UNIVERSIDADE FEDERAL DE VIÇOSA

TOMÁS GOMES REIS VELOSO

EFEITOS AMBIENTAIS NO MICROBIOMA: TRÓPICOS E ANTÁRTICA

VIÇOSA – MINAS GERAIS
2021

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Tese apresentada à Universidade Federal de Viçosa como parte das exigências do Programa de Pós-Graduação em Microbiologia Agrícola, para obtenção do título de *Doctor Scientiae*.

Orientadora: Maria Catarina Megumi Kasuya

Coorientadores: Carlos Ernesto G. Reynaud Schaefer
Mateus Ferreira Santanna

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Tomás Gomes Reis Veloso

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Autor

Maria Catarina Megumi Kasuya

Maria Catarina Megumi Kasuya
Orientadora

A Deus,

A mimha família,

Dedico

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RESUMO

VELOSO, Tomás Gomes Reis, D.Sc., Universidade Federal de Viçosa, maio de 2021. **Efeitos ambientais no microbioma: trópicos e Antártica**. Orientadora: Maria Catarina Megumi Kasuya. Coorientadores: Carlos Ernesto Gonçalves Reynaud Schaefer e Mateus Ferreira Santana.

Os microganismos desepenham papel fundamental nos ciclos biogeoquímicos, como os ciclos do carbono, nitrogênio, enxofre, fósforo, hidrogênio e oxigênio. Quando presentes na rizosfera ou no interior de tecidos, podem atuar na promoção do crescimento das plantas, no controle de fitopatógenos, como fitorreguladores e na disponibilização de água e nutrientes essenciais. Estas comunidades são diretamente afetadas pelas condições edafoclimáticas. Assim, no atual cenário das mudanças climáticas, conhecer a diversidade microbiana edáfica e os fatores que afetam são essenciais para entender o potencial de cada solo, tanto para uso agrícola, como seu papel no atual cenário das mudanças climáticas. Dentre as diversas formas possíveis de avaliar a diversidade microbiana, o sequenciamento de DNA de alto rendimento vem sendo uma das técnicas mais utilizadas. Dessa forma, esta tese apresenta três capítulos nos quais esta ténica foi utilizada para avaliar a diversidade em cada solo. O primeiro capítulo, intitulado "Effects of environmental factors on microbiota of fruits and soil of *Coffea arabica* in Brazil" tem como objetivo avaliar os efeitos das diferentes condições edáficas e topográficas presentes no cultivo de cafeeiro sobre as comunidades bacterianas e fúngicas em solos e frutos. O segundo e terceiro capítulo abordam, respectivamente, a diversidade de comunidades bacterianas e fúngicas em solos recentemente expostos pela deglaciação e em solos de pinguineiras. Para o cafeeiro, observou-se que as comunidades microbianas presentes nos frutos são afetadas principalmente por fatores topográficos (altitude, face de exposição ao sol e radiação), enquanto que as comunidades presentes no solo, embora também influenciadas por estes fatores, são fortemente modadas pelas variáveis edáficas. Na Antártica marítima, nos solos recentemente expostos pela retração (solos jovens) observou-se uma maior variabilidade da diversidade beta, o que indica uma sucessão microbiana mais intensa do que solos mais desenvolvidos. Por fim, obervou-se que em solos ornitogênicos a comunidade bacteriana e fúngica é fortemente alterada após o solo ser abandonado pelos pinguins. Solos ornitogênicos em áreas abandonadas apresentaram maior abundância predita para genes relacionados nitrificação e ao metaboilsmo de enxofre. Em suma, tanto em solos tropicais como em solos antárticos, os fatores edafoclimáticos e temporais afetam a estrutura da comunidade microbiana.

Palavras-chave: Microbiota. Café. Solos ornitogênicos. Geleiras. Sequenciamento de nova geração.

ABSTRACT

VELOSO, Tomás Gomes Reis, D.Sc., Universidade Federal de Viçosa, May, 2021**.** **Environmental effects on microbiome: tropics and Antarctic**. Advisor: Maria Catarina Megumi Kasuya. Co-advisors: Carlos Ernesto Gonçalves Reynaud Schaefer and Mateus Ferreira Santana.

