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SOIL MICROBIOLOGY

Fungal, Bacterial, and Archaeal Diversity in Soils Beneath Native and Introduced Plants in Fiji, South Pacific

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Abstract

The Fiji Islands is an archipelago of more than 330 islands located in the tropics of the South Pacific Ocean. Microbial diversity and biogeography in this region is still not understood. Here, we present the first molecular characterization of fungal, bacterial, and archaeal communities in soils from different habitats within the largest Fijian island, Viti Levu. Soil samples were collected from under native vegetation in maritime-, forest-, stream-, grassland-, and casuarina-dominated habitats, as well as from under the introduced agricultural crops sugarcane, cassava, pine, and mahogany. Soil microbial diversity was analyzed through MiSeq amplicon sequencing of 16S (for prokaryotes), ITS, LSU ribosomal DNA (for fungi). Prokaryotic communities were dominated by Proteobacteria (~ 25%), Acidobacteria (~ 19%), and Actinobacteria (~ 17%), and there were no indicator species associated with particular habitats. ITS and LSU were congruent in β-diversity patterns of fungi, and fungal communities were dominated by Ascomycota (~ 57–64%), followed by Basidiomycota (~ 20–23%) and Mucoromycota (~ 10%) according to ITS, or Chytridiomycota (~ 9%) according to LSU. Indicator species analysis of fungi found statistical associations of *Cenococcum*, *Wilcoxina*, and *Rhizopogon* to *Pinus caribaea*. We hypothesize these obligate biotrophic fungi were co-introduced with their host plant. *Entoloma* was statistically associated with grassland soils, and *Fusarium* and *Lecythophora* with soils under cassava. Observed richness varied from 65 (casuarina) to 404 OTUs (cassava) for fungi according to ITS region, and from 1268 (pine) to 2931 OTUs (cassava) for bacteria and archaea. A major finding of this research is that nearly 25% of the fungal OTUs are poorly classified, indicative of novel biodiversity in this region. This preliminary survey provides important baseline data on fungal, bacterial, and archaeal diversity and biogeography in the Fiji Islands.

Keywords Fiji Islands · Soil microbiome · Mycorrhizal fungi · ITS MiSeq · UPARSE · LSU rDNA

Introduction

The Fiji Islands are an archipelago of 332 islands ranging in size from (10,390 to < 1 km^2) and spread over an area of approximately 18,300 km^2 in the tropical South Pacific (17.7134° S, 178.0650° E) [1]. The geological history of Fiji is complex owing to its proximity to the Australian–Pacific plate boundary. The islands are of volcanic origin and began to form in the Late Eocene [2]. Floristic studies have shown that the majority of plant species in Fiji are native, 25% of which are endemic to the islands [3]. Many plant species such as cassava (*Manihot esculenta* Crantz) and sugarcane (*Saccharum officinarum* L.) have been introduced into Fiji for agriculture. Cassava was introduced around the 1800s, while sugarcane is thought to be indigenous to the islands of the South Pacific, where several of the world's principal commercial varieties originated, with commercial farming ongoing since 1862 (http://www.fao.org, http://www.fsc.com.fj). These crops typically rely upon traditional animal and plant-based organic amendments (e.g., for cassava) or conventional fertilizers (in the case of sugarcane) to maintain soil fertility and productivity. Lower input perennial plants were later introduced for forestry, including pine

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(*Pinus caribaea* Morelet) introduced in 1955 [4] and mahogany (*Swietenia macrophylla* King) introduced in 1935 (http://www.fao.org/docrep/005/Y7207E/y7207e06.htm). In contrast to what is known about Fiji's macrodiversity [3, 5], little is known about fungal and other microbial diversity of these islands, or how introduced plant hosts impact microbial communities.

Mycological studies in Fiji began in the early 1900s and were primarily aimed at documenting terrestrial macrofungi on the islands. Expeditions made by the Australian mycologist J.B Cleland and US mycologists H.E. Parks and W.A. Setchell in the 1920s, and A.C. Smith and others in the 1940s and 1950s were among the earliest documentation of the macrofungi in Fiji (a Database of fungal collection data for specimen that have been made in Fiji and accessioned into fungal herbaria collections is provided, please see the Electronic supplementary material Section). Given the importance of agriculture to these island economies, there have been many surveys of economically important plant pathogenic fungi in the South Pacific [6]. A number of new fungal species have been described from Fiji, including *Polyporus fiji* Lloyd and *Paracercospora fijiensis* (M. Morelet) Deighton, the causal agent of "black Sigatoka of banana," which devastated the banana industry across the island in the early 1900s [7]. In the 1980s and later, J.J. and B. Kohlmeyer carried out extensive surveys of marine fungi in Fiji [8, 9]. Collections of fungal fruiting bodies and infected plants of Fiji were originally maintained at the Herbarium of the University of the South Pacific, Suva. They have since been transferred to the Herbarium at the University of California, Berkeley CA, USA, partly due to the expense and challenge of maintaining a fungarium in a tropical developing country. Most prokaryotes known from Fiji are associated with animal and plant disease. The *Pacific Islands Pest List* (http://www.spc.int/pld/) is a database compiled from reports, surveys, and publications that includes all known fungal, insect, bacterial, and viral plant pathogens in the South Pacific. Still, there are large gaps in our understanding on microbial and fungal taxa across different habitats of Fiji, and other Pacific islands.

