

# DNA Metabarcoding Reveals Cryptic Geographically-influenced Microbial Diversity After Anthropogenic Impact in Original Forest Soils on the Isolated Trindade Island, South Atlantic

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## Research Article

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## Abstract

Located 1,140 km from the South American coastline in the South Atlantic Ocean, and with an age of 4 million years, Trindade Island is the most recent volcanic component of Brazilian territory. Its vegetation was severely damaged by human influence in particular through the introduction of exotic grazing animals such as goats. However, since the complete eradication of goats and other feral animals in the late 1990s, the island's vegetation has been recovering and even some endemic species that had been considered extinct have been rediscovered. In this study we set out to characterize the contemporary microbial diversity of Trindade Island forest soils using metabarcoding by High Throughput Sequencing (HTS). Sequences of representative of two domains (Bacteria and Archaea) and five kingdoms (Fungi, Metazoa, Protozoa, Chromista and Viridiplantae) were identified. Bacteria were represented by 20 phyla and 116 taxa, while Archaea by only one taxon. Fungi were represented by seven phyla and 250 taxa, Viridiplantae by five phyla and six taxa, Protozoa by five phyla and six taxa, Metazoa by three phyla and four taxa and Chromista by two phyla and two taxa. Even after the considerable anthropogenic impacts and devastation of the island's natural forest, our sequence data revealed the presence of a rich, diverse and complex diversity of microorganisms, invertebrates and plants.

## Introduction

Located 1,140 km from the South America coastline in the South Atlantic Ocean, Trindade Island is the most recent volcanic component of Brazilian territory, with an age of approximately 4 million years [1,2,3]. Discovered in 1501 by the navigator João da Nova [4], Trindade has been sporadically settled over the last five hundred years. The first official record of landing on the island dates to the year 1700 with the arrival of English astronomer Edmund Halley. Disputed by the United Kingdom and Portugal throughout the 18<sup>th</sup> century, Trindade was occupied in 1783 by Portuguese military and civilian personnel (including six Azorean couples), but was later abandoned [4]. Since then, the island has only been occupied for short periods, mostly by slave traders and pirates, and during the two world wars [5]. In 1957, the Brazilian Navy constructed a garrison on the island, and the island has been continuously occupied since then. Currently, the island hosts a military meteorological station, the Trindade Island Oceanographic Post (POIT), which also includes a science research station, and has been officially declared a federally protected area.

Little is known of the original native vegetation of Trindade Island as it was severely damaged by the introduction of exotic animals during the earlier phases of human occupation. The expedition led by Edmund Halley, for example, introduced goats to the island, which were abandoned, became feral and subsisted on native vegetation [6]. Today, most land below 400 m a.s.l. is characterized by open grasslands (about 60% of the area is dominated by Poaceae and Cyperaceae), with higher altitudes hosting the native "giant fern forest" dominated by the single species *Cyathea delgadii* Sternb. [7], analog to the high-altitude Cycad forests found in Tristan da Cunha [8].

Alves (2006) [9] reported 130 species of vascular plant, including five endemics, while Dantas et al. (2017) [10] described one additional endemic species of *Peperomia* from the island. Faria et al. (2012) [11] investigated the bryophytes, listing 32 species of liverwort, 11 species of moss and one hornwort. Twelve species of freshwater algae were reported by [6]. The island is surrounded by a rich marine environment with many endemic gastropods and fish and it is an important breeding area for the marine turtle *Chelonia mydas* [6, 12, 13, 14, 15, 16]. Virtually no data are available on most groups of soil microorganisms (Bacteria, Fungi, Protista) from Trindade Island.

Since the complete eradication of goats and other feral animals in the late 1990s, the island's vegetation has been recovering [17, 18, 19]. This vegetation recovery has likely relied on seeds, spores and propagules already present in the soil which, if viable, could develop under favorable conditions, or by new long-distance colonization events with propagules carried by agents such as wind, humans, and other organisms. Biological connections between Trindade and mainland South America have been demonstrated [20, 21, 7].

Most studies of Trindade Island biodiversity have relied on traditional morphological identification, with the exception of a small number of phylogenetic studies [10, 7]. Morphological studies have important limitations due to the inherent difficulties of identification of resting stages, spores, and small propagules. Recent developments in molecular biology, such as DNA metabarcoding by High Throughput Sequencing (HTS), have provided a promising and powerful tool to access previously unrecognized biodiversity (e.g., [22, 23]), and in some cases with application to very old samples [24]. The recent use of such approaches in studies of Antarctic soils has yielded evidence of the presence of much greater diversity of algae and fungi than studies based only on morphological approaches (e.g., [25, 26, 27]). DNA metabarcoding has not previously been applied in investigation of soil biological diversity in Atlantic islands such as Trindade Island. In this study we set out to describe the contemporary microbial diversity present in the Giant Fern Forest soils in Trindade Island using metabarcoding.

## Methods

### Study area

Trindade Island is located at 20°29'-20°32'S and 29°17'-29°21'W (Figure 1) in the South Atlantic Ocean. Rising 5,500 m from the surrounding ocean floor, Trindade has an area of 13.5 km<sup>2</sup> and is predominately rugged, being considered the most heterogeneous and topographically varied Brazilian volcanic island [1, 28]. It includes the only volcanic crater the currently exists in Brazil (extinct).

Trindade Island comprises ultramafic to intermediate volcanic rocks of alkaline composition [29], formed during five volcanic episodes: i) Trindade Complex (3.9-2.5 Ma) with nephelinitic to phonolitic pyroclastic deposits; ii) Desejado Sequence (2.5-1.6 Ma) composed of silica sub-saturated alkaline rocks intercalated with pyroclastic sequences of equivalent composition; iii) Morro Vermelho Formation (no age available) with effusive deposits and

pyroclastics of ankaratritic composition; iv) Valado Formation (no age available), with pyroclastic sequences and lava flows of melanephelinitic olivine composition, and v) Vulcão do Paredão Formation (ca 0.25 Ma) with pyroclastic deposits intercalated with minor volcanic effusive units of ankaratritic composition [31, 31, 1, 32, 33, 2, 3, 29]. Holocene deposits (modern sediments) comprising eolian, beach, alluvial and slope deposits, are also present, along with raised coral reefs [34].

Trindade Island soils are shallow, including Leptosols, Regosols, Cambisols, Andosols and endemic Histosols [35, 36, 37]. The latter are organic soils, formed by association between the volcanic (phonolites) and organic (primarily vegetation) parent materials. Its main characteristic is the presence of a superficial organic horizon, formed by vegetable fibers or decomposed organic materials, called the H horizon (predominance of fibers) or O horizon (predominance of humified organic matter) [29]. On Trindade Island, these have been described as acidic, nutrient-poor soils exclusively associated with giant fern forests [35, 38, 36, 39, 40].

Influenced by the South Atlantic Subtropical Anticyclone (SASA), which contributes to intense oceanic evaporation in this area [1, 41] the climate in Trindade Island varies with altitude, with some areas showing semiarid characteristics and other areas characterized by higher humidity, such as Desejado Hill (620 m altitude), where the Histosols are located [25].

### Soil sampling

Based on previous studies [35, 36, 39], three soil samples were collected from the organic horizons of Trindade Histosols in October 2018 using spatulas previously disinfected with 70% alcohol and stored in sterilized WhirlPak bags (Sigma-Aldric, USA). Two samples were obtained from Desejado Hill (PD5 and PD6; Figure 1E, F), and one from Fazendinha (PF7; Figure 1G) (Table 1). The organic horizons in PD5, PD6, and PD7 were sampled, respectively, to depths of 10, 5 and 25 cm.

