host-specific interactions with plants as symbionts, pathogens and saprotrophs compared with bacteria (Heinemeyer et al., 2004; Christian L Lauber, Strickland, Bradford, & Fierer, 2008).

4.2. Changes in soil properties due to land use change impacted the soil microbial compositions

The changes in microbial community composition shown in our study are partly explained by the change in soil properties caused by the land use change. Land use change and management practices are the predominant driving factors of changes in soil attributes, such as soil organic carbon, soil nitrogen, soil phosphorus, soil pH and soil aggregate stability (Delelegn et al., 2017; Sharma, Rai, Sharma, & Sharma, 2004). The soil C and N variations determined in areas with different land uses are an effect of erosion and soil organic matter decomposition presumably due to the changes in vegetation and litter quality. Among the soil factors, soil pH was determined to be the second main factor shaping the microbial community, especially for bacteria; however, the small differences in the soil pH determined between the land uses make the effect of pH not dominant, as found in many other studies (Rousk et al., 2010). Hydroxide-reactive P was a strongly influential soil parameter in the eucalyptus stands, explaining the difference in microbial community compositions in this stand which could be associated with the limited supply of P relative to plant requirements for growth (Crous, Osvaldsson, & Ellsworth, 2015), where fertilization is not commonly practiced in plantation forests (Figure 3).

The chemoheterotrophic bacterial distribution has been shown to be related to soil N, C, hydroxide-reactive P, pH and soil aggregate stability. Chemoheterotrophic bacteria include a wide range of heterotrophic bacterial communities that derive energy from chemicals, thus presumably corresponding to different soil parameters. Several studies have reported soil pH as the main factor shaping bacterial community compositions (Christian L Lauber et al., 2008; Rousk et al., 2010). Nevertheless, in our study, among the studied bacterial functional groups, nitrogen cycling and sulfur cycling bacteria exhibited no significant correspondence. Other bacterial functional groups, including aromatic compound degradation, carbon cycling, chemoheterotrophic, and predatory/exoparasitic bacteria, showed a strong correspondence with soil pH (Christian L. Lauber, Hamady, Knight, & Fierer, 2009). We found that soil N and C were the best predictors for plant pathogenic fungi, while soil N and soil aggregate stability were the best predicting factors for saprotrophic fungal community composition (Table S16). The clustered pattern of forested land use types shown in our results (Figure 3B) could indicate vegetation composition as an additional factor that drives the divergence of the microbial community, especially mycorrhizae, which are symbiotically associated with vegetation clustered to vegetation types.

4.3. Land use change altered the composition of specific fungal functional groups in different land use types

In the eucalyptus plots, a net gain in fungal functional groups, specifically ectomycorrhizal and ericoid mycorrhizal fungi, was observed. Eucalyptus is known to form ectomycorrhizas in many types of ecosystems. The presence of ericoid mycorrhizal fungi in the eucalyptus stand is unexpected, although it is known that these fungi also associated with non-ericaceous host taxa (Chambers, Curlevski, & Cairney, 2008). The ericoid mycorrhizal fungal species detected only in the eucalyptus land use type were exclusively in the *Myxotrichaceae* family, including *Oidiodendron maius*, *O. griseum*, and *O. echinulatum* (Table S17). The ground vegetation in the eucalyptus stands is sparse and dominated by grasses with no obvious presence of ericaceous plants. The presence of ericoid mycorrhizal fungal richness in eucalyptus soil could be associated with the low decomposability of eucalyptus leaves (allelopathic effect of eucalyptus) (Briones & Ineson, 1996; Cornelissen, Aerts, Cerabolini, Werger, & Van Der Heijden, 2001). Ericoid mycorrhizal fungi have been reported to have a high degrading potential for complex organic N (Wurzburger & Hendrick, 2007), as would be expected to be present in the leaf litter of eucalyptus.