Recent advances in high throughput amplicon sequencing have revolutionized the ability to detect and discern microbial diversity on the planet [10]. With these tools, biogeographic patterns in fungal, bacterial, and archaeal diversity have been detected at continental and global scales [11–14]. In this research, we leverage high throughput amplicon sequencing of the ITS, LSU, and 16S ribosomal RNA (rDNA) to generate a baseline molecular inventory and characterization of fungal and prokaryotic communities in soils from the capital Fijian island, Viti Levu. Our goal was to assess whether fungal, bacterial, and archaeal diversity in soils differed between habitats dominated by native or introduced plant species. Thus, we sampled soils beneath economic crops including annual sugarcane and cassava, as well as perennial pine and mahogany stands. We also sampled soils in diverse native habitats that included perennial maritime, forest, stream bank, and grassland habitats. Previous studies have shown that exotic plants tend to diminish invaded plant community diversity [15]. Similarly, the strong influence of plants on associated microbial communities is well documented [16, 17]. While there is extensive literature on invasive species impacts on soils and plant communities, and exotic plants are known to alter soil-plant feedbacks (Callaway et al. 2004), the organisms involved in this biogeochemical re-wiring are not known. Next-generation sequencing can help to assess impacts of introduced plants on soil microbial communities [18]. We hypothesized that soil microbial diversity would differ between vegetation types, and that higher levels of fungal and prokaryotic diversity would be found under native host plants compared to introduced host plants. We also expected the composition of microbes would differ between native and introduced hosts, and we expected to detect introduced (and native) ectomycorrhizal fungal (EMF) species in soils beneath the introduced obligate ectomycorrhizal host plant *Pinus caribaea*.

Materials and Methods

Sampling and Sample Preparation and Storage

Soil samples were collected in both natural and agricultural habitats in Fiji (Fig. 1). Natural habitats that were sampled included beach intertidal zones at the forest edge (2 sites), a grassland (1 site), stream banks (3 sites), dense native forests (2 sites), and forests with a Casuarina canopy (*Casuarina equisetifolia* L., 2 sites). These sites were dominated by perennial overstory vegetation. Agricultural habitats that were sampled consisted of the annual species cassava (*Manihot esculenta* Crantz, 2 sites) and sugarcane (*Saccharum officinarum* L., 2 sites), as well as forest stands established with perennial mahogany (*Swietenia macrophylla* King, 3 sites), and pine (*Pinus caribaea* Morelet, 1 site) species. For each site, 3 soil cores were collected to a 15 cm depth under the target host plant, and were dried and preserved in silica gel. Samples were stored at room temperature prior to DNA extraction.

Soil Analyses

Soil samples for each site were analyzed for chemical analysis. Zinc (Zn), manganese (Mn), and iron (Fe) cations were measured with an AAnalyst 400 Atomic Absorption Spectrometer (PerkinElmer Inc., USA). Soil phosphorus (P), nitrate (NO_3), and ammonium (NH_4) were measured on a QuikChem 8500 Series 2 (Lachat Instruments, USA) attached to an ASX-520 Autosampler (Teledyne CETAC, USA). Soil

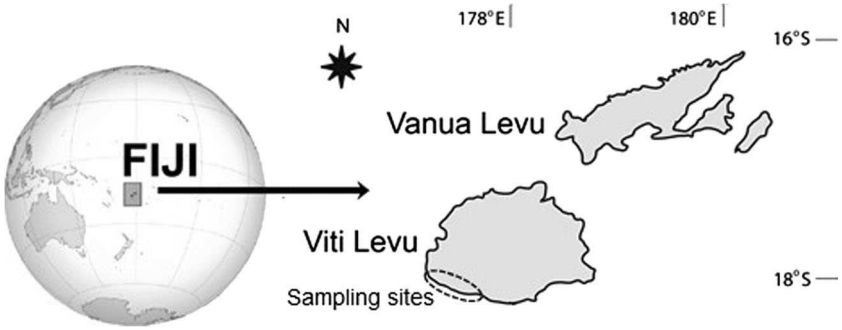

Fig. 1 Map of Fiji Islands

magnesium (Mg), calcium (Ca), and potassium (K) were measured with an AutoAnalyzer AA3 (SEAL Analytical, USA). All the analyses were performed by the Soil and Plant Nutrient Laboratory, Michigan State University, USA.

DNA Extraction, PCR Amplification, and Amplicon Library Preparation

DNA was extracted from ~0.5 g of dried and homogenized soils with the PowerMag® Soil DNA Isolation Kit (Qiagen, Carlsbad, CA) following manufacturer's recommendations. Extracted DNA was amplified using DreamTaq Green DNA Polymerase (Thermo Fisher Scientific, USA) with the following primer sets: ITS1f-ITS4 and LROR-LR3 for fungi and 515F-806R for bacteria and archaea, following a protocol based upon the use of frameshift primers, modified from [19]. PCR products were stained with ethidium bromide, separated by gel electrophoresis, and visualized under UV light. Amplicons were normalized between samples with SequalPrep Normalization Plates (Thermo Fisher Scientific, USA) and were then pooled. The amplicon library was concentrated 20:1 with Amicon Ultra 0.5 mL 50 K filters (EMD Millipore, Germany), purified with Agencourt AMPure XP magnetic beads (Beckman Coulter, USA), and subsequently sequenced on an Illumina MiSeq analyzer with the v3 600 cycle kit (Illumina, USA). Sequence reads have been submitted to NCBI SRA archive with the accession number SRP102954, BioProject ID: PRJNA380966.

Bioinformatic Data Analysis

Sequence quality was evaluated for raw forward and reverse Illumina 16S, ITS, and LSU reads with FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). 16S reads were merged with PEAR [20]. For ITS and LSU only, the forward reads were used in downstream analyses given insufficient overlap needed for assembly and lower quality of reverse reads. Selected reads were demultiplexed in QIIME according to sample barcodes [21] and Cutadapt was used to remove Illumina adapters and sequencing primers [22]. Sequences were quality filtered, trimmed to equal length [23, 24], de-replicated, removed from singleton sequences, and clustered into operational taxonomic units (OTUs) based on 97% similarity following the UPARSE algorithm [25]. Taxonomic assignments were performed in QIIME with the RDP naïve Bayesian Classifier [26]. The UNITE sequence database [27] version 7.1 2016-08-22, the RDP Fungal LSU training data, and the Greengenes database [28] version gg_13_8 were used as ITS, LSU, and 16S rDNA reference sequence databases, respectively. OTUs of putative mycorrhizal, endophytic, and plant pathogenic fungi were also extracted from the ITS dataset with FUNGuild [29], checked manually, and analyzed separately.