**Table 1.** Location of sampled soil profiles from Trindade Island, South Atlantic Ocean.

Soil Profile	Latitude	Longitude	Altitude (m)	Profile depth (cm)	Location	Local relief and vegetation
PD5	20°30'43.82"S	29°19'31.78"W	552	0-65+	Desejado Hill	Steep slope, with <i>Cyathea delgadii</i> and <i>Peperomia glabella</i>
PD6	20°30'42.17"S	29°19'31.10"W	568	0-55+	Desejado Hill	Steep slope, <i>Cyathea delgadii</i> , <i>Peperomia glabella</i> and <i>Cyperus atlanticus</i>
PF7	20°30'36.72"S	29°19'39.82"W	484	0-87+	Fazendinha	Medium slope, with <i>Cyathea delgadii</i> , <i>Peperomia glabella</i> , <i>Pityrogramma calomelanus</i> and mosses

### Soil analyses

Soil physical and chemical analysis were performed following Teixeira et al. [42]. Granulometry was performed using the pipette method (50 rpm, 16 h). pH was determined using a 1:5 soil:deionized water ratio. Potential acidity (H+Al) was extracted with 0.5 mol·L<sup>-1</sup> Ca(OAc)<sub>2</sub> buffered to pH 7.0 and quantified by titration with 0.0606 mol·L<sup>-1</sup> NaOH. Exchangeable Ca<sup>2+</sup>, Mg<sup>2+</sup>, and Al<sup>3+</sup> were extracted with 1 mol·L<sup>-1</sup> KCl, and Na<sup>+</sup>, K<sup>+</sup> and P<sup>+</sup> were extracted with Melich-1 [42]. The element levels in the extracts were determined by ICP (Al<sup>3+</sup>), flame emission (Na<sup>+</sup> and K<sup>+</sup>) and photocolormetry (P) by the ascorbic acid method. Organic matter content (OM) was quantified by wet oxidation with 0.167 mol·L<sup>-1</sup> of K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> with sulfuric acid with external heating [43]. All analyses were performed in triplicate. Total cation exchange capacity (CEC) was calculated as the sum of the bases (Ca<sup>2+</sup>, Mg<sup>2+</sup>, Na<sup>+</sup>, K<sup>+</sup>), and potential acidity (H<sup>+</sup>+Al<sup>3+</sup>).

### DNA extraction, Illumina library construction and sequencing

Total DNA was extracted using the QIAGEN Power Soil Kit (QIAGEN, Carlsbad, USA), following the manufacturer's instructions. DNA quality was analyzed by agarose gel electrophoresis (1% agarose in 1×Trisborate-EDTA) and then quantified using Quanti-iT™ Pico Green dsDNA Assay (Invitrogen). The internal transcribed spacer 2 (ITS2) of the nuclear ribosomal DNA was used as a DNA barcode for molecular species identification of Chromista, Protozoa, Viridiplantae and Fungi [44, 45] using the universal primers ITS3 and ITS4 [46]. For Bacteria and Archaea, we used the 16S rRNA gene V3-V4 region [47, 48]. Library construction and DNA amplification were performed using the Library kit Herculase II Fusion DNA Polymerase Nextera XT Index Kit V2, following Illumina 16S Metagenomic Sequencing Library Preparation Part #15044223 Rev. B protocol. Paired-end sequencing (2 × 300 bp) was performed on a MiSeq System (Illumina) by Macrogen Inc. (South Korea). Library construction and DNA amplification were performed using the Library kit Herculase II Fusion DNA Polymerase Nextera XT Index Kit V2, following Illumina 16S Metagenomic Sequencing Library Preparation Part #15044223 Rev. B protocol. Paired-end sequencing (2 × 300 bp) was performed on a MiSeq System (Illumina) by Macrogen Inc. (South Korea).

### Data analyses

Rarefaction calculations were carried out using the rarefaction analysis command in the software MOTHUR, where we clustered sequences into OTUs by setting a 0.03 distance limit. Quality analysis was carried out using BBDuk version 38.34 (BBMap – Bushnell B. – sourceforge.net/projects/bbmap/) with the following parameters: Illumina adapter removal (Illumina artefacts and the PhiX Control v3 Library); ktrim = l; k = 23; mink=11; hdist=1; minlen = 75; –tpe; –tbo; qtrim = rl; trimq = 20; ftm = 5; maq = 20. The remaining sequences were imported to QIIME2 version 2020.8 (<https://qiime2.org/>) for

bioinformatics analyses [49]. The qiime2-dada2 plugin is a complete pipeline that was used for filtering, dereplication, turn paired-end fastq files into merged, and remove chimeras [50]. Taxonomic assignments were determined for amplicon sequence variants (ASVs) using the qiime2-feature-classifier [51] classify-sklearn against SILVA 138 Ref NR 99 [52] for the 16S rRNA gene. Unknown ASVs from the last classification were classify-sklearn against the NCBI non-redundant nucleotide sequences (nt) database (August 2020) using BLASTn [53] with default parameters. Taxonomy assignments were performed using MEGAN6 [54]. For the ITS sequences, ASVs were classify-sklearn against the UNITE Fungal ITS database version 8.2 (Abarenkov et al. 2020a). Unknown ASVs from the last classification were classify-sklearn against UNITE Eukaryotes ITS database version 8.2 (Abarenkov et al. 2020b). Finally, remaining ASV sequences were aligned against the NCBI non-redundant nucleotide sequences (nt) database (August 2020) using BLASTn [53] with default parameters. The taxonomy assignments were performed using MEGAN6 [54]. All classification used standard texts for these taxonomic groups: Garrity et al. [57] and Yilmaz et al. [58] for Bacteria; Leliaert et al. [59] for Viridiplantae; Cavalier-Smith [60] for Chromista, Protista and Metazoa; Kirk et al. [61], Tedersoo et al. [62], MycoBank (<http://www.mycobank.org>) and the Index Fungorum (<http://www.indexfungorum.org>) for Fungi.

## Results

### Forest soil properties

The analyzed soils were acidic, with a pH in H<sub>2</sub>O < 4 (Table 2). Despite the high values of CEC, pH was controlled by the potential acidity (H+Al), which indicates a large reserve of H<sup>+</sup> protons. This is confirmed by the low base saturation values (PBS < 50%), which characterize all organic horizons as dystrophic. The most exchangeable cation was Ca, which may have its origin associated with geological substrates, but also with the decomposition of organic matter, mainly from fern stalks. The organic matter content in the fine earth was high, although mainly in the form of vegetable fibers. Among micronutrients, Fe stood out, whose main source is the weathering of ferromagnesian minerals in the geological substrate. Physically, clay and silt fractions predominated in the fine earth (< 2 mm) composition, characterizing the texture of all horizons as clay-loam.

**Table 2.** Physicochemical properties of forest soil from the organic horizons of Trindade Island, South Atlantic Ocean.

Profile	pH	P	K	Na	Ca	Mg	SB	H+Al	CEC	Fe	Mn	Zn	Cu	PBS	OM	< 2 mm soil fraction				Textural Class
																CS	FS	S	C	
		mg/dm <sup>3</sup>	cmol/dm <sup>3</sup>						mg/dm <sup>3</sup>					%	dag/Kg	(%)				
PD5	3.90	157.60	0,29	0,36	5.76	1.45	7.86	80.85	88,71	278.10	6.70	3.20	0.30	8.90	13.33	7	32	28	33	Clay loam
PD6	4.10	101.00	0,37	0,60	9.94	2.54	13.45	43.89	57,34	179.32	2.80	0.20	0.00	23.50	11.69	27	4	31	38	Clay loam
PF7	4.20	191.10	0,77	0,47	1.21	1.59	4.04	64.02	68,06	272.10	12.40	2.00	0.40	5.90	12.09	14	22	37	27	Clay loam

SB: sum of bases; H+Al: potential acidity; CEC: cation exchangeable capacity; PBS: percent of base saturation; OM: organic matter; CS: coarse sand; FS: fine sand; S: silt; C: clay.

### Diversity

The calculated rarefaction curves for all taxa investigated approached a plateau, indicating that the reads gave an accurate representation of the local sequence diversity (Fig. 2). A total of 594,009 reads were generated of which 111,760 (19%) remained after quality filtering. Considering each marker for 16S (Bacteria), a total of 433,579 DNA reads was generated and 82,306 reads (19%) remained after quality filtering. For ITS (Fungi and Plant) a total of 160,430 reads was generated and 29,454 reads (18%) remained after quality filtering. Sequences from two prokaryotic domain (Bacteria and Archaea) were detected, representing 25 taxa in four phyla. Sequences representative of five Eukaryota kingdoms were detected, of which Fungi included seven phyla and 255 taxa, Chromista one phylum and 28 taxa, Protozoa three phyla and four taxa, Metazoa three phyla and four taxa and Viridiplantae four phyla and nine taxa (Suppl. Table 1). In total, sequences of 294 taxa were detected, of which 104 were shared between both sampling locations (Fig. 3). Relative abundances are shown in Fig. 4

### Bacteria and Archaea

The analysis showed the presence of 116 taxa belonging to the domain Bacteria and 1 taxon to the domain Archaea. The latter belonged to the class Thermoplasmata and were only represented in samples PD5 (0.08%) and PF7 (0.12%). The Bacteria domain was represented by 20 phyla, 35 classes, 67 orders and 75 families (Suppl. Table 1). Actinobacteria was the most abundant bacterial phylum presenting 22% of all sequences, followed by the phyla Proteobacteria (19.12%), Acidobacteria (13.58%), Verrucomicrobia (8.72%) and Chloroflexi (8.63%). Relative abundance of the dominant phyla in each sample varied. Actinobacteria presented highest relative abundance in sample PD5 (24.88%), Proteobacteria (20.79%) and Verrucomicrobia in sample PD6 (13.46%), and Acidobacteria (17.33%) and Chloroflexi (11.40%) in sample PF7 (Figures 5, 6).