The microorganisms play a key role in the in the main biogeochemical cycles, such as Carbon, nitrogen, sulfur, phosphorus, hydrogen and oxygen. In the rhizosphere and inside plant tissues, they can enhance plant growth, phytopathogen control, act as phytoregulators and increasing water and nutrient availability. These communities are directly affected by edaphoclimatic conditions. Thus, in the current scenario of climate change, the knowledge about the edaphic microbial diversity and the factors that shape it are essential to understand the potential of each soil, either for agricultural use or to understand the role of the soil face the climate change. There are many methods to study the microbial diversity, among them, the high-throughput DNA sequencing is the one of the most commonly used. This thesis is composed of three chapters where this technique was used to evaluate the diversity in each soil. The first title, entitled "Effects of environmental factors on microbiota of fruits and soil of *Coffea arabica* in Brazil" aimed to evaluate the effect of edaphic and topographic coditions in coffee crops on bacterial and fungal communities in soil and fruits. The second and third chapters addresses, respectively, the diversity of bacteria and fungi in soils recently exposed by deglaciation and ornithogenic soils. The microbial communities of fruits are affected mainly by tophographic factors (Altitude, facing slope and radiation), whereas microbial communities of soil, although also influenced by topographic factors, are strongly shaped by edaphic factors. Soils recently exposed by glacier retreat (young soils) displayed a greater variation in beta diversity than more developed soils. Lastly, the bacterial and fungal communities in the ornithogenic soils undergoes a substantial change after the abandonment by the penguins. Abandoned ornithogenic soils showed a greater predicted abundance of genes related to nitrification and sulfur metabolism. In short, both in tropical and antarctic soils, edaphoclimatic and temporal factors affect the structure of the microbial community.

Keywords: Microbiota. Coffee. Ornithogenic soils. Glaciers. Next generation sequencing.

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INTRODUÇÃO GERAL

Os microganismos desepenham papel fundamental nos principais ciclos biogeoquímicos, como os ciclos do carbono, nitrogênio, enxofre, fósforo, hidrogênio e oxigênio (Gougoulias et al. 2014; Hofer 2018; Kuypers et al. 2018). Pela decomposição microbiana da matéria orgânica, os nutrientes tornam-se novamente disponíveis para utilização por outros organismos. O funcionamento e a estabilidade desses processos dependem de uma série de interações entre a microbiota e fatores edafoclimáticos presentes em cada local (Islam et al. 2020). Além disso, comunidades microbianas habitam a rizosfera e o interior dos tecidos vegetais, atuando no controle de fitopatógenos, na promoção do crescimento das plantas por meio da produção de fitorreguladores, e disponibilização de água e nutrientes essenciais, como: ferro, fósforo, nitrogênio, entre outros (Trivedi et al. 2020).

Com o atual cenário das mudanças climáticas, o entendimento do funcionamento da comunidade microbiana nos solos é essencial, uma vez que estes podem atuar como bombas de carbono, fixando e estabilizando-o na matéria orgânica do solo, por meio da atividade anabólica, ou lançando-o na atmosfera, pelo processo catabólico (Liang et al. 2017). Além disso, comunidades microbianas em solos agrícolas desempenham papéis essenciais na fixação biológica de nitrogênio, aquisição de água e nutrientes (como as micorrízas) e modulação do crescimento de plantas (como as bactérias promotoras de crescimento vegetal).

Solos são ambientes complexos que apresentam minerais, gases, líquidos, matéria orgânica e organismos vivos em sua constituição. Diversos atributos ambientais e do hospedeiro modulam a composição e dinâmica das comunidades microbianas presentes no solo, e também no interior de tecidos de hospedeiros. Assim, esta tese é divida em três capítulos, nos quais são estudadas comunidades bacterianas e fúngicas em solos agrícolas tropicais e da Antártica sob diferentes condições ambientais. No primeiro capitulo é retratado um estudo com o café, intitulado *"Effects of environmental factors on microbiota of fruits and soil of Coffea arabica*