Statistical Analyses

The *otu_table.biom* [30] and *metadata.txt* files for each marker gene were imported into R statistical environment for analysis [31]. Before proceeding with analyses, *otu_table.biom* files were quality filtered removing OTUs with less than 10 total sequences [32] across samples and with < 5 sequences in a given sample [33]. Observed OTU richness [34] and Shannon's diversity index [35] were calculated using the function "plot_richness" in the R package *phyloseq* [36] and used as α-diversity metrics. Before calculating β-diversity, OTUs were normalized by dividing their number by the total number of sequences per sample, and Hellinger transformed to compensate for highly abundant OTUs, providing a compromise between linearity and resolution [37]. Non-metric multidimensional scaling (NMDS) was used to investigate community β-diversity with the functions "metaMDS" and "ordinate" from *vegan* and *phyloseq* packages. To test the influence of soil parameters on fungal and prokaryotic community structure, we first selected the subset with Euclidean distances of scaled environmental variables having maximum Pearson correlation with community dissimilarities, as suggested by Clarke and Ainsworth [38] and implemented in the "bioenv" function in *vegan*. Then, we used canonical analysis of principal coordinates (CAP) [39] constrained ordination to display differences in community structure related to specifically identified environmental soil variables. Model and diversity patterns were tested for statistical differences

across sites in the *vegan* package with the PERMANOVA function "adonis." Homogeneity of variance was assessed using the function "betadisper." NMDS ordination patterns of ITS and LSU rDNA genes were compared with the "protest" function [40]. A taxon-group association analysis was used to assess the degree of preference and significance of each OTU for the target group in relation to other groups using function "multipatt" in the *indicspecies* R package [41].

Results

Community Composition

In this study, fungal, bacterial, and archaeal soil community structure in different sites on the Fiji Island of Viti Levu were investigated with high-throughput amplicon sequencing. A total of 54 soil samples (3 replicates for each of the 18 sites across 9 different habitats) were analyzed for 16S and ITS rDNA, and 51 (3 replicates for each of the 18 sites across 9 different habitats) for the LSU rDNA diversity following DNA extraction, PCR amplification, and sequencing on Illumina MiSeq DNA analyzer. In total, we obtained 3,596,552, 1,929,546, and 3,414,801 raw sequence reads for 16S, ITS, and LSU libraries. After quality filtering, we obtained 5790, 1259, and 1061 OTUs for 16S, ITS, and LSU, respectively (Supplemental Datasets 1, 2, and 3).

Overall, the most abundant prokaryotic phyla were Proteobacteria 25.4%, Acidobacteria 19.1%, Actinobacteria 16.7%, Verrucomicrobia 10.4%, Planctomycetes 8.2%, Chloroflexi 7.6%, Nitrospirae 3.3%, and Crenarchaeota (Archaea) 2.0%. Barplots showing relative abundances at ordinal level for ITS, LSU, and 16S OTUs are reported in Fig. S1. A list of the top 10 most abundant OTUs for each of the 18 sites sampled is reported in Table S1. The most abundant fungal phyla were Ascomycota 57.4%, Basidiomycota 23.5%, Mucoromycota 9.9%, Glomeromycota 0.81%, Rozellomycota 0.51%, and Chytridiomycota 0.48% according to the ITS rDNA. A total of 7.3% of the ITS-based OTUs were unclassified at phylum-level. Based on LSU rDNA OTUs, there were Ascomycota 64.5%, Basidiomycota 20.5%, Chytridiomycota 9.1%, and Glomeromycota 0.30%, with a total 5.7% of unclassified OTUs at phylum-level. Barplots showing relative abundance at ordinal level for ITS, LSU, and 16S OTUs are reported in Fig. S1. A list of the top 10 most abundant OTUs for each of the 18 sites sampled is reported in Table S1.

Analysis of Different Fungal Guilds

FUNGuild analyses allowed us to quantify the relative abundance of putative mycorrhizal (362 OTUs, 32 genera, and 6.5% of the total relative abundance), endophytic (86 OTUs, 20 genera, and 2.1% of the total relative abundance), and plant pathogenic fungi (156 OTUs, 50 genera, and 14.0% of the total relative abundance) in each sampled habitat. Relative abundances of putative mycorrhizal (including arbuscular, ectomycorrhizal, ericoid, and orchid mycorrhizas) fungal genera extracted from the ITS OTU dataset are summarized in Fig. 2 barplots. Among the ectomycorrhizal (EM) fungi, *Tomentella* (14.1%) was the most abundant, followed by *Entoloma* (11.6%), *Chloridium* (10.7%), *Cenococcum* (8.5%), *Amanita* (7.7%), and *Inocybe* (2.7%). *Entoloma*, a genus that includes both mycorrhizal and saprotrophic species, was most abundant in the grassland site (Fig. 2a). *Oidiodendron* (6.7%) and *Serendipita* (0.2%) were the single representatives of ericoid and orchid mycorrhizal/endophytic fungi, respectively. Among arbuscular mycorrhizal fungi (AMF), the most abundant was *Glomus* (7.7%), followed by *Rhizophagus* (2.9%), *Scutellospora* (1.4%), *Acaulospora* (0.7%), and *Septoglomus* (0.48%). Among endophytes, *Lecythophora* (4.3%) was the unique dark septate endophyte (DSE) detected, mostly in sugarcane, pine forest, and grassland habitats. *Camarosporium* was the most abundant (29%) endophyte and was found mostly in grassland, beach intertidal, and cassava habitats. The plant endophyte *Phomopsis* (18.3%) was abundant in casuarina habitats. *Trichoderma*, a lineage of fungal endophytes and mycoparasites, was detected at highest levels (13%) in mahogany, native forest, and stream bank habitats (Fig. 2b). The most abundant plant pathogenic fungal genera were represented by *Haematonectria* (34.5%), *Fusarium* (24.0%), and *Cylindrocarpon* (= *Ilyonectria*) (10.1%), found in high abundance in native forest and stream bank habitats, and *Ganoderma* (8.3%) in habitats with woody trees (native forests, stream banks, pine, and mahogany). *Phoma* (3.5%) was most dominant in the sugar cane habitat (Fig. 2c). Indicator species analysis (Table 1) showed statistically significant associations of specific OTUs to a specific habitat. For example, *Cenococcum*, *Wilcoxina*, and *Rhizopogon* genera were statistically associated to the pine habitat, *Fusarium* to cassava, and *Entoloma* to the grassland habitat.