The dominant ectomycorrhizal fungal species identified were *Descomyces* sp.*, Pisolithus albus Hysterangium inflatum*, and *Tomentella pilosa* (Table S17). Another species identified, *Ruhlandiella peregrine,* is reported to be disseminated worldwide through the cross-continental human-driven dispersal of eucalyptus or melaleuca tree species of Australasian origin for commercial plantations (Kraisitudomsook et al., 2019). According to a previous study (Lu, Malajczuk, Brundrett, & Dell, 1999), the genera *Laccaria, Pisolithus and Scleroderma* are typically dominant in young eucalyptus plantations, whereas the genera *Inocybe, Descomyces, Hysterangium,* and *Cortinarius* were reported to be found only in older plantations. Interestingly, we found all of the stated ectomycorrhizal fungal species in the soils of the old eucalyptus plantation (> 30 years) in this study (Table S17A). Although the surrounding agricultural landscape and the natural forest are dominated by arbuscular mycorrhizal fungi, the origin of either the ectomycorrhizal or ericoid mycorrhizal species is unknown. However, the high species richness in the eucalyptus stands of these fungi suggests that within the surrounding landscape, there are as-yet unknown sources.

The lower species richness of fungal plant pathogens and saprotrophic fungi in eucalyptus plantations could be due to the antagonistic effect of ectomycorrhizal fungi (Smith & Read, 1997). The ectomycorrhizal fungi, e.g., *Laccaria* species, detected in the eucalyptus stand could have contributed to the reduction of common plant pathogens (Smith & Read, 1997). Eucalyptus was also the only land use that showed lower saprotrophic fungal OTU

richness than the natural forest. This could be explained by both the low dead wood levels in the eucalyptus stand (Zhou, Wang, Ren, & Sun, 2020) and by ectomycorrhizal suppression of saprotrophic fungi (Fernandez & Kennedy, 2016; Leake, Donnelly, & Boddy, 2002). The composition and richness of fungal functional group showed distinct correlation with the key soil enzyme activities. The detailed explanation on these correlations can be found online in the Supplementary Information (S7).

4.4. The fate of arbuscular mycorrhizal fungi under land use changes

Arbuscular mycorrhizal fungi are ubiquitous in most terrestrial ecosystems and are associated with more than 80% of all plant species (Davison et al., 2015) with low endemism. Our investigation shows a clear separation of the arbuscular mycorrhizal fungal community between forested land uses and non-forested land uses (i.e., cropland and grassland) as well as similarity between natural forest and exclosure areas. This pattern shows a potential separation between woody and nonwoody vegetation but also between continuous vegetation cover and systems with soil disturbance (Lumini, Orgiazzi, Borriello, Bonfante, & Bianciotto, 2010). The arbuscular mycorrhizal fungal species richness in the exclosure was not significantly different from that of the natural forest (Figure 3B). This suggests that the recovery of woody vegetation promotes a rapid recovery of arbuscular mycorrhizal fungal species richness, even though the exclosure has a lower plant species diversity and lacks the majority of the native tree species found in the natural forest (Birhane, Gebremedihin, Tadesse, Hailemariam, & Solomon, 2017; Delelegn et al., 2018). In contrast, the other woody plant system, eucalyptus, had low arbuscular mycorrhizal fungal species richness, which could be due to the density of understory vegetation and the potential suppression by ectomycorrhizal and ericoid mycorrhizal species (Becklin, Pallo, & Galen, 2012; Zobel & Öpik, 2014).

4.5. Conversion of natural forest to cropland and grassland leads to enriched fungal plant pathogens

Notably, our results showed the highest species richness of plant pathogenic fungi in the croplands and grasslands. Studies also reported that the conversion of natural forest to commercial monoculture led to higher pathogen pressure (Brinkmann et al., 2019). The increase in plant pathogenic fungal species could be due to a number of reasons, including soil disturbance and/or fertilizer inputs suppressing beneficial fungi (Borowicz, 2001; Edy, Zakaria, & Lakani, 2019; Wehner, Antunes, Powell, Mazukatow, & Rillig, 2010) as well as the low plant species diversity of

these land uses (Banerjee et al., 2019; McDonald & Stukenbrock, 2016). Moreover, the increase in newly detected pathogenic fungal richness in cropland and grasslands was perhaps facilitated by the induced spread of pathogens mediated by traditional seed exchange systems between smallholder farmers in different agroecological zones and spreading by grazing animals (Buddenhagen et al., 2017; McGuire, 2008).