in Brazil" e está vinculado ao projeto "Efeito das condições edafoclimáticas na composição da microbiota endofítica do café e seus respectivos impactos na qualidade" do Laboratório de Associações Micorrízicas – DMB/Bioagro/UFV em convênio com o Laboratório de Análise e Pesquisa em Café (LAPC)/ UFES Venda Nova, e objetivou avaliar os efeitos de fatores ambientais na população de bactérias e fungos presentes em solos e frutos de cafeeiro. Esta pesquisa está publicada na revista Scientific Reports (Veloso et al. 2020). O segundo e o terceiro capítulos retratam pesquisas em solos da Antártica Marítima e estão vinculadas ao gupo de pesquisa TERRANTAR (Ecossistemas Terrestres da Antártica) e ao PROANTAR (Programa Antártico Brasileiro). A pesquisa apresentada no segundo capítulo, intitulada "*The microbiota playing "musical chairs" in a glacier retreat zone of Maritime Antarctica*" avalia as populações fúngicas e bacterianas em solos presentes em áreas recentemente expostas pela deglaciação. O terceiro apresenta um estudo relacionado a solos ornitogênicos presentes em áreas de nidificação antigas e atuais e teve como objetivo avaliar os perfis das comunidades bacteriana e fúngica em cada tipo de solo.

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CAPÍTULO 1: Effects of environmental factors on microbiota of fruits and soil of *Coffea arabica* in Brazil

Effects of environmental factors on microbiota of fruits and soil of *Coffea arabica* in Brazil

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Effects of environmental factors on microbiota of fruits and soil of *Coffea arabica* in Brazil

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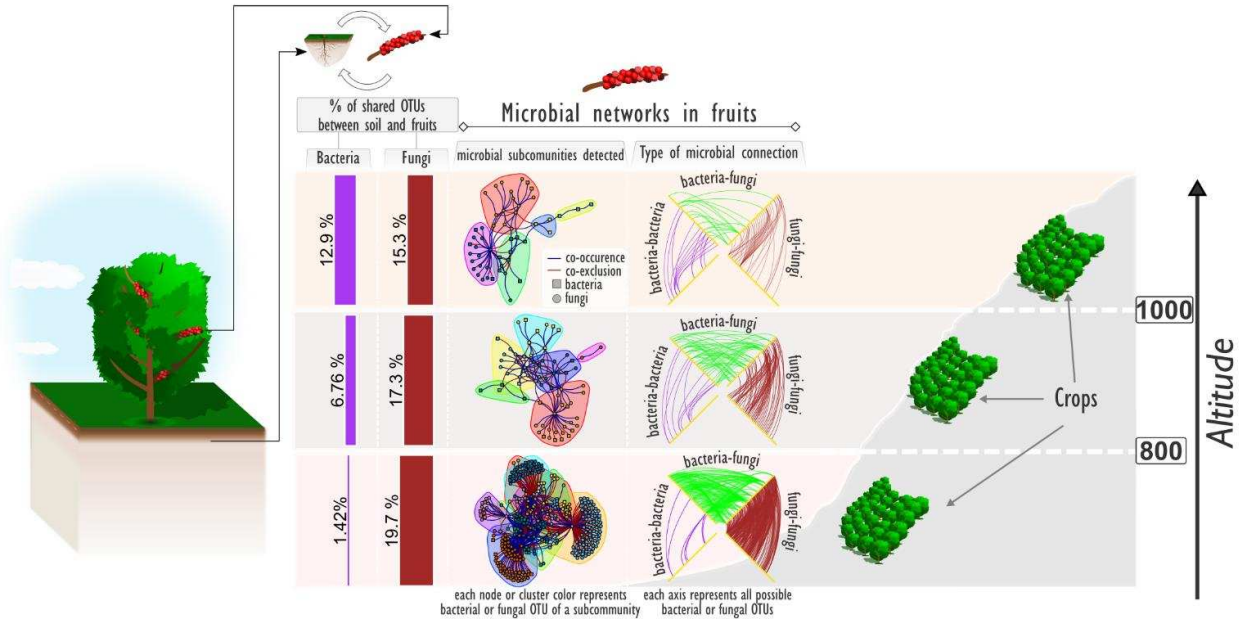
Conflit of interest

We have no conflict of interest to declare.

Graphical abstract



Abstract

In recent years, several studies have been developed to understand the impact of fermentation on the final quality of coffee and have indicated that postharvest processing could be a determinant of quality. However, a trend has appeared as a scientific counterpoint, indicating that the interactions between soil, fruit, altitude, and slope exposures with respect to the Sun are important to understand the behavior of the microbiome in coffee. Studies on the microbiota of coffee have addressed its role during the fermentation process, however the knowledge of indigenous microorganisms harbored in fruits and soil of coffee trees growing in fields are essential, as they can contribute to fermentation. Therefore, the aim of this work was to evaluate the influence of topographic and edaphic factors on the bacterial and fungal communities present in the soil and in the fruits of *Coffea arabica* trees. Samples of fruits and soil were collected from different growing areas at different altitudes and soil conditions. The microbial DNA was extracted and sequenced. The results showed the contribution of environmental factors in the structure of bacterial and fungal communities. The richness, evenness and diversity of the mycobiome and bacteriome were higher in the soil than in the fruits, independent of altitude. In addition, coffee trees at higher altitudes tended to have more bacteria shared between the soil and fruits. The co-occurrence/co-exclusion network showed that bacteria-bacteria connections were greater in higher altitudes. On another hand, fungi-fungi and fungi-bacteria connections were higher in low altitudes. This was the first study that evaluates