### Fungi

From the three soil samples we detected 416,408 DNA reads distributed in 250 fungal ASVs (Suppl. table 1), belonging to the phyla *Ascomycota*, *Basidiomycota*, *Mortierellomycota*, *Mucoromycota*, *Glomeromycota*, *Chytridiomycota* and *Kickxellomycota*, in rank order of diversity (Figure 7). Nineteen taxa were dominant ( $\geq 1\%$  of relative abundance), representing the phyla *Ascomycota*, *Basidiomycota*, *Mortierellomycota* and unknown fungi within the fungal community. Among them, *Sclerotiniaceae* sp., *Antarctomyces psychrotrophicus*, *Pseudogymnoascus* sp. (*Ascomycota*), *Apiotrichum* sp. (*Basidiomycota*) and *Mortierella humilis* (*Mortierellomycota*) were the most abundant fungal taxa detected. However, the majority of the sampled fungal communities comprised intermediate and rare taxa. Fifty-three (21.2%) of the ASVs could be identified to higher taxonomic levels (kingdom, phylum, class, order, family) and may therefore represent taxa not present in the databases consulted or be currently undescribed species (Suppl. Table 1).

The fungal assemblages of the three soils from giant fern forest differed (Figures 8-10). The greatest number of fungal ASVs was detected in Pico Desejado 1 (156), followed by Pico Desejado 2 (137) and Fazendinha (98). Among them, 46 (18.4%) were detected across both sampling locations, including the most dominant taxa.

## Metazoa

Sequences of only four species of Metazoa were detected, representing three Phyla (Arthropoda, Nematoda, Plathyelminthes). Arthropoda was only detected at PD6 (52.7% of total Metazoa reads), Nematoda at PD5 (10.8% of total Metazoa reads) and Plathyelminthes at PF7 (36.4% of total Metazoa reads). Fig 11 shows the relative abundance of the sequences detected.

## Protozoa and Chromista

Sequences representing five phyla were detected, Bacillariophyta, Cercozoa, Ciliophora, Heterolobosea and Imbricatea. The Bacillariophyta was represented by one species (*Sellaphora pupula*) present only at PD5 (with 23.5% of total Protista reads). Representing Cercozoa two taxa were found, Order Cercomonadida at PD6 (5.0% of total Protista reads) and *Eocercomonas echina* at PF7 (3.9% of total Protista reads). Only one species of Ciliophora was found, *Pseudocohnilembus* sp, which was only detected at PF7 (15.1% of total Protista reads). Heterolobosea was represented by one species, *Vahlkampfia* sp, present at both PD6 and PF7 (30.3% of total Protista reads). Finally, *Thaumatomonas* sp. was the sole representative of Imbricatea, present only at PF7 (21.9% of total Protista reads). Figure 12 shows the relative abundance of the sequences obtained.

## Viridiplantae

Sequences of four phyla were detected, Anthophyta, Bryophyta, Chlorophyta and Monilophyta. Among the Anthophyta two species were found, *Begonia cathayana* present at PD5 and PF7 (21.2% of total Viridiplantae reads) and *Vachellia gummifera* present at PD6 (3.6% of total Viridiplantae reads). The sole Bryophyta detected, *Campylopus oerstedianus*, was present only at PD6 (11% of total Viridiplantae reads). The most diverse group was Chlorophyta with 6 taxa: Family Chlamydomonadaceae present only at PD6 (8.7% of total Viridiplantae reads), *Asterochloris* sp. present only at PD6 (6.3% of total Viridiplantae reads), *Chlamydomonas* sp. present only at PD5 (3.4% of total Viridiplantae reads), *Chloromonas* sp. present only at PD6 (.7% of total Viridiplantae reads), *Eremochloris sphaerica* present only at PD6 (10.4% of total Viridiplantae reads) and *Trebouxia* sp. present at PD5 (2.2% of total Viridiplantae reads). The sole fern detected was *Alsophila gigantea*, which was found at PD5 and PF7 (24% of total Viridiplantae reads). Figure 13 shows the relative abundances of the sequences detected. At Desejado Hill a total of 260 taxa were detected, with a lower number of 138 at Fazendinha, although a high number of taxa occurred at both sampling locations (Figure 14).

## Discussion

Our DNA sequence data point to the presence of diverse and abundant assemblages of organisms previously not recorded on Trindade Island. The presence of some organisms may indicate the consequences of human influence while others are consistent with the known local diversity. However, we also recognize that the detection of fragments of an organism's DNA does not confirm the presence of living organisms or viable propagules, as the finding can be related to encysted forms, spores, pollen or even single cells, while sometimes dead tissue can also provide detectable DNA. Sequence assignment also relies on the quality and completeness of data available in existing sequence databases.

## Bacteria and Archaea

The present study detected few sequences representing the domain Archaea in samples PD5 and PF7, belonging to the class Thermoplasmata, phylum Euryarchaeota. Recent studies have shown that the relative abundance of these Archaea among all archaeal sequences in soil can be higher than previously reported [63]. Thermoplasmatales is the order of Archaea with most organisms in culture and the only one validated under the rules of the International Code of Nomenclature of Bacteria. They comprise extreme acidophiles that lack a cell wall [64] and are usually found in volcanic continental areas; they derive their energy from aerobic respiration but can grow anaerobically using iron or sulfur in their metabolism, which contributes to their prevalence in acid mine drainage communities [65]. The sequences identified here could not be classified at the order level, and the physiology of these microbes remains unknown.

The bacterial community was dominated by Actinobacteria, Proteobacteria, Acidobacteria, Chloroflexi and Verrucomicrobia. This dominance is generally typical of soil bacterial community composition in soil samples [66].

Among the representatives of the phylum Actinobacteria, the class Acidimicrobiia stands out, as it is typically not as dominant as the classes Actinobacteria and Thermoleophilia in soils. Most cultured members of the class Acidimicrobiia are extreme acidophiles, originally obtained from geothermal or iron-rich mining sites and belonging to the family Acidimicrobiaceae [67]. However, these taxa were not detected in the current study and

most sequences belonging to this group could not be further classified, indicating that they belong to unknown groups. The bacterium IMCC26256 was the most abundant identified sequence of Acidimicrobiia found in the Trindade Island samples, a taxon first described in a freshwater study and that forms a separate branch within this class [67]. Among the genera identified here were *Luedemannella* and *Mycobacterium*, taxa found in soil, and *Acidothermus*, found in acidic geothermal springs [67].

Only the classes alpha and gamma proteobacteria were identified in Trindade Island soil samples. However, it should be noted that the classification system used in the present study combines the class formerly known as betaproteobacteria with the gamma proteobacteria. Members of alpha proteobacteria are metabolically very diverse and include nitrogen-fixing bacteria [68]. Although the majority of the sequences assigned here belong to the order Rhizobiales, none of the identified taxa are nitrogen-fixing, but are genera found in soil and the rhizosphere. Sequences assigned to the orders Elsterales and Micropepsales were also abundant, but there are no cultured representatives of these orders and their physiology is unknown. Members of the gamma proteobacteria mostly represented the orders Burjikhorderiales and Pseudomonadales, groups that are common in soils. All three soil samples examined here contained high numbers of the genus *Acidibacter*, an acidophile known from iron-rich mine sites [69].

The Acidobacteria sequences assigned in this study belong to taxa usually found in acidic environments, such as members of the class Acidobacteriia (former subgroups 1, 2, 3, 5, 11, 12, 13, 14, 15, 24) [70]. Trindade Island soil samples included members of the class Acidobacteriia along with the Class Vicinamibacteria, which are heterotrophic aerobic bacteria, and class Holophagae, an anaerobic group. Their presence is consistent with bacterial communities from acidic, iron-rich soils, and possibly associated with geothermal sources. Many of the soil bacterial taxa assigned here are unknown and further research is necessary to understand their roles in Trindade Island soils.