Comparisons of Fungal Communities Using ITS and LSU rDNA

Knowing that every primer set and locus has inherent biases, we characterized fungal communities with both ITS and LSU rDNA. We found that total observed OTU richness (200–500 OTUs) (Fig. 3) and beta diversity patterns (Fig. 4) were consistent between these two loci. However, many OTUs across both markers were poorly classified; either these consisted of novel sequences or sequences were most similar to unclassified organisms known only from other environmental sequences (Fig. S1). There were,

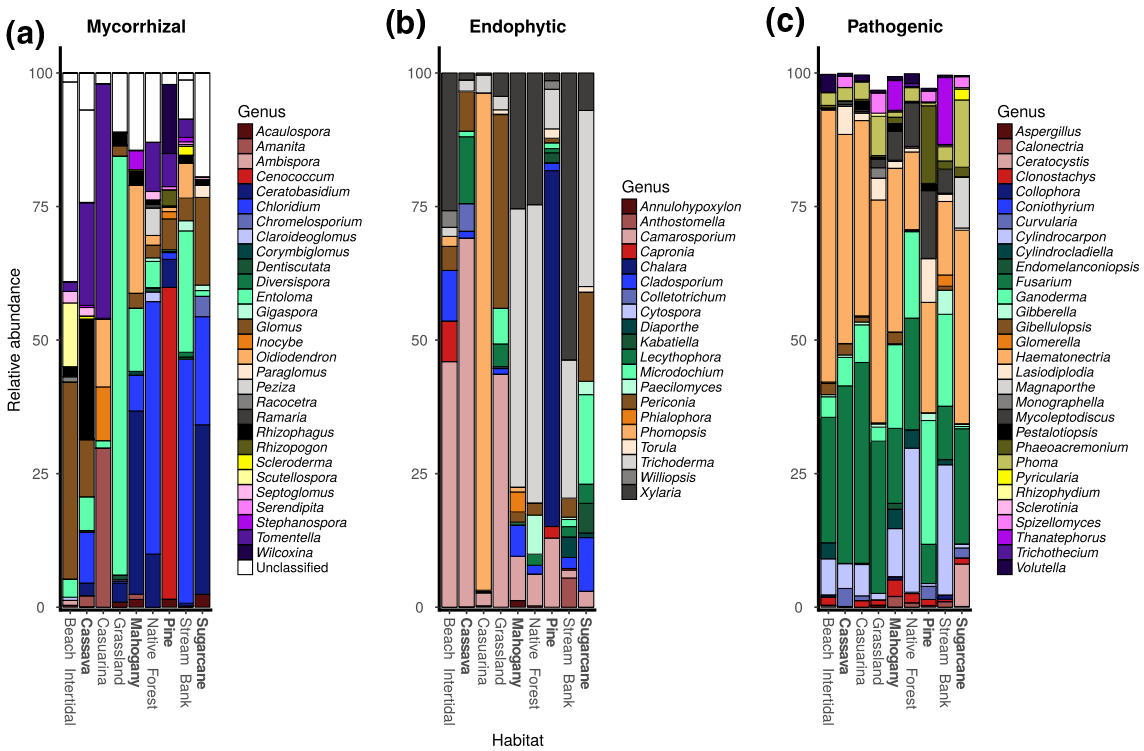

Fig. 2 Barplots of fungal genera relative abundance based on the ITS rDNA region and divided by ecological guild. (**a**) Putative mycorrhizal (including ecto, arbuscular, ericoid, and orchid mycorrhizas); (**b**) endophytic (top 30 genera) and (**c**) pathogenic (top 30 genera) fungi. Habitats dominated by introduced plant hosts are denoted in bold

however, discrepancies between the classification or detection capability of ITS and LSU amplicons. Specifically, orders of fungi detected in ITS data but not in LSU data included Corticiales, Geminibasidiales, Mortierellales, Onygenales, Orbiliales, Rhizophlyctidales, Russulales, Sporidiobolales, Trichosphaeriales, and Trichosporonales. Conversely, orders of fungi detected in LSU data, but missing in ITS datasets, included Blastocladiales, Boliniales, Cladochytriales, Classiculales, Coronophorales, Erythrobasidiales, Geastrales, Gomphales, Helicobasidiales, Lobulomycetales, Melanosporales, Paraglomerales, Phyllachorales, Sebacinales, and Septobasidiales. However, aside from Mortierellales, most taxa in these aforementioned orders were low in abundance. In every case, there were fewer unclassified OTUs for ITS compared to LSU amplicons.