in deep the influence of environmental factors in the microbiota habiting fruits and soil coffee trees, which may affect the coffee beverage quality.

Introduction

Coffee is one of the most important agricultural commodities in the world. Brazil is the largest exporter and second largest consumer of coffee, producing more than 56 million coffee bags, approximately one third of all coffee exported in the world[1]. The two main coffee species cultivated in Brazil are *Coffea arabica* (75 %) and *Coffea canephora* (25 %)[1]. While *C. canephora* is cultivated in altitudes ranging from 50 to 550 m, *C. arabica* crops are present in altitudes from 600 to 1200 m.

The quality of coffee trees as well as their beverages rely on a suitable combination of climatic and edaphic factors[2–5]. Some of these factors are well known to influence the quality of the beverage; for instance, it is well known that higher elevations produce dense beans with higher quality[5]. If the environment affects the final quality, both processing and environment may be influencing the microbial community structures and hence the chemical composition of the final coffee beans. The studies of coffee ecosystems contribute to a better understanding of a state-of-the-art framework for the further analysis and subsequent control of this complex biotechnological process[6] since coffee pulp and mucilage are natural substrates for the growth of microorganisms, such as bacteria and fungi, which have been shown to be implicated in coffee quality[7].

Studies have tried to describe the dynamics of microbiota in coffee crops and processing[8–10], however, to the best of our knowledge, no published research has investigated in depth the influence of topographic factors such as altitude and sun face exposition on the microbiota of coffee. The microbiota associated with coffee plants may play a critical role in the final quality of coffee, however, the microbial diversity in coffee cherries is still poorly characterized[11]. In fact, most studies on the microbiota in coffee have addressed its role during the fermentation process[6,12,13] and not the microbiota related to coffee trees growing in fields; it is known that after harvesting, coffee fruits are processed to allow for spontaneous fermentation by

indigenous microbiota[13]. Therefore, it is necessary to understand the impact of environmental factors on the indigenous microbiota that inhabits coffee beans, because these microorganisms can develop an important role in the fermentation of coffee beans (e.g. yeasts and lactic acid bacteria)[14].

Soil microbiota plays an important role in nutrient cycling by making available the required mineral nutrition available for the root system[15]. On the other hand, the microbiota in fruits play an important role during coffee fermentation by degrading the mucilage and impacting the beverage flavor[16]. That the microbial interactions are neither known nor controlled during the fermentation process[17]. Furthermore, there are no studies that compare the shared microbiota between these two niches.

Considering that soil chemical and topographical factors may influence the microbial composition in coffee crops, the aim of this study was to evaluate the influence of these factors on the bacterial and fungal communities in the soil and fruits of *C. Arabica*.

Material and Methods

Study areas and sampling of soil and fruits

As the Espírito Santo is the second largest producer of coffee in Brazil and has many small farms that produce coffee with high quality beverages, in a wide range of environmental factors, it was chose to develop this study. The samplings were conducted on eight small agricultural farms, with altitudes ranging from 735 to 1,078 m (see Supplementary Fig. S1). Red Catuaí 81 was selected because this variety is used by all the farms included in this study.

From each farm, three composite samples of fruits were sampled (Fig. 1); each sample comprised 30 fruits from three coffee plants and three composite samples of soil (about 300 g each), which came from three randomly points inside an area of 1 m² and 10 cm of depth[18], and

under the canopy projection of coffee trees (Fig. 1). All the samples were stored in sterile plastic bags and carried to the laboratory under refrigeration and kept at -20 ºC.