## Fungi

The most dominant sequences assigned in the giant fern forest soils were *Sclerotiniaceae* sp., *Antarctomyces psychrotrophicus*, *Pseudogymnoascus* sp., *Apiotrichum* sp. and *Mortierella humilis*, which represent fungi from different phyla. The family *Sclerotiniaceae* includes 47 genera and 284 species, many of which are pathogenic or saprophytic taxa, which are able to infect various plant species and tissues. These fungi are characterized by the formation of sclerotia and stalked apothecia located within the colonized host plant tissue [61]. The genus *Antarctomyces* includes only two known species, *A. psychrotrophicus* and *A. pellizariae*, which were originally described from Antarctica. *Antarctomyces psychrotrophicus* was originally described in soil samples from King George Island [71] and later reported from other Antarctic habitats [72] but not elsewhere, this is the first record outside Antarctica. *Pseudogymnoascus* (anamorphic form-genus *Geomyces*) has a wide distribution globally [73, 74] and has been reported in soils from Arctic, alpine, temperate, and Antarctic regions [75, 72]. *Pseudogymnoascus* taxa are capable of colonizing and utilizing different carbon sources and can be particularly abundant at lower temperatures [76]. *Pseudogymnoascus* has received attention due to the pathogenic species, *P. destructans*, the causative agent of white-nose syndrome (WNS) in bats in temperate regions [77].

*Apiotrichum* is an anamorphic basidiomycetous yeast genus containing 20 species distributed widely globally, including a number of soil-associated species [78, 79]. The genus *Mortierella* includes approximately 100 species [80], occurring mainly in different soil types [61]. Some members of *Mortierella* appear to assist crops and mycorrhizal fungi in phosphorus acquisition [81]. In addition, they can decompose plant debris and degrade polyaromatic hydrocarbons [82]. *Mortierella humilis* has been isolated from plant tissues, the rhizosphere and roots in different tropical and temperate ecosystems and is reported as a producer of fatty acids useful in medicine, pharmacology, cosmetics, agriculture and in the food industry [83]. In addition, *M. humilis* produces enzymes capable of degrading xylans, sugars (such as sucrose, galactose, fructose, mannose and maltose), paraffin, chitin, cellulose and lignin [84, 85, 86].

## Metazoa

None of the Metazoa sequences detected here were found in both sampling locations. Desejado Hill samples generated the greatest diversity, but we suggest that other markers more widely used in animal studies, such as COX1 would identify a more diverse community of metazoans living in Trindade Island soils. The Collembola (springtails) are one of the most common, widespread and abundant arthropod groups, with a wide global distribution [87], and are particularly abundant in leaf litter and soil. Despite their low biomass, Collembola do influence soils. Kubiens (1953) [88] reported a layer of humus 15-20 cm deep composed entirely of their feces and Lagerlöf & Andren (1991) [89] highlighted the importance of Collembola in agricultural soils. *Desoria* is a genus of about 100 species, with a wide distribution in the Northern Hemisphere, including in glacial-influenced habitats, while some species have affinity to anthropogenically disturbed areas. Having limited dispersal abilities, the genus may have been introduced in Trindade by humans, as is the case for *D. trispinata*, which is found in the Azores [90] and colonized the island in the 18th century. *Lepidocyrtus* is one of the largest collembolan genera worldwide [90]. *Lepidocyrtus koreanus* is an Asiatic species, but this specific assignment is likely an instance of database incompleteness. The presence of Collembola on Trindade Island was cited by Alves [6], but this may represent an error as the species cited is a true insect rather than a collembolan. However, it is also extremely unlikely that the island does not host a native, and likely highly endemic, collembolan community, as do other remote volcanic islands in the Atlantic [91, 92, 93, 94]. Both of the taxa assigned here were obtained from the Desejado Hill sample (PD6) and have not been recorded from Trindade Island previously.

The nematode genus *Heterocephalobellus* includes only three species worldwide, all terrestrial, and was originally described from Brazil [95]. To date, only marine nematodes have been investigated in Trindade Island [96]. In the current study, assigned sequences were only obtained from Desejado Hill (PD5). The platyhelminth *Rhynchoscolex simplex* is a common species and has been recorded from São Paulo, mainland Brazil [97]. The assigned sequence was obtained only from Fazendinha (PF7). Both the nematode and platyhelminth are new records for Trindade Island, but appear likely candidates as

anthropogenic introductions. Again, certainly the nematodes are likely to have a diverse native community on Trindade Island, requiring application of both appropriate survey techniques and molecular probes.

## Chromista and Protozoa

Most of the reads representing these groups were assigned to Kingdom rank, excepting that of *Sellaphora pupula*, a cosmopolitan freshwater diatom [98], including in Brazil, Africa and the Azores. In Brazil this species is found in coastal regions and the Atlantic Rainforest [99]. The assignment here is the first record from Trindade Island, where it was found only at Desejado Hill (PD5).

*Cercomonadida* was found only at Desejado Hill (PD6) and is the second most commonly recorded zooflagellate in soils globally [100, 101]. Within this group, *Eocercomonas* is a less known genus segregated from the widespread *Cercomonas* [101]. *Eocercomonas echina* is a freshwater and soil species previously reported only from the United Kingdom and South Korea [102] and was found here only at Fazendinha (PF7). *Vahlkampfia* is a poorly known genus including about 12 species that is abundant worldwide in a wide variety of aquatic and terrestrial habitats [103] and potentially pathogenic for humans [104]. It was found at both Desejado Hill (PD6) and Fazendinha (PF7). *Thaumatomonas* is another poorly known Northern Hemisphere genus commonly found in lake sediments [105], and assigned here only at Fazendinha only (PF7). All the protist sequences assigned in this study have not been previously reported from Trindade Island.

## Viridiplantae

The four green algae (Chlorophyta) reported have not previously been recorded from Trindade Island [98]. *Eremochloris sphaerica* is a North American species common in brackish waters. *Trebouxia* is a cosmopolitan and widespread genus including more than 40 species [98] and has been found in almost every environmental condition including terrestrial and aerial. It is commonly found on tree bark in humid forests and is also a common photobiont of lichens [106]. *Chlamydomonas* is a genus including more than 200 species and is widely distributed in both fresh and sea water as well as in soil and snow [98]. *Asterochloris* is a genus with more than 19 cosmopolitan species and is one of the most common lichen photobionts [98]. All the green algae recorded here were present only at Desejado Hill (PD5 and PD6).

The moss *Campylopus oerstedianus* (Bryophyta) is a Central and North American species not previously reported from Trindade Island. Faria et al. [11] recorded two other species in this genus from Trindade Island, with further discussion of the identify of these representatives given by Gama et al. [20]. We recorded it only at Desejado Hill.

The fern *Alsophila gigantea* (= *Cyathea gigantea*) is an Asiatic species. Alves et al. [21] reported spores of a different species of *Cyathea* in air samples obtained on the island. However, the assignment generated in the current study is likely to reflect a lack of completeness in the available database, and mostly likely refers to *C. delgadii*, the single and dominant tree fern on the island. It is important to note that GenBank contain only 8 ITS sequences of *Cyathea*, not including *C. delgadii*, and that some of these sequences are very short (less than 300 bp). This further reinforces the importance of well curated and complete databases being available to support metabarcoding studies. Fern sequences were obtained from both sampling locations, Desejado Hill (PD5) and Fazendinha (PF7).

The two flowering plants reported here have not previously been reported from Trindade. *Begonia cathayana* (Begoniaceae) is an ornamental Chinese species, however other members of the genus are common in the Brazilian Atlantic Rainforest [107]. *Begonia* propagules could reach the island by means of aerial transfer or with human assistance. In the current study on the sequences were detected at both Desejado Hill (PD5) and Fazendinha (PF7). *Vachellia gummiifera* (Fabaceae) is a leguminous plant native to Morocco and the Mediterranean Sahara. Again, some related species, *V. caven*, *V. farnesiana*, *V. ibirocayensis* and *V. seyal*, are native to Brazil, where they occur in coastal regions [108, 109]. The sequence was identified only at Fazendinha (PF7). It is important to note that, over time, many species have been introduced to Trindade Island with human assistance [6]. The number of these still present is not clear, but there remains the possibility that detectable DNA could remain and be detected using metabarcoding approaches.

## Conclusions

Despite the history of considerable anthropogenic impact and devastation of pre-existing native ecosystems, the application of DNA metabarcoding revealed the presence of sequences indicating the presence of a rich, diverse, and complex DNA sequence diversity of microorganisms, invertebrates and plants in soils from the native fern forests of Trindade Island. A large majority of the identified diversity represented bacterial and fungal groups, and the use of further target genes in future studies is likely to identify further diversity of soil invertebrates and plants. Assignment of putative identities based on sampling of environmental DNA does not, however, confirm the presence of active or viable organisms or their propagules, and the precision of identifications relies heavily on the quality and completeness of available databases. Further studies will be required to better understand the importance of these complex biological webs for forest soil health, conservation management and habitat restoration on Trindade Island. The study also highlights the potential for further application of metabarcoding approaches to elucidate the currently less-known elements of microbial, invertebrate, and lower plant diversity of other remote and often hard to access mid- and South Atlantic islands, many of which share the volcanic history of Trindade Island, such as the Cape Verde Islands, St. Helena, the Tristan da Cunha archipelago, and South Georgia.