The abundant plant pathogenic fungi found in cropland were *Gibberella intricans, Edenia gomezpompae*, and *Lectera longa*, while those in grasslands were *Gibberella intricans, Ascochyta rabiei,* and *Macrophomina phaseolina*. The newly detected plant pathogenic fungal species in both cropland and grassland was *Macrophomina phaseolina* (Tassi). The fungus *M. phaseolina* is one of the most harmful and economically important seed- and soil-borne pathogenic fungi, causing charcoal rot disease (Pandey, Burlakoti, Rathore, & Nair, 2020). This plant pathogen is widely adapted to more than 500 cultivated and wild plant species, including corn (*Zea mays* L.), sorghum (*Sorghum bicolor* [L.] Moench), wheat (*Triticum aestivum* L.), soybean (*Glycine max* [L.] Merrill), cotton (*Gossypium hirsutum* L.), and vegetables and other wild crops (Khan, 2007; Su, Suh, Schneider, & Russin, 2001).

4.6. Land use changes altered the composition and richness of bacterial functional groups

Soil bacterial community compositions (both total and specific functional groups) were significantly changed with land use types, especially in the eucalyptus plantation, croplands and grasslands, compared with natural forests. This can be explained by the alteration of litter and organic matter and land use intensity (Jangid et al., 2011; Suleiman, Manoeli, Boldo, Pereira, & Roesch, 2013; J. Zeng et al., 2016). However, the similarity of the total and specific bacterial functional group composition between the exclosure and natural forest was similar to that shown for arbuscular mycorrhizal fungi. As bacteria are less plant dependent than arbuscular mycorrhizal fungi, this suggests that a driving factor could be the recovery of soil chemical properties after exclosure (da C Jesus, Marsh, Tiedje, & de S Moreira, 2009; Q. Zeng, An, & Liu, 2017). Among the detected carbon cycling bacteria, the genus *Acidothermus* of the phylum *Actinobacteria* was the uniquely dominant bacterial OTU detected in the eucalyptus land use. The dominant occurrence of the genus *Acidothermus* in the eucalyptus plantation could be associated with its ability to play important ecological roles, including recycling and degradation of complex polymers (Alvarez et al., 2017). Its dominance in this land use type could therefore be explained by the phenolic polymers of the eucalyptus litter and their potential role in lignocellulose degradation, as the self-pruning nature of eucalyptus

contributes to the accumulation of lignocellulosic material in the forest floor (Větrovský, Steffen, & Baldrian, 2014). Interestingly, our results showed the loss in total native aromatic compound-degrading bacteria in all land use types compared with the natural forest. This bacterial composition in the natural forest can be attributed to the higher vegetation diversity and the possible accumulation of complex organic compounds (aromatic compounds) in the old forest floor compared with anthropogenically influenced land use types (Guggenberger, Pichler, Hartmann, & Zech, 1996; Obrist, Zielinska, & Perlinger, 2015). The composition and richness of bacterial functional group showed distinct correlation with the key soil enzyme activities. The detailed explanation on these correlations can be found online in the Supplementary Information (S7).

4.7. Exclosure areas of degraded ecosystems facilitate recovery of native soil microbiomes

It is now obvious that natural forest ecosystems provide refuge to native soil microbial communities (Guo et al., 2016; Haichar et al., 2008; Muñoz−Arenas et al., 2020); thus, to restore the native soil microbiomes lost to deforestation, promoting regeneration could be an ideal approach. This study demonstrated the role of exclosure areas in restoring soil fungal and bacterial community compositions (both total and specific functions), with the exception of fungal plant pathogens. Exclosure practices are reported to promote the natural regeneration of indigenous trees and shrubs, fostering vegetation diversity (Birhane et al., 2017; Mekuria & Aynekulu, 2013). In addition to the regeneration potential of exclosure practices, the change in soil physicochemical parameters (Birhane et al., 2017; Mekuria & Aynekulu, 2013) could have contributed to the shift in soil microbial community compositions (Guo et al., 2016; Li et al., 2014). Overall, it has been suggested that the depauperation of microbial communities through the loss of natural forest cannot easily be reversed (Sui et al., 2019); however, in our study, we could show a clear recovery of the microbial community in the exclosure to one similar to the native forest.