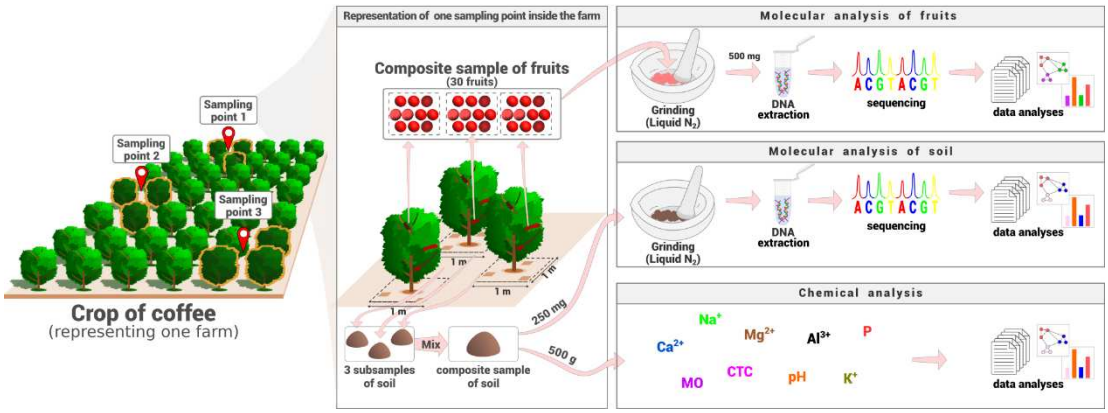


Figure 1: Sampling design of soil and fruits in each crop of coffee. From each farm we collected soil and fruits from three sampling points. The molecular analyses were performed for bacteria and fungi communities.

A total of 250 mg of the composite samples was used to soil analysis (Laboratory of Analysis of Soil in Viçosa, MG, Brazil). Soil pH was measured in water (ratio soil:water = 1:2.5). The potential acidity (H + Al) was determined with calcium acetate at pH 7.0. The acid solution Mehlich-1 was used as the extractor of P and K. Ca, Mg and Al were extracted with KCl solution (1 M) and quantified by atomic absorption spectrophotometry. The results allowed the determination the sum-of-bases (SB), base saturation (V), aluminum saturation (m), and potential cation-exchange-capacity (CTC)[19].

DNA extraction, PCR and sequencing

DNA extraction from the soil and fruits was performed using NucleoSpinSoil (MACHENEREY-NAGEL) extraction kit. For the soil, 250 mg was used for extraction according to the manufacturer's instructions. The fruits were first smashed using a sterile pistil and 250 mg of the resulting homogenate was used for the extraction process according manufacturer's instructions.

To evaluate the mycobiome profile, polymerase chain reactions (PCR) were performed using the primer pairs ITS1F (5' CTTGGTCATTTAGAGGAAGTAA 3') and ITS2 (5' GCTGCGTTCTTCATCGATGC 3 ') to amplify the region ITS1 (Internal Transcribed Spacer 1) region of the rDNA of the fungal community, while the bacteriome was evaluated by amplification of V4 subregion of the 16S rDNA with the primers 515F (5' GTGYCAGCMGCCGCGGTAA 3') and 806R (5' GGACTACNVGGGTWTCTAAT 3')[20]. The PCR libraries were quantified using Qubit hs-DS-DNA kit (Invitrogen, Carlsbad CA) on a Tecan Infinite F200 Pro plate. The results were used to normalize all the libraries in a equimolar concentration of 2 nM, which were pooled for sequencing. The sequencing of the 16S and ITS1 libraries were performed using the platforms Illumina MiSeq 2 x 150 bp and Illumina MiSeq 2 x 250 bp, respectively.

Processing of 16S amplicons

The reads from sequencing were processed according the pipeline proposed by other authors[21] using the softwares USEARCH v.11[22] and QIIME 1.9.1[23]. The reads with an expected error greater than 0.5 were removed using the command *fastq_filter* of the USEARCH v.11. This software was also used to remove the singletons by the command *sortbysize* (minsize = 2)[24], to remove chimeras by the command *uchime2_ref* and to cluster the remaining sequences in Operational Taxonomic Units (OTUs) using the command *cluster_otus* with a threshold of 97 % of similarity. Each OTU was annotated against the SILVA database (Release 132)[25] using the script *assign_taxonomy.py* available in QIIME 1.9.1. Additionally, a reference tree was reconstructed by the script *make_phylogeny.py*. This tree was used to calculate the distance matrix based in the Unifrac distance[26].