Table 1 Statistically significant associations of fungal operational taxonomic units (OTUs) to a specific habitat. Habitats dominated by introduced plant hosts are denoted in bold

	OTU_ID	Stat	*p* val	Taxon	Guild
Pine	OTU 271	0.621	0.0282	*Wilcoxina*	Mycorrhizal
	OTU 63	0.58	0.0282	*Cenococcum*	Mycorrhizal
	OTU 1645	0.579	0.0282	*Rhizopogon*	Mycorrhizal
	OTU 144	0.688	0.03	*Chalara*	Endophytic
Native forest	OTU 1928	0.685	0.0282	*Peziza*	Mycorrhizal
Grassland	OTU 124	0.706	0.0224	*Entoloma*	Mycorrhizal
	OTU 249	0.709	0.004	*Periconia*	Endophytic
Beach intertidal	OTU 3452	0.634	0.0496	*Racocetra*	Mycorrhizal
Cassava	OTU 663	0.61	0.0385	*Lecythophora*	Endophytic
	OTU 23	0.593	0.03	*Camarosporium*	Endophytic
	OTU 83	0.727	0.0156	*Lasiodiplodia*	Pathogenic
	OTU 5	0.716	0.0234	*Fusarium*	Pathogenic

Alpha and Beta Diversity

Alpha diversity showed variation in OTU richness and evenness across the different habitats, with the presence of outlier samples (Fig. 3). ITS (Fig. 3a) and LSU (Fig. 3b) rDNA datasets were consistent with each other in terms of observed richness and Shannon diversity index. Greatest fungal richness and diversity were recorded in cassava (highest sample replicate = 404 OTUs stream bank (395 OTUs), grassland (370 OTUs), and native forest (368 OTUs) habitats. In contrast, the lowest fungal richness and diversity was detected in casuarina (lowest sample replicate = 65 OTUs), mahogany (77 OTUs), beach intertidal (116 OTUs), and pine (149 OTUs) habitats. The highest prokaryotic richness and diversity was recorded (Fig. 3c) in cassava (highest sample replicate = 2931 OTUs), stream bank (2888 OTUs), and grassland (2715 OTUs) habitats, while the lowest diversity was detected in pine (1268 OTUs), casuarina (1493 OTUs), and sugarcane (1498 OTUs) habitats.

Non-metric multidimensional scaling (NMDS) graphs were used to show β-diversity patterns of fungal and prokaryotic communities across the different sampling sites (Fig. 4). Defined clusters of samples are shown for sugarcane, beach intertidal, and mahogany (site 1 and 2 in particular) for the ITS (Fig. 4a), LSU (Fig. 4b), and 16S (Fig. 4c) datasets (Shepard diagrams of stress are reported in Fig. S2). In particular, sugarcane samples (upper-left), beach intertidal (lower-left), and mahogany samples (right-middle) and highland casuarina are most distinguishable in ordination space. Native forest and stream bank samples occupied the center of the multidimensional space, while pine samples were closer to grassland and lowland casuarina samples. Procrustes analysis confirmed that the ITS and LSU NMDS ordination were similar in configuration ($m_{12} = 0.11$, $r = 0.94$, $p = 0.0001$, perm. = 9999) as is shown in the Procrustes superimposition and ordination residuals plots (Fig. S3).

PERMANOVA results show that approximately half of the variance is explained by site effects, and in particular, 52.3% of the sums of squares can be explained by the sampling site in the ITS, 52.6% in the LSU, and 63.5% in the 16S dataset, respectively ($p \leq 0.01$, perm. = 9999). Homogeneity of variance test indicates non-significant differences in dispersion within group samples at $p \leq 0.05$ for ITS (ANOVA, $p = 0.078$, perm. = 9999), LSU (ANOVA, $p = 0.053$, perm. = 9999), and 16S (ANOVA, $p = 0.39$, perm. = 9999), confirming the robustness of the location effect detected by PERMANOVA (distribution of distances of group samples from each relative group centroid in multivariate space as provided in Fig. S4).

Soil parameters (Table 2) that showed the highest correlations with community dissimilarities were K, Ca, Mn, and NH_4 (correlation $r = 0.56$) for fungi, and P, K, Ca, Mn, Fe, and NH_4 (correlation $r = 0.47$) for prokaryotes. CAP analysis performed using the two subsets of soil parameters as model constraints (Fig. 5) showed how environmental variables (the environmental scores were plotted onto the ordination) determined changes in community composition. CAP ordination of fungi (Fig. 5a) explained 26.2% of the total variance across four axes (ANOVA, perm. 9999, $F_{4,49} = 4.286$, $p = 0.001$). Native forest and stream bank samples clustered on the top of the ordination space. Increased NH_4 was associated to mahogany 2 and 3 as well as casuarina 2 which clustered on the right top corner of the ordination while beach intertidal and cassava samples, which clustered in the opposite side of the ordination, were driven by Ca. Pine and sugarcane samples lied along the K arrow and clustered in the lower corner of the ordination space. CAP ordination of prokaryotes (Fig. 5b) explained the 34.08% of the total variance across 6 axes (ANOVA, perm. 9999, $F_{6,47} = 4.050$, $p = 0.001$). Differential effects of Ca and NH_4 on the community composition are similar to what was obtained for fungi to explain variation along the CAP1 axis. Prokaryotes in the beach intertidal samples clustered in the top left ordination graph, while mahogany 2 and 3 and casuarina 2 along the top left. The other site and habitats appear intermediate and influenced by a mixture of K, Fe, and Mn variables.