## Declarations

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## Conflicts of interest

The authors declare no conflict of interest

## Ethics approval

Not needed

## Consent to participate for publication

All authors consent its participation and publication

## Availability of data and material

All data will be made available once the paper get accepted and prior to its publication if required by the journal

## Authors' contributions

PEASC and LHR designed the study and secured funds; FLVB and FACL performed the bioinformatic analyses; FSO and LPC did fieldwork and collecting; PEASC, LHR and DKH performed lab work; CCB worked the bacteria data; LHR worked the fungi data, MCS and PEASC worked on the plant data, PEASC and PC worked on the remaining groups. FSO and LPC worked on the soil data; PEASC, LHR and PC wrote the manuscript and all authors revised it.

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## Figures

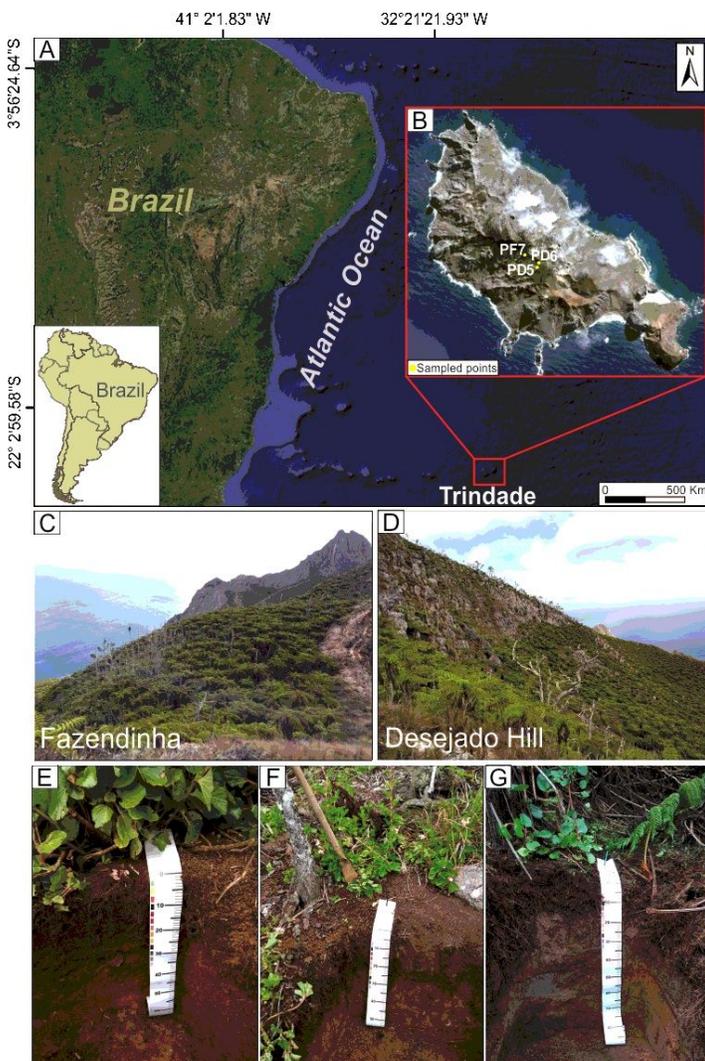
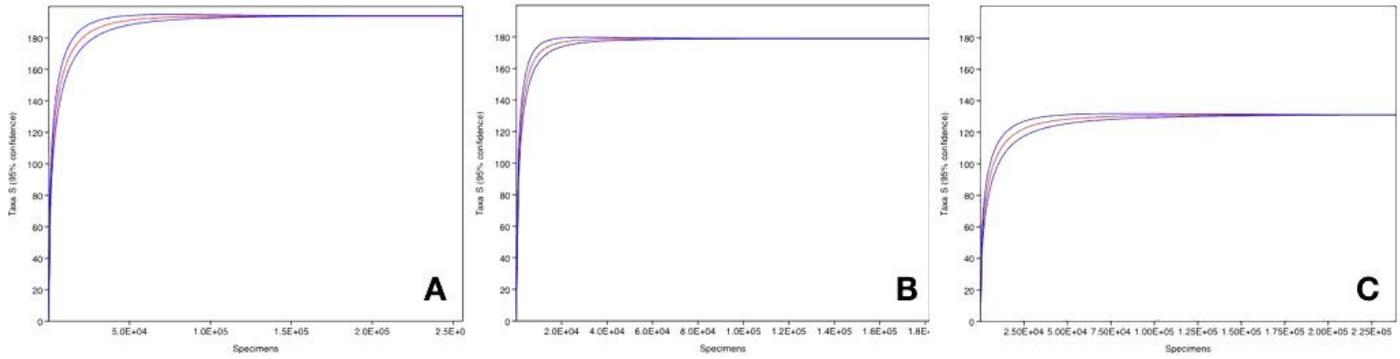


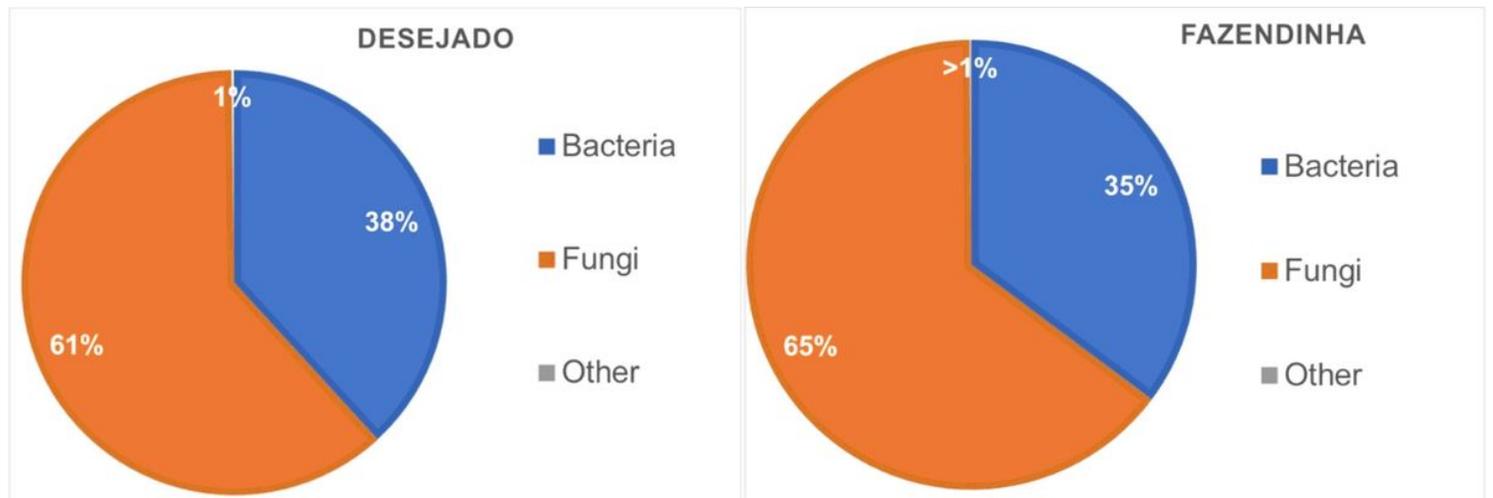
Figure 1

Localization of Trindade Island relative to Brazil in the South Atlantic Ocean (A), sampled points (B; upper red rectangle) in Fazendinha (C) and in Desejado Hill (C), and the soil profiles PD5 (E), PD6 (F), and PF7 (G). Modified from Google®.