5. Conclusion and outlook

In this study, we showed that land use changes significantly altered the soil fungal and bacterial taxonomic and functional group richness and community compositions. Lower plant diversity and higher land use intensity in the grazing land and cropland were linked to newly detected fungal plant pathogens in soil. Ericoid mycorrhiza-forming fungi were found in the soil of eucalyptus plantations, suggesting that when grown as an exotic species, eucalyptus

may use symbiotic fungi outside the normally considered spectrum. However, we do not know for certain whether ericoid mycorrhiza-forming fungi are directly associated with eucalyptus or from where they originate in the landscape. The strategy of excluding human and grazing animals from degraded lands leads not only to recovery of the aboveground vegetation but also to enrichment of soil microbial communities that overlap with the communities of the natural forest. This indicates the potential of soil microbial communities to recover faster than aboveground vegetation, suggesting that soil factors rather than vegetation factors are the driving force of microbial community recovery. Clearly, native forests act as refugia for microbial communities, but the restoration of microbial communities and concomitant recovery of ecosystem function via deintensification of land use are possible. Our results highlight the need for further studies to deeply understand the belowground ecosystem dynamics and the above- and belowground interactions in different land use types, including the exclosure of degraded lands at the landscape level. Moreover, in view of the changing land management policy and the increasing rate of deforestation, particularly in Ethiopia and generally in Africa, we suggest undertaking a coordinated regional study on the impact of exclosure practices that could help to recover native soil microbial communities to ensure sustainable ecosystem function.

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Data accessibility

The bacterial 16S and fungal ITS2 raw read sequence datasets are deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) under BioProject number PRJNA667338.

Authors contributions

Y.T.D., T.W., D.L.G., and H.S. conceived the project. Y.T.D., D.L.G., and H.S. planned the field sampling and collected samples. T.W. and A.N. analyzed the sequence data. W.P. and Y.T.D. led the statistical analysis and prepared the first draft of the manuscript, and A.N., T.W., D.L. G and H.S. read and commented on the manuscript.

Conflict of interests

We have no conflict of interests.

Figure legends

Figure 1. Comparison of total and specific fungal functional group OTU richness across different land uses (A – F). Different letters indicate significant differences between land uses.

Figure 2. Comparison of total and specific bacterial functional group OTU richness across different land uses (A – F). Different letters indicate significant differences between land uses.

Figure 3. Nonmetric dimensional scaling (NMDS) ordinations showing factors corresponding to total and specific fungal (A – D) and bacterial (E and F) functional group community compositions distributed across five different land uses. The stress values of all NMDS ordinations are less than 0.2 based on Jaccard distance measures and 999 permutations, where SAS = soil aggregate stability; BP = bicarbonate-reactive P; HP = hydroxide-reactive P; C = organic carbon; and N = total soil nitrogen.

Tables

Table 1. Fungal and bacterial OTUs gained and lost due to land use changes. The fungal and bacterial OTUs were classified based on their ecological functions. Gain, loss and net balance were also calculated, where NF = natural rainforest; EX = exclosure area; CL = cropland; GL= grassland; and EU = eucalyptus. (**Fungal functional groups:** AMF = arbuscular mycorrhizal fungi; EM = ectomycorrhizal fungi; EriM = ericoid mycorrhizal fungi; PP = plant pathogen; SAP = saprotrophs; **bacterial functional groups:** AD = aromatic-compound-degrading bacteria; CC = carbon-cycling bacteria; CH = chemoheterotrophic bacteria; NC = nitrogen-cycling bacteria; and SC = sulfur-cycling bacteria).