Discussion

Microbial Diversity on Islands in the South Pacific

Soil microbial communities live in a heterogeneous environment and are complex in function [42]. Here, we show that fungal, bacterial, and archaeal communities in Fiji are diverse and distinguished by habitat. We used both ITS and LSU rDNA to characterize fungal communities and found that independent characterizations of the fungal community were largely consistent between loci, as has been found previously [17]. Although taxonomy could be assigned to a greater proportion of ITS OTUs compared to those of LSU, many OTUs for both loci were either novel or most similar to unclassified organisms known only from environmental sequences. For example, > 5% of fungal OTUs could not be classified at the ordinal level for either rDNA marker, indicative of novel fungal phylogenetic diversity in need of characterization and representation in public databases. There were discrepancies between the detection or possible classification of fungi by these two loci. This is likely due to biases in reference databases for taxa from the Northern Hemisphere and temperate environments, as there has been less research done on tropical fungi in the Southern Hemisphere and South Pacific [27, 43]. Further, the reference sequence library for ITS is more expansive and better curated than that of LSU, yet both are lacking in their representation of tropical and Southern Hemisphere fungi. This is particularly true for early diverging lineages of fungi,

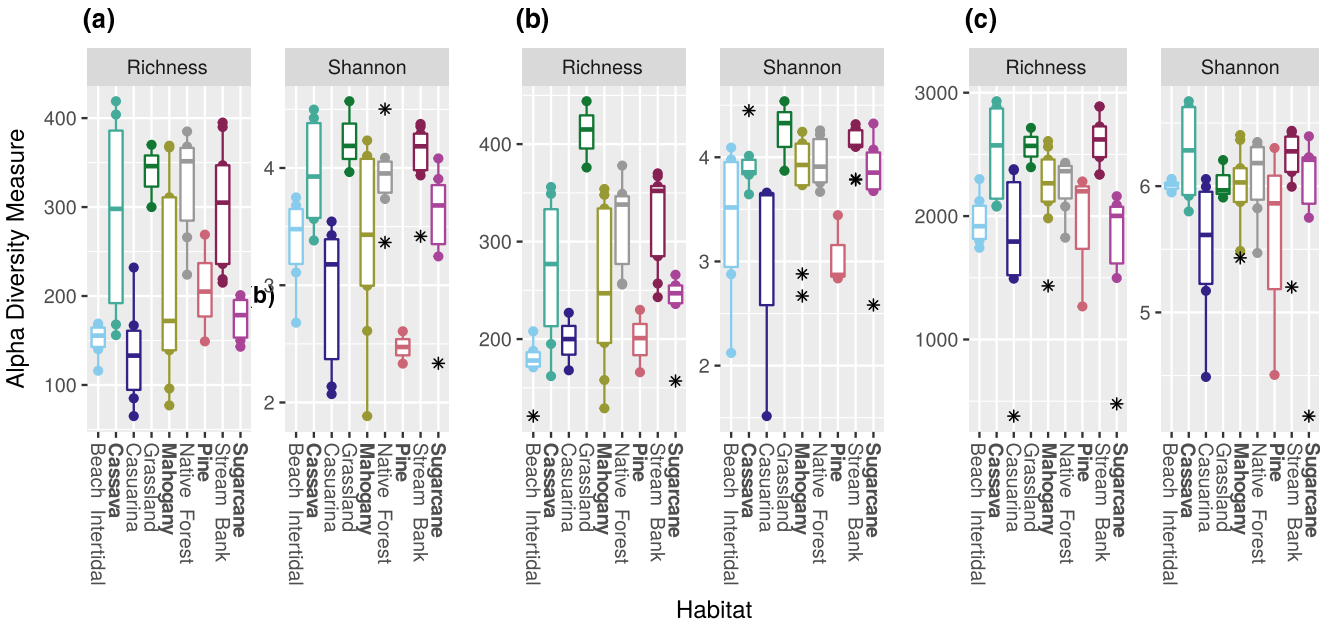

Fig. 3 Alpha diversity boxplots of observed OTUs Richness and Shannon index showing median, upper, and lower quartiles, and maximum and minimum values as well as outliers of the soil sample distribution of each of the 9 habitats. (**a**) ITS rDNA, (**b**) LSU rDNA, and (**c**) 16S rDNA gene datasets. Habitats dominated by introduced plant hosts are denoted in bold

such as the Mortierellales, which are not well represented or classified in LSU reference libraries. In contrast, most prokaryotic sequences were classified at a reasonable confidence interval, and richness based on 16S rDNA (1500–2800 OTUs) was nearly an order of magnitude higher than fungal diversity.

Microbial Differences Between Unmanaged and Agricultural Ecosystems

Our hypothesis that native habitats would host more fungal and prokaryotic diversity than agricultural ecosystems was not supported by these data. While grasslands, native forests, and stream bank soils did consistently contain among the highest diversity of fungi, bacteria, and archaea, the beach intertidal zone was characterized by a distinct community of fungi and low OTU richness, but high prokaryotic diversity. We suspect this is due to the relatively low availability of carbon for fungi in this habitat compared to the other habitats, limiting heterotrophic niches, but may also arise from low levels of iron, manganese, and zinc co-factors, or high levels of calcium and phosphorus. Prokaryotic richness in the beach intertidal zone (~2000 observed OTUs) was similar as that of pine and sugarcane habitats, yet each habitat was comprised of a different community of species. In fact, cassava had among the highest diversity of observed fungal, bacterial, and archaeal OTUs of any habitat sampled in this study. This could be a result of the traditional agricultural system by which cassava is grown in Fiji, in small plots