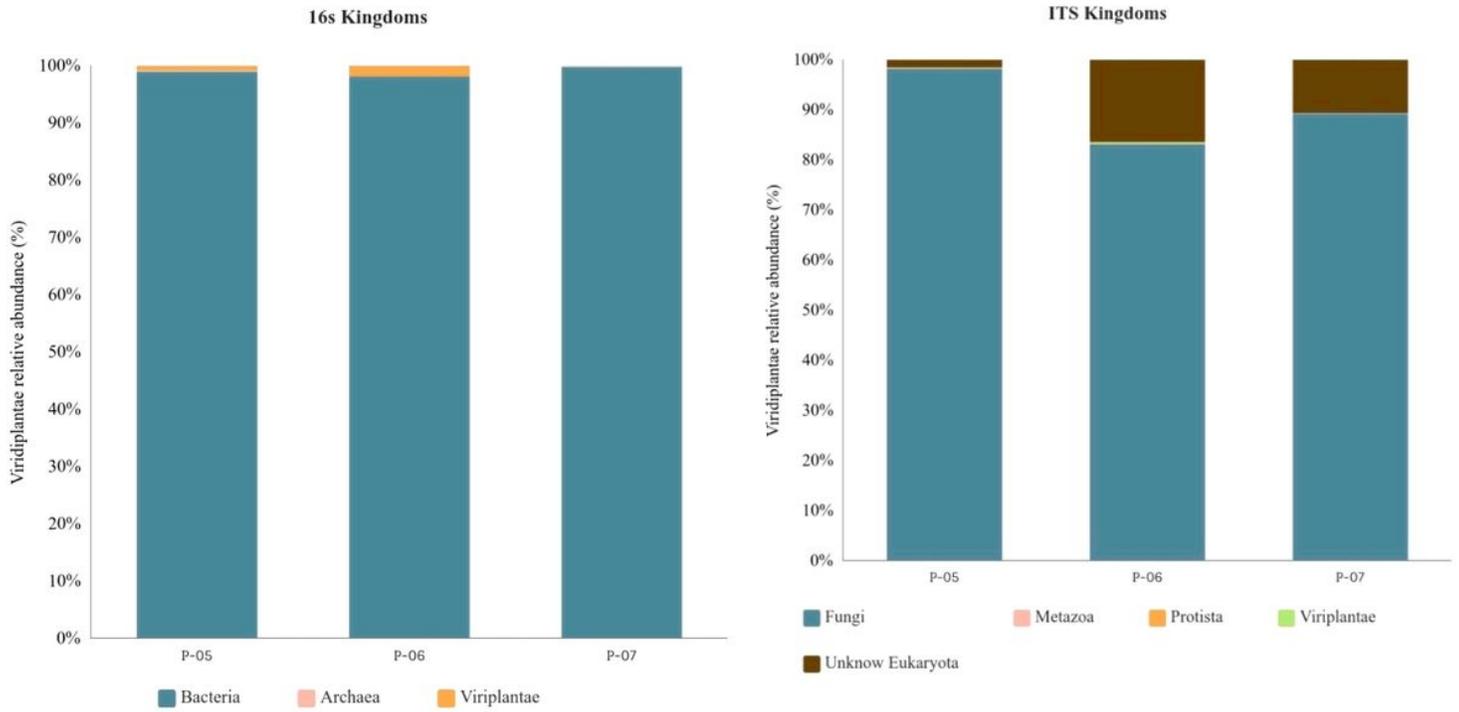
**Figure 2**

Rarefaction curves obtained from the three samples, (a) P-05, (b) P-06 and (c) P-07.



**Figure 3**

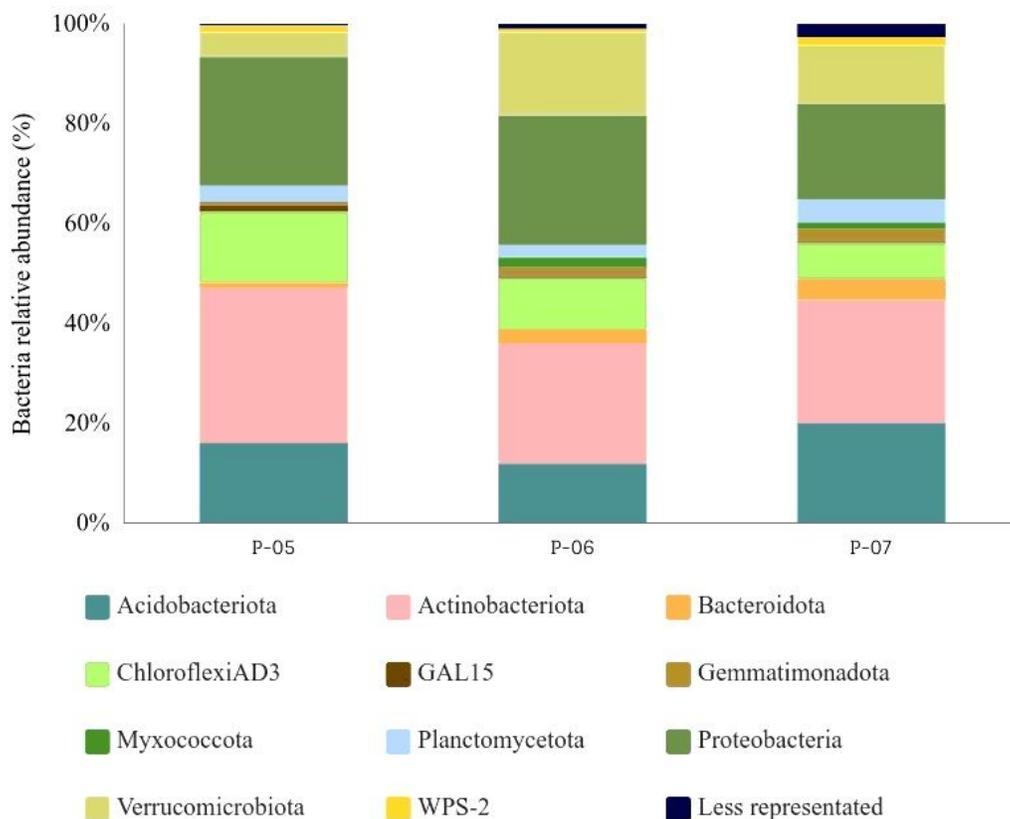
Relative abundance of taxa found in both sampling locations. Taxa referred as 'Other' include Protozoa, Chromista, Viridiplantae and Metazoa.



**Figure 4**

Relative abundance of kingdoms found in Trindade Island soils sampled in 2018. Taxonomic assignments were determined using the following databases: SILVA 138 Ref NR 99, and NCBI. ITS sequences were clustered into OTUs by setting a 0.03 distance limit.