	Treatments	Total fungi	AMF	EM	EriM	PP	SAP
Fungal Community Composition	Fungi shared by all land uses (NF, EX, CL, GL, and EU)	282	25	0	0	23	36
	Fungi in NF partly shared with at least one land use	2208	155	7	0	169	291
	Native fungi lost from all land uses due to land use change	1689	83	2	1	77	211
	Newly detected fungi that did not appear in the NF	8586	233	24	628	392	818
	Sum	**12765**	**496**	**33**	**629**	**661**	**1356**
	Gain/loss of fungi compared with NF	**Total fungi**	**AMF**	**EM**	**EriM**	**PP**	**SAP**
	Net balance EX (%)	0.14	0.2	-12.12	0.16	-6.96	-2.95
	Net balance CL (%)	-2.64	-17.74	-27.27	0.32	3.33	-6.93
	Net balance GL (%)	-0.62	-25	-6.06	0.32	10.89	-0.66
	Net balance EU (%)	-4.15	-29.64	51.52	98.89	-31.01	-21.76
		Total Bacteria	**AD**	**CC**	**CH**	**NC**	**SC**
Bacterial Community Composition	Bacteria shared by all land uses (NF, EX, CL, GL, and EU)	2088	19	10	287	98	27
	Bacteria in NF partly shared with at least one land use	3598	26	9	463	162	42
	Native bacteria lost from all land uses due to land use change	683	2	2	60	29	8
	Newly detected bacteria that did not appear in the NF	5956	16	43	658	252	51
	Sum	**12325**	**63**	**64**	**1468**	**541**	**128**
	Gain/loss of bacteria compared with NF	**Total Bacteria**	**AD**	**CC**	**CH**	**NC**	**SC**
	Net balance EX (%)	3.59	-14.29	12.5	2.04	3.14	0
	Net balance CL (%)	3.59	-11.11	23.44	-2.59	5.55	-3.13
	Net balance GL (%)	-0.24	-11.11	10.94	3.27	-1.66	-7.81
	Net balance EU (%)	-4.05	-15.87	29.69	-1.57	-5.91	-6.25

Table 2. Analysis of similarity (ANOSIM) based on Jaccard distance measures using abundance data comparing total and specific fungal functional group community compositions among land uses, where AM: arbuscular mycorrhizal fungi; EM: ectomycorrhizal; EriM: ericoid mycorrhiza; PP: plant pathogen; SAP: saprotrophs; AD: aromatic compound degrading bacteria, CC: carbon cycling bacteria, CH: chemoheterotrophic bacteria, NC: nitrogen cycling bacteria, and SC: sulfur cycling. Significant factors ($P < 0.05$) are indicated in bold.