Processing of ITS1 amplicons

For the ITS1 dataset, only de forward reads were used since this region is variable in length. The softwares USEARCH v.11[27], QIIME 1.9.1[23] and ITSx[28] were used to process the reads. As performed for 16S sequences, USEARCH v.11 was used to remove low quality sequences and singletons, using the commands *fastq_filter* (-fastq_maxee = 0.5) and *sortbysize* (minsize = 2), respectively. Before clustering the sequences in OTUs, the fungal ITS1 region was extracted. This step ensured the removal of conserved flanking regions 18S and 5.8s and the removal of ITS sequences from other eukaryotes that are not fungi. The remaining sequences were clustered in OTUs at 97 % of similarity with the command *cluster_otus* of USEARCH v.11. The taxonomic assignment was performed using the script *assign_taxonomy.py* of QIIME 1.9.1 with the UNITE (Version 7.2)[29] database as reference. Due to the high variability of the ITS1 region, which makes the multiple alignment of highly divergent sequences difficult, it was not built a phylogenetic tree, as it was done for 16S.

Statistical analysis

The Principal coordinate analysis (PCoA) built for the 16S and ITS datasets were based on Bray-Curtis dissimilarity and weighted UNIFRAC indexes, respectively. The topographic and edaphic factors were fitted to the ordination by the *envfit* function available on vegan package 2.5.6[30]. The significance of each edaphic and topographical factor was assessed by PERMANOVA using the function *adonis* in R v.3.5.1[31]. To evaluate the contribution of each group of factors, i.e. edaphic and topographic factors, a RDA variance partitioning[32] was performed using the function *varpart* of vegan package 2.5.6.

The estimator of richness (Chao1 index), evenness (Pielou's evenness) and diversity (Simpson index) were calculated using the vegan package v. 2.5.6[30]. To evaluate how these indexes were related with altitude of the crops we fitted linear regressions models using the built-in function *lm* of R v.3.5.1. On another hand, to compare the difference of the values of

these indexes among the slope exposures we used the Kruskal-Wallis test using the function *kruskalmc* of package pgirmess v1.6.9 in R v.3.5.1. The putative functions were predicted using FAPROTAX v.1.1 [33].

SparCC correlations[34] were used to estimate the cooccurrence (positive) and co-exclusion (negative) relationships between bacteria and fungi in different environmental conditions using python scripts with 100 bootstraps using the software fastspar[35]. The cooccurrence networks were built using only significant correlations (p-value $\leq$ 0.01 and absolute correlation $\geq$ 0.7). The topology of each network was evaluated using the igraph package[36].

Results

Influence of edaphic and topographical factors on the microbial communities inhabiting fruits and soil of coffee trees

A total of 1 342 396 and 3 135 343 sequences were obtained from the 16S (Archaea and Bacteria) and ITS1 (Fungi) regions, respectively. After cleaning the data, 666 585 16S reads and 1 017 668 ITS1 reads were retained. The raw reads were submitted to the the NCBI-SRA archive and are available in the BioProject PRJNA626678.

The principal coordinate analysis (PCoA) and a permutational multivariate analysis of variance (PERMANOVA) showed a significant influence of the slope side (p-value = 0.001) and altitude (p-value = 0.001) in bacteria inhabiting fruits (Fig. 2A). Solar radiation influenced the bacterial community in the soil (Fig. 2A) but not in the fruits (Fig. 2B).

The redundancy analysis (RDA) variance partition showed the relative contribution of environmental factors in the structure of bacterial and fungal communities (Fig. 2). In the fruits, the topographical factors influenced the bacterial (Fig. 2A) and fungal (Fig. 2C) communities more than the edaphic factors. On the other hand, in soil, the bacterial community was more

influenced by the edaphic factors than the topographical factors (Fig. 2B), whereas the fungal community was equally influenced by both (Fig. 2D). However, in all the cases a high percent of the variance was not explained by these two groups of factors (residuals). It indicates that other factors, which were not considered here, could also be shaping the microbial communities, mainly the bacterial community of soil (residuals = 90.6 %).

To evaluate the differences in richness, evenness and diversity among different altitudes and slope exposures with respect to the Sun, the Chao1 estimate of richness, the Pilou evenness and the Simpson diversity index were calculated for each sample. These three indexes of the bacteriome and mycobiome were higher in the soil than in the fruits in most altitudes (Fig. 3A). The bacterial diversity in the fruits increased with the altitude (p-value < 0.001), which was explained by the increase of evenness (p-value < 0.01) and richness (p-value < 0.01).