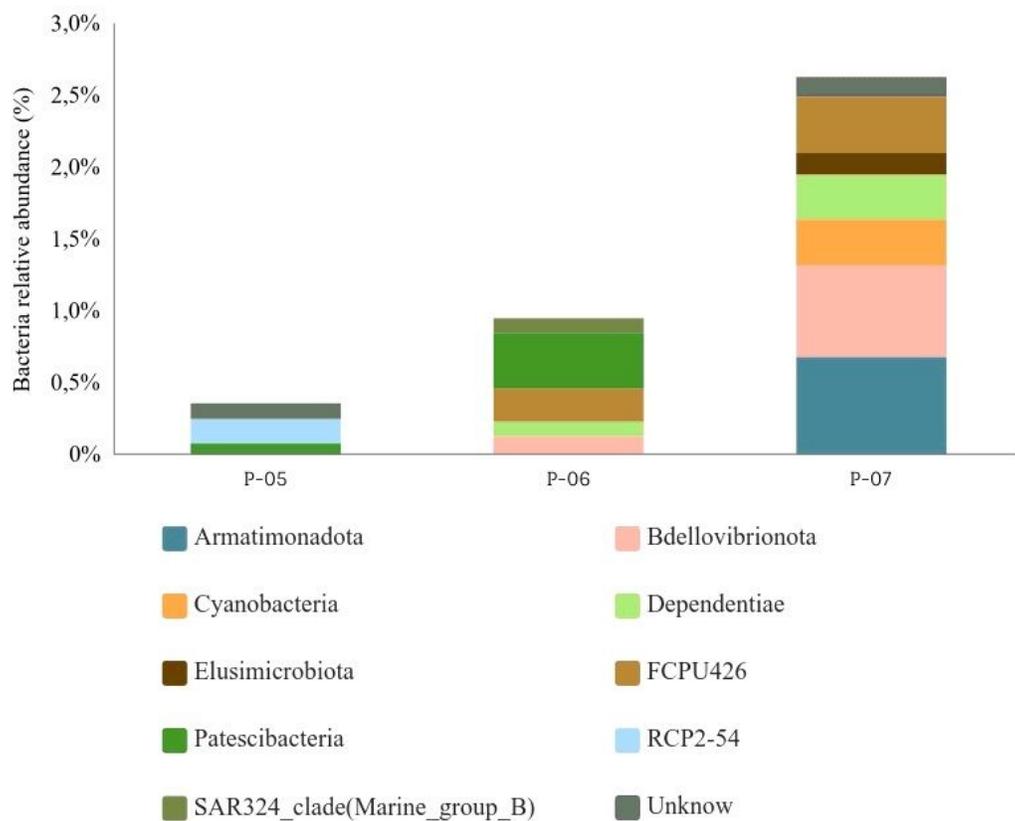
### Most represented Bacteria



**Figure 5**

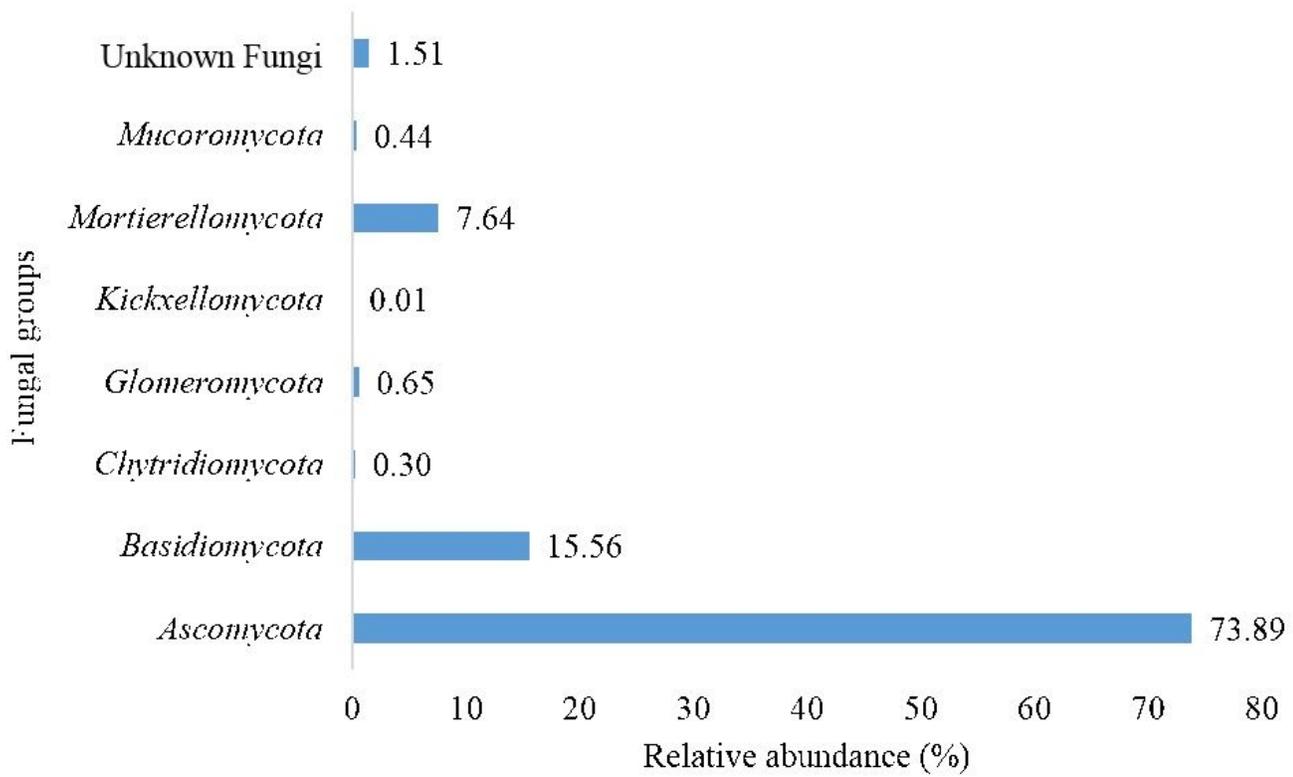
Relative abundance of the most represented bacterial phyla found in Trindade Island soils sampled in 2018. Taxonomic assignments were determined using the following databases: SILVA 138 Ref NR 99, and NCBI.

### Less represented Bacteria



**Figure 6**

Relative abundance of the least represented bacterial phyla found in Trindade Island soils sampled in 2018. Taxonomic assignments were determined using the following databases: SILVA 138 Ref NR 99, and NCBI.



**Figure 7**

Relative abundance (%) of the fungal groups detected in the giant fern forest soil samples obtained on Trindade Island in 2018.

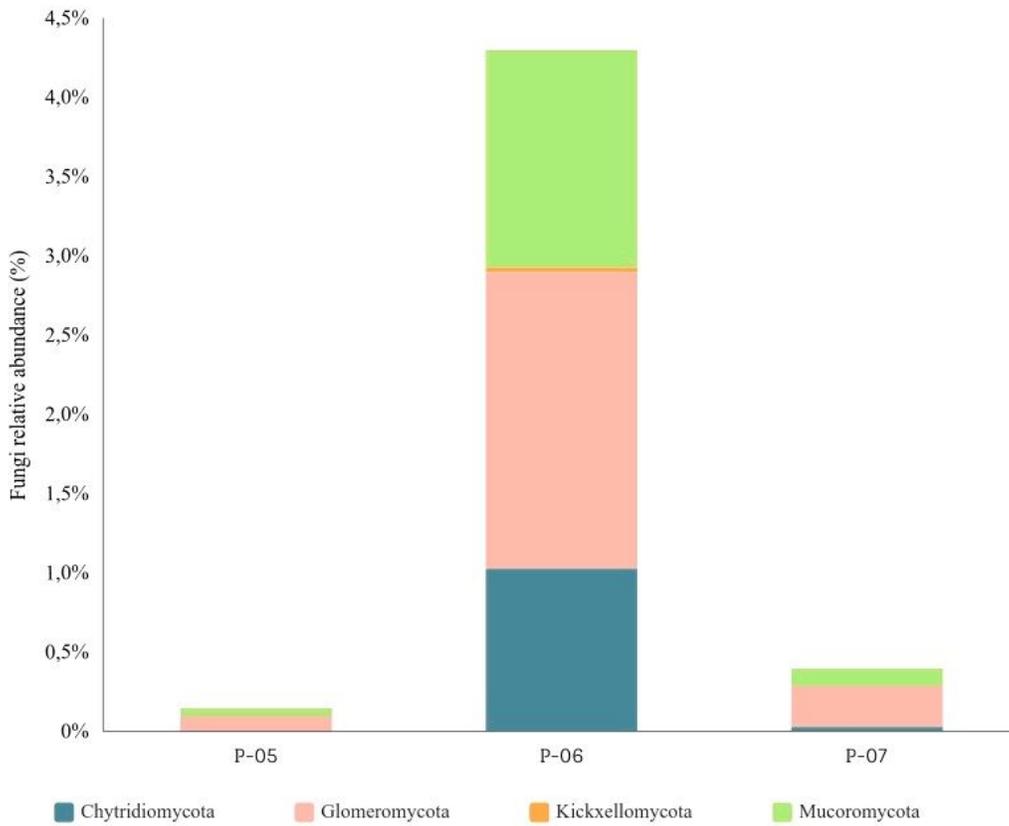
**Figure 8**

Venn diagram showing the fungal amplicon sequence variants (ASVs) detected in the three giant fern forest soil samples obtained on Trindade Island, South Atlantic Ocean, in 2018.

**Figure 9**

Relative abundances of the most represented fungal sequences found in Trindade Island soil samples obtained in 2018. ITS sequences were clustered into OTUs by setting a 0.03 distance limit. Taxonomic assignments were determined using the following databases: UNITE Fungal ITS database 8.2, UNITE Eukaryotes ITS database 8.2, and NCBI.

### Less represented Fungi



**Figure 10**

Relative abundances of the less represented fungal sequences found in Trindade Island soils sampled in 2018. ITS sequences were clustered into OTUs by setting a 0.03 distance limit. Taxonomic assignments were determined using the following databases: UNITE Fungal ITS database 8.2, UNITE Eukaryotes ITS database 8.2, and NCBI.