Soil microbes	Land Use Categories (LU1 Vs. LU2)	*R (P)*	*R (P)*	*R (P)*	*R (P)*	*R (P)*	*R (P)*
		All Fungi	**AMF**	**EM**	**EriM**	**PP**	**SAP**
Fungal Functional Groups	Overall	**0.67(0.001)**	**0.46(0.001)**	**0.13(0.001)**	**0.20(0.001)**	**0.57(0.001)**	**0.67(0.001)**
	Natural Forest – Eucalyptus	**0.74 (0.02)**	0.18 (0.16)	**0.31 (0.04)**	**0.50 (0.01)**	**0.72 (0.02)**	**0.68 (0.01)**
	Natural Forest – Exclosure	0.41 (0.07)	0.28 (0.11)	0.07 (1.00)	0.00 (1.00)	**0.34 (0.03)**	0.42 (0.06)
	Natural Forest – Grassland	**0.80 (0.02)**	**0.72 (0.02)**	-0.01 (1.00)	0.00 (1.00)	**0.69 (0.02)**	**0.75 (0.02)**
	Natural Forest – Cropland	**0.76 (0.01)**	**0.59 (0.02)**	0.02 (1.00)	0.02 (1.00)	**0.63 (0.02)**	**0.68 (0.03)**
	Eucalyptus – Exclosure	**0.56 (0.02)**	0.24 (0.12)	**0.36 (0.01)**	**0.5 (0.01)**	0.32 (0.12)	**0.63 (0.02)**
	Eucalyptus – Grassland	**0.83 (0.01)**	**0.44 (0.02)**	**0.32 (0.01)**	**0.46 (0.03)**	**0.85 (0.02)**	**0.91 (0.02)**
	Eucalyptus – Cropland	**0.94 (0.01)**	**0.37 (0.02)**	**0.50 (0.01)**	**0.52 (0.02)**	**0.80 (0.01)**	**0.85 (0.01)**
	Exclosure – Grassland	**0.58 (0.02)**	**0.56 (0.01)**	-0.07 (1.00)	0.00 (1.00)	**0.38 (0.04)**	**0.48 (0.03)**
	Exclosure – Cropland	**0.66 (0.01)**	**0.56 (0.01)**	0.05 (1.00)	0.00 (1.00)	0.35 (0.05)	**0.56 (0.02)**
	Grassland – Cropland	**0.47 (0.01)**	**0.69 (0.02)**	0.02 (1.00)	0.02 (1.00)	**0.50 (0.03)**	**0.55 (0.01)**
		All Bac	**AD**	**CC**	**CH**	**NC**	**SC**
Bacterial Functional Groups	Overall	**0.58(0.001)**	**0.29(0.001)**	**0.19(0.001)**	**0.55(0.001)**	**0.62(0.001)**	**0.34(0.001)**
	Natural Forest – Eucalyptus	**0.65 (0.01)**	0.23 (0.33)	**0.31 (0.03)**	**0.70 (0.01)**	**0.74 (0.02)**	**0.73 (0.01)**
	Natural Forest – Exclosure	0.23 (0.32)	0.12 (0.97)	0.04 (1.00)	0.29 (0.23)	0.38 (0.05)	0.11 (1.00)
	Natural Forest – Grassland	**0.72 (0.02)**	**0.39 (0.02)**	0.21 (0.20)	**0.69 (0.02)**	**0.74 (0.02)**	**0.68 (0.02)**
	Natural Forest – Cropland	**0.59 (0.04)**	**0.43 (0.03)**	0.16 (0.57)	**0.55 (0.03)**	**0.53 (0.04)**	**0.65 (0.02)**
	Eucalyptus – Exclosure	**0.52 (0.01)**	0.13 (1.00)	**0.37 (0.02)**	**0.50 (0.01)**	**0.64 (0.01)**	0.20 (0.29)
	Eucalyptus – Grassland	**0.77 (0.01)**	**0.51 (0.01)**	0.35 (0.06)	**0.84 (0.01)**	**0.74 (0.00)**	0.15 (0.84)
	Eucalyptus – Cropland	**0.66 (0.02)**	**0.31 (0.04)**	0.20 (0.32)	**0.61 (0.01)**	**0.68 (0.01)**	**0.50 (0.02)**
	Exclosure – Grassland	**0.72 (0.01)**	**0.43 (0.01)**	0.22 (0.06)	**0.66 (0.01)**	**0.72 (0.01)**	0.26 (0.21)
	Exclosure – Cropland	**0.54 (0.01)**	0.22 (0.31)	0.00 (1.00)	**0.42 (0.02)**	**0.45 (0.01)**	0.20 (0.36)
	Grassland – Cropland	**0.56 (0.02)**	0.26 (0.10)	0.10 (1.00)	**0.39 (0.01)**	**0.57 (0.01)**	0.06 (1.00)

Note: *R* degree of separation between test groups ranging from − 1 to 1; $R = 0$, not different; $R = 1$, completely different (i.e., where the *R* - value between 0 – 0.299 "no separation/overlapping"; 0.300 – 0.749 "different but with some overlapping"; and > 0.750 "well separated"); N = population size. *P* values were based on 999 permutations, and Bonferroni correction was applied in all cases.