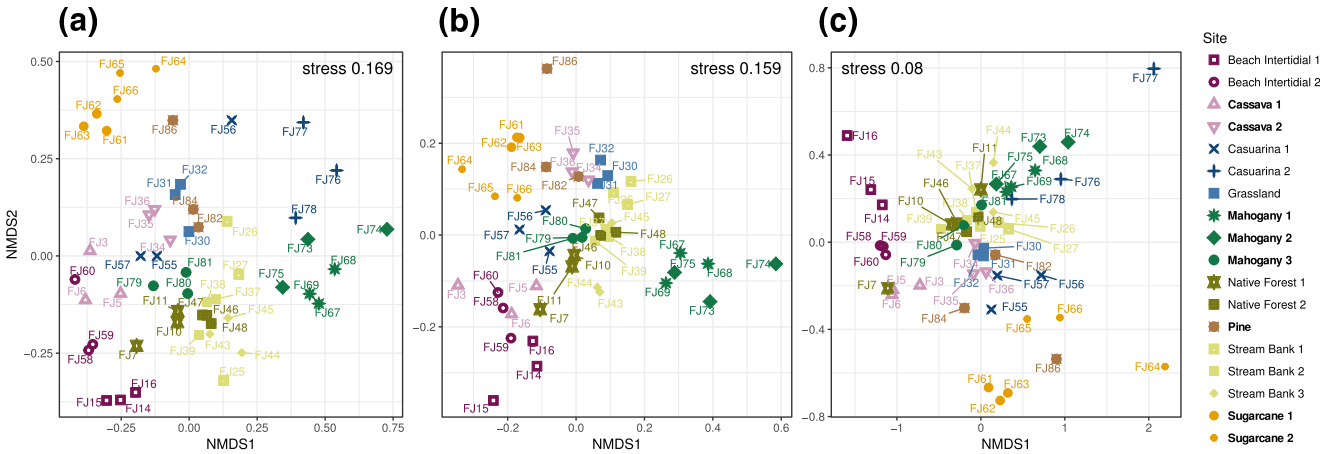

Fig. 4 Bi-dimensional ($k = 2$) non-metric multidimensional scaling ordination graphs of Hellinger transformed data (**a**) ITS rDNA, (**b**) LSU rDNA, and (**c**) 16S rDNA gene datasets. Shepard diagrams of goodness of fit are reported in Fig. S2. Sites dominated by introduced plant hosts are denoted in bold

Table 2 Soil analysis of the sampled site. Values are in ppm. Habitats dominated by introduced plant hosts are denoted in bold

Site	P	K	Ca	Mg	Zn	Mn	Fe	NO_3	NH_4
Beach intertidal 1	3	108	4108	492	1.0	26.6	7.0	2.7	21.6
Beach intertidal 2	5	39	4898	466	0.7	25.2	3.6	2.2	24.1
Cassava 1	64	51	4738	189	13.1	78.6	5.8	1.6	14.4
Cassava 2	11	264	2060	2286	4.8	226.4	220.0	4.1	14.6
Casuarina 1	17	165	3690	2484	4.4	298.0	220.0	1.0	13.5
Casuarina 2	9	68	524	173	3.9	66.2	126.0	2.1	33.2
Grassland	10	271	2233	2898	3.4	165.0	84.0	0.9	16.4
Mahogany 1	13	90	1091	167	2.3	73.2	170.0	1.3	46.7
Mahogany 2	7	118	1069	169	2.7	188.2	146.0	1.3	52.9
Mahogany 3	16	196	2984	1204	12.1	215.6	188.0	1.2	18.0
Native forest 1	19	133	5565	879	5.7	130.0	8.0	1.4	24.4
Native forest 2	4	62	5000	1300	2.5	390.0	400.0	1.1	20.0
Pine	5	362	4923	870	2.9	294.8	340.0	1.4	41.0
Stream bank 1	11	215	1685	978	6.8	369.2	124.0	0.8	29.4
Stream bank 2	4	148	2976	2232	20.9	401.2	240.0	1.8	14.9
Stream bank 3	5	44	1861	486	4.0	232.8	380.0	1.4	31.5
Sugarcane 1	3	123	4710	720	2.1	177.2	240.0	1.0	30.5
Sugarcane 2	14	327	1652	464	4.3	211.6	280.0	3.5	40.3

established adjacent to forests and unmanaged vegetation, and often utilizing plant and animal-based organic amendments. Microbial diversity of sugarcane, grown in extensive monoculture, was intermediate to that of other sampled habitats. Interestingly, we found that it was the habitats with ectomycorrhizal trees, casuarina, and pine that the lowest richness of fungi and prokaryotes was detected, indicating that mycorrhizal affinity of the plant community is an important driver in the assembly, maintenance, and functioning of both fungal and prokaryotic communities in soils [44].

How Are Microbial Communities Impacted by Introduced Plants?

Introduced plants may establish and persist in exotic locations through the establishment of belowground symbioses [45]. There are numerous cases whereby non-native EMF fungal species have been co-introduced with host species through transplantation [46, 47]. Here, we assessed the fungal, bacterial, and archaeal communities underneath introduced timber species of pine and mahogany, and the agricultural crops sugarcane and cassava. The habitat with *Pinus caribaea* showed a low species richness, even though *Pinus* spp. can associate with hundreds to thousands of taxa in their native range [12, 48]. The pine habitat was unique in its association with the ectomycorrhizal fungal genera *Cenococcum*, *Wilcoxina*, and *Rhizopogon*, common members of ECM fungal communities in the Northern Hemisphere. These indicator species, in addition to the endophyte *Chalara*, were abundant under *Pinus caribaea* but were not detected in any of the other habitat. We posit these fungi may have been co-introduced with their host plants, as have been reported previously for non-native ectomycorrhizal trees and ectomycorrhizal fungi [46, 47, 49, 50]. We did not detect any species of *Pisolithus*, which were reported as the most abundant ectomycorrhizal fungi associated with *Pinus caribaea* introduced to Seychelles [48]. This may imply that each introduction imposes its own selection pressures on the limited set of taxa in the gene pool of founder populations.

Mahoganies are pan-tropical species in the Maliaceae that are grown in agro-forestry systems worldwide [51]. It has been noted that mahogany can lower native plant diversity in habitats where they have been introduced, and have a prevalence to invade non-native habitats when there is an absence of natural enemies [52]. Although extracts of mahogany plant tissues and those of their foliar endophytic fungi have been shown to have antimicrobial properties [53, 54], we are not aware of previous studies assessing the impacts of mahogany introduction on biological communities in non-native soils. We found that fungal, bacterial, and archaeal communities in soils under mahogany stands were intermediate in OTU richness, but they were distinct in composition compared to other habitats sampled in this study. Mahogany soils contained many endophytic and ectendomycorrhizal fungal OTUs classified as *Ceratobasidium*, *Oidiodendron*, *Chloridium*, and *Stephanospora* [55]. Arbuscular mycorrhizal fungi belonging to *Acaulospora* and *Glomus* were also detected in the mahogany habitat. These fungi have also been reported from mahogany habitats in Mexico [56]. Prokaryotic communities under mahogany were dominated by Chthoniobacterales and Rhizobiales. However, none of these groups of fungi, bacteria, or archaea were unique to mahogany.