### Metazoa

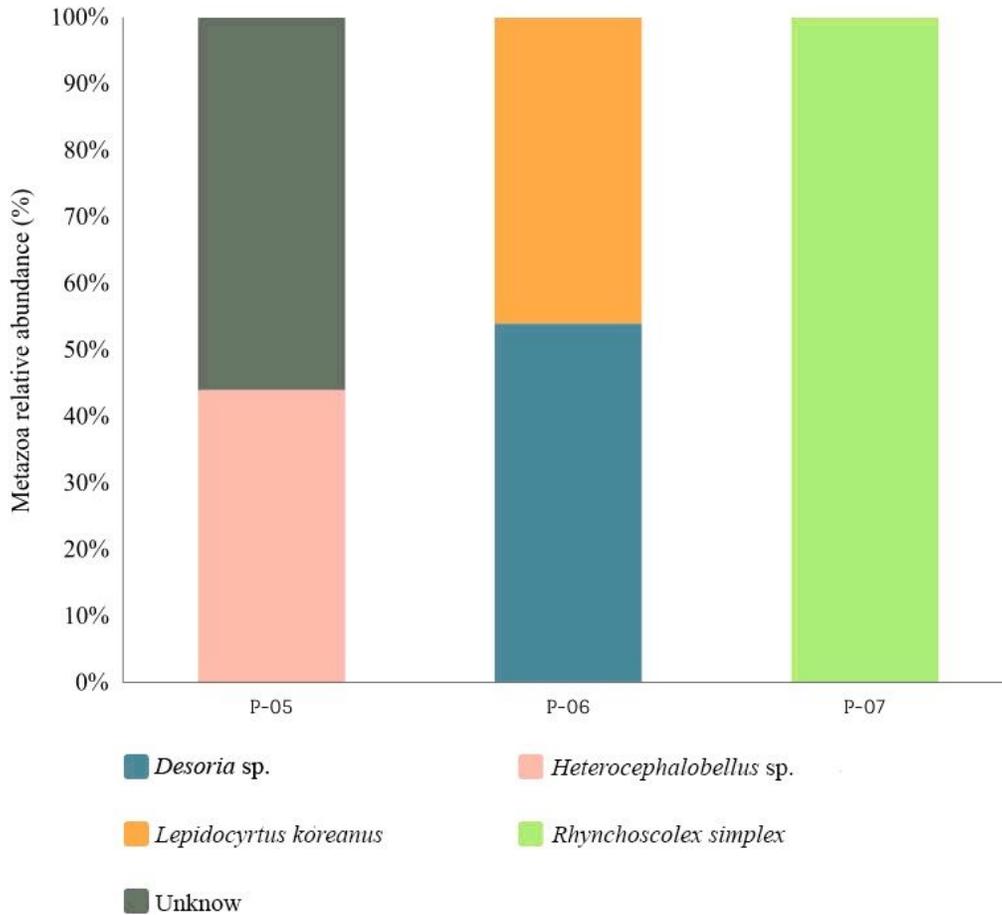


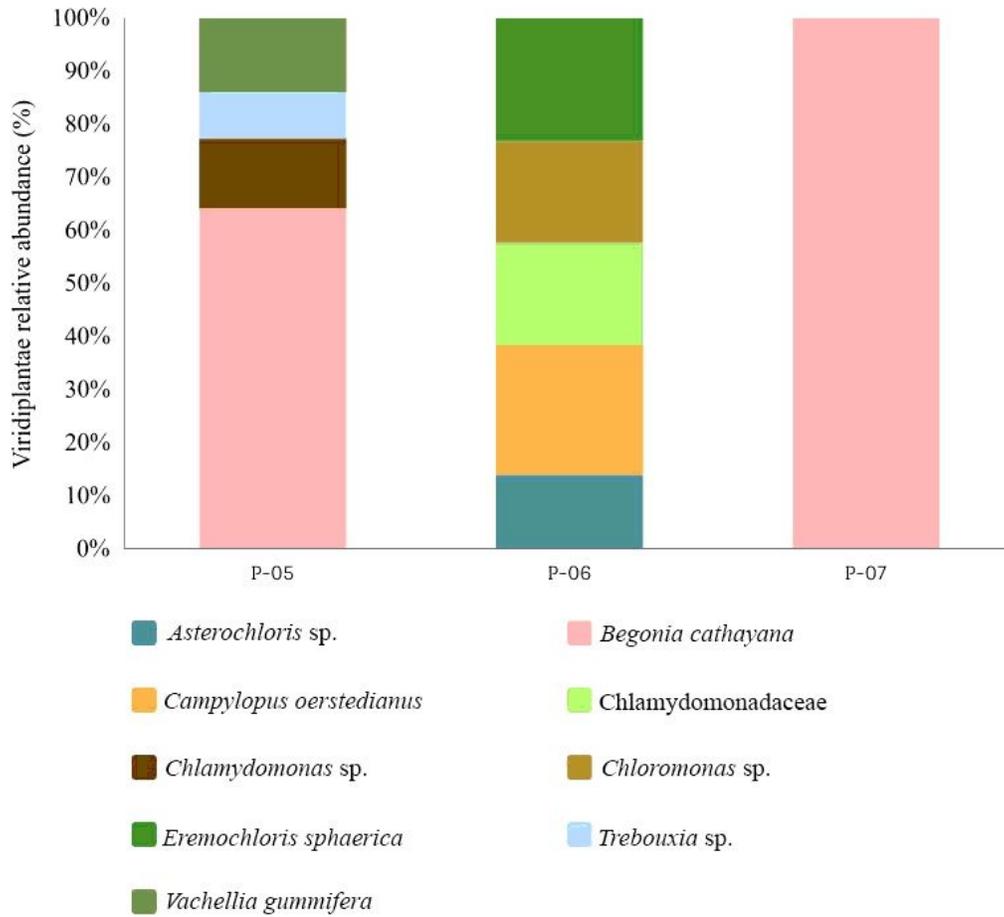
Figure 11

Relative abundances of the metazoan sequences detected in Trindade Island soil samples obtained in 2018. ITS sequences were clustered into OTUs by setting a 0.03 distance limit. Taxonomic assignments were determined using the following databases: UNITE Fungal ITS database 8.2, UNITE Eukaryotes ITS database 8.2, and NCBI.

Figure 12

Relative abundances of Protista sequences detected in Trindade Island soil samples obtained in 2018. ITS sequences were clustered into OTUs by setting a 0.03 distance limit. Taxonomic assignments were determined using the following databases: UNITE Fungal ITS database 8.2, UNITE Eukaryotes ITS database 8.2, and NCBI.

### Viridiplantae



**Figure 13**

Relative abundances of sequences of Viridiplantae found in soils sampled on Trindade Island in 2018. ITS sequences were clustered into OTUs by setting a 0.03 distance limit. Taxonomic assignments were determined using the following databases: UNITE Fungal ITS database 8.2, UNITE Eukaryotes ITS database 8.2, and NCBI.

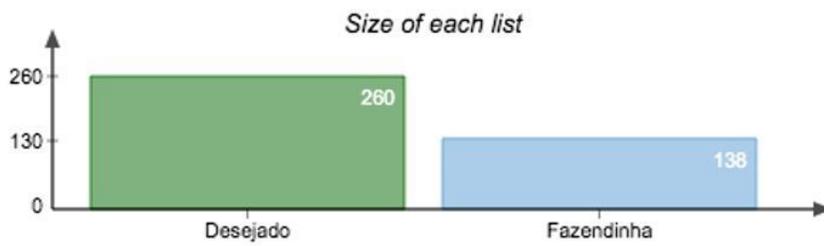
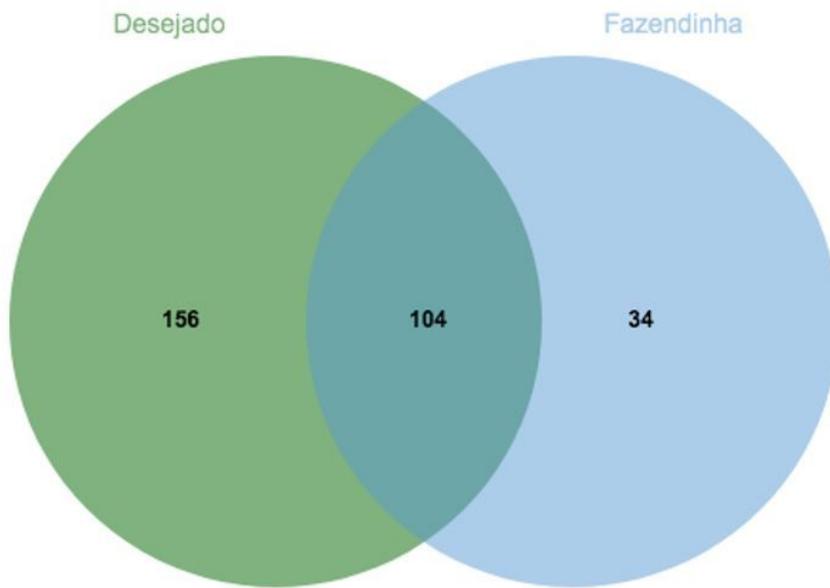


Figure 14

Venn diagram showing Viridiplantae sequence distribution across the two sites sampled.

## Supplementary Files

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