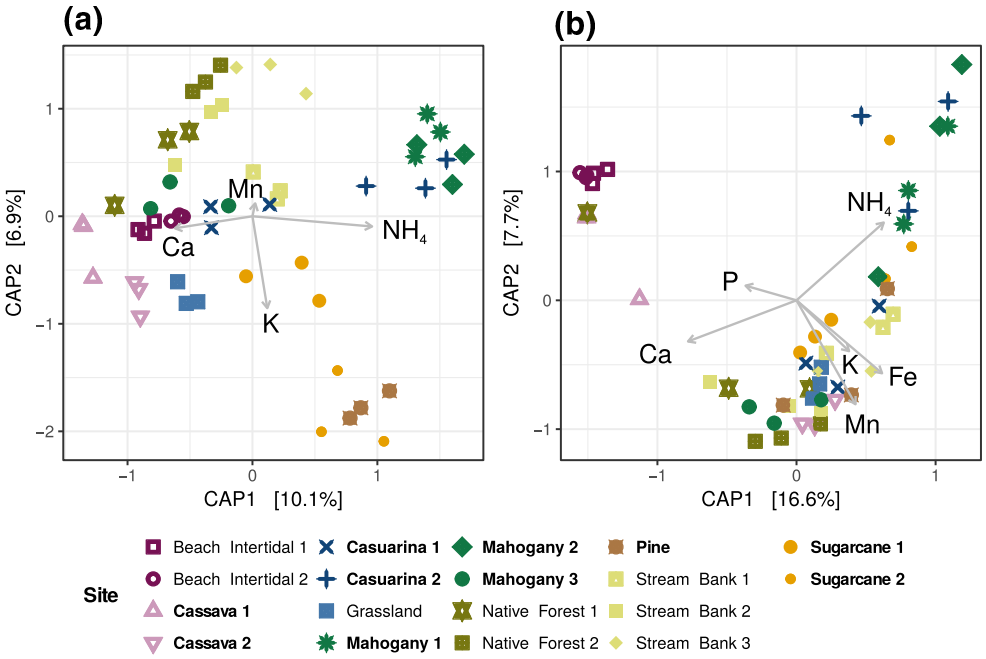

Fig. 5 Canonical analysis of principal coordinates (CAP) of fungal (**a**) and prokaryotic communities (**b**). Environmental soil variables (used as ordination constrains) scores were plotted onto the ordination as arrows. Sites dominated by introduced plant hosts are denoted in bold

Previous studies assessing the sugarcane microbiome and microbial communities in bulk soil in which it was grown identified a core set of OTUs and found that plant organ microbiomes are a select subset of taxa that are derived from the soil microbial species pool [57]. Fungi belonging to Capnodiales, Eurotiales, and Polyporales, and bacteria within Rhodospirillales and Saprospirales were found to be enriched in the soil and rhizosphere compartments of sugarcane; however, in their study most of the fungi and prokaryotes remained poorly classified [57]. In our study, we found OTUs belonging to these orders in sugarcane soils of Fiji; however, fungi in the Hypocreales, Sordiales, and Tremellales were more abundant. Our results indicate that the core microbiome of sugarcane may be geographically dependent.

Cassava is one of the main sources of human calorie intake in Fiji and many parts of the world; thus, it is a crop of broad interest [58, 59]. In our study, we found that OTUs of *Lecythophora*, *Camarosporium*, *Lasiodiplodia*, and *Fusarium* were statistically associated with soils where cassava is cultivated. While these fungi can be pathogens in some plants, it is unknown how they interact or function in cassava, a crop that is largely clonally propagated. Cassava has been found to associate with arbuscular mycorrhizal fungi and responds favorably to inoculations with these fungi [60]. We detected OTUs belonging to *Rhizophagus* and *Glomus* in cassava soils, but these OTUs were at a low relative abundance. Ammonia-oxidizing Archaea belonging to *Candidatus* Nitrososphaera were dominant in soils under cassava. Continued research is needed to tap the microbiome of cassava to harness these organisms for sustainable agricultural practices.

In summary, through high-throughput amplicon sequencing we were able to characterize fungal, bacterial, and archaeal communities in native and agricultural habitats in Fiji. In addition to identifying taxa that are likely to have been introduced into Fiji with *Pinus* hosts, our data show that soils under ectomycorrhizal tree hosts were lower in fungal and prokaryotic diversity. Contrary to our hypothesis, microbial communities under the agricultural crop cassava were as diverse as those under native vegetation. A significant amount of fungal and prokaryote diversity (> 10%) could be explained by differential effects of soil calcium and ammonium levels. This study provides important baseline data on fungal, bacterial, and archaeal communities in soils of agricultural, forestry, and natural habitats in Fiji.

Conclusion

Characterizing microbiomes is the first step to link microbial composition to ecosystem functions. The Fijian archipelago provides a variety of terrestrial habitats in a tropical marine climate for which the soil microbial biodiversity remains poorly studied. Here, we present the first molecular characterization of fungal, bacterial, and archaeal communities in Fijian soils beneath diverse native and human-modified habitats. This study provides important baseline data on microbial diversity in native maritime, forest, stream bank, and grassland habitats, as well as in introduced agricultural crops including sugarcane, cassava, pine, and mahogany, and the impact of soil chemistry on microbial diversity.

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Author Contribution G. B. and V. B. designed the research, sampled the soils in Fiji, and wrote the manuscript. G. M. N. B. extracted DNA, prepared the Illumina library, analyzed the data, and wrote the manuscript. All authors approved the final version for submission.

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