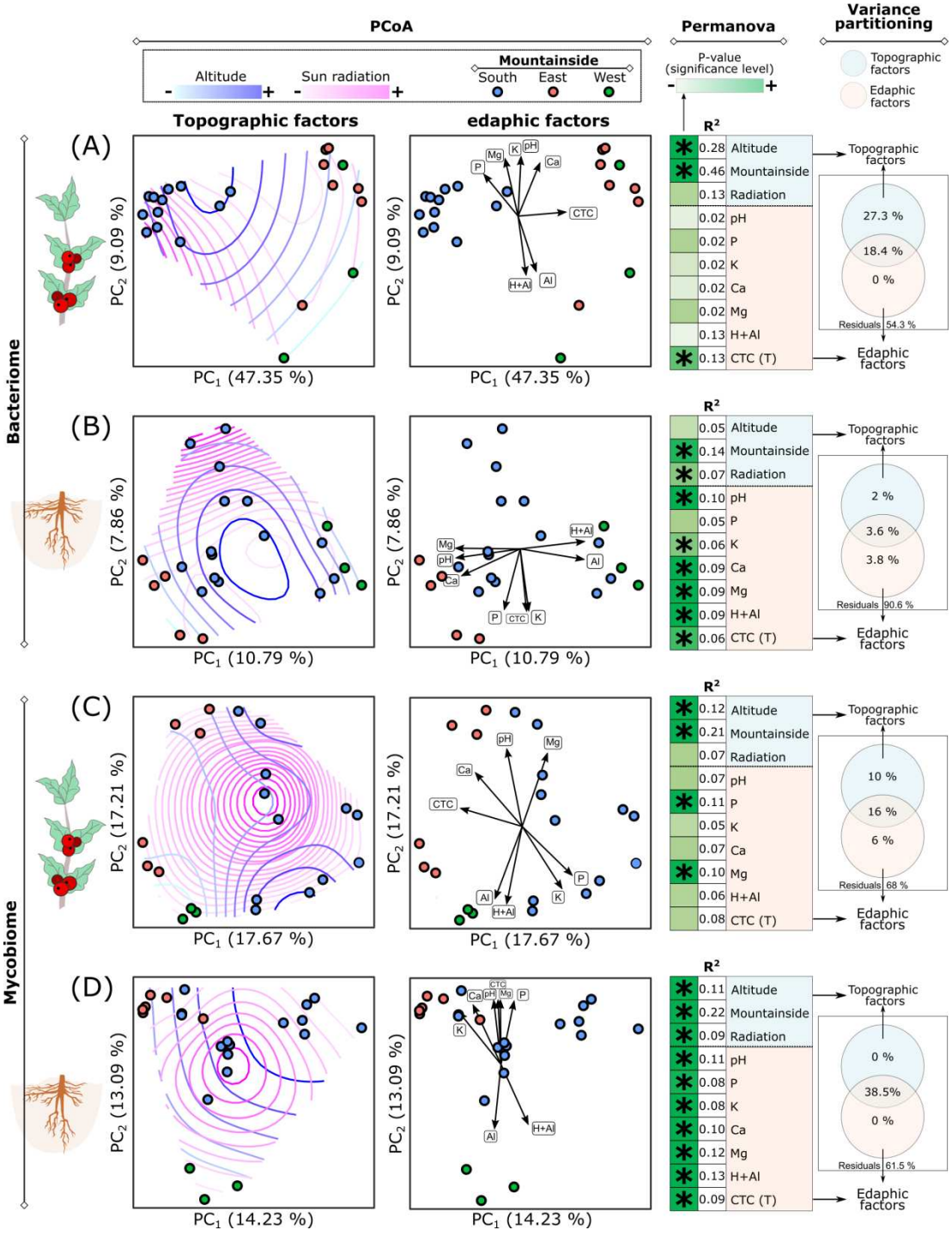


Figure 2: Effect of topographic and edaphic factors on bacteriome and mycobiome of fruits and soil of coffee trees. Analysis of bacteriome (A and B) and mycobiome (C and D) in the fruits (A and C) and soil (B and D) in the *Coffea arabica* crop. Ordination analysis using Principal coordinate analysis (PCoA) based on Bray-Curtis dissimilarity of OTU abundances. The *envfit* function was used to calculate the fitted curves of topographic and edaphic variables. The significance of edaphic and topographical factors were calculated by PERMANOVA, and the variables assigned by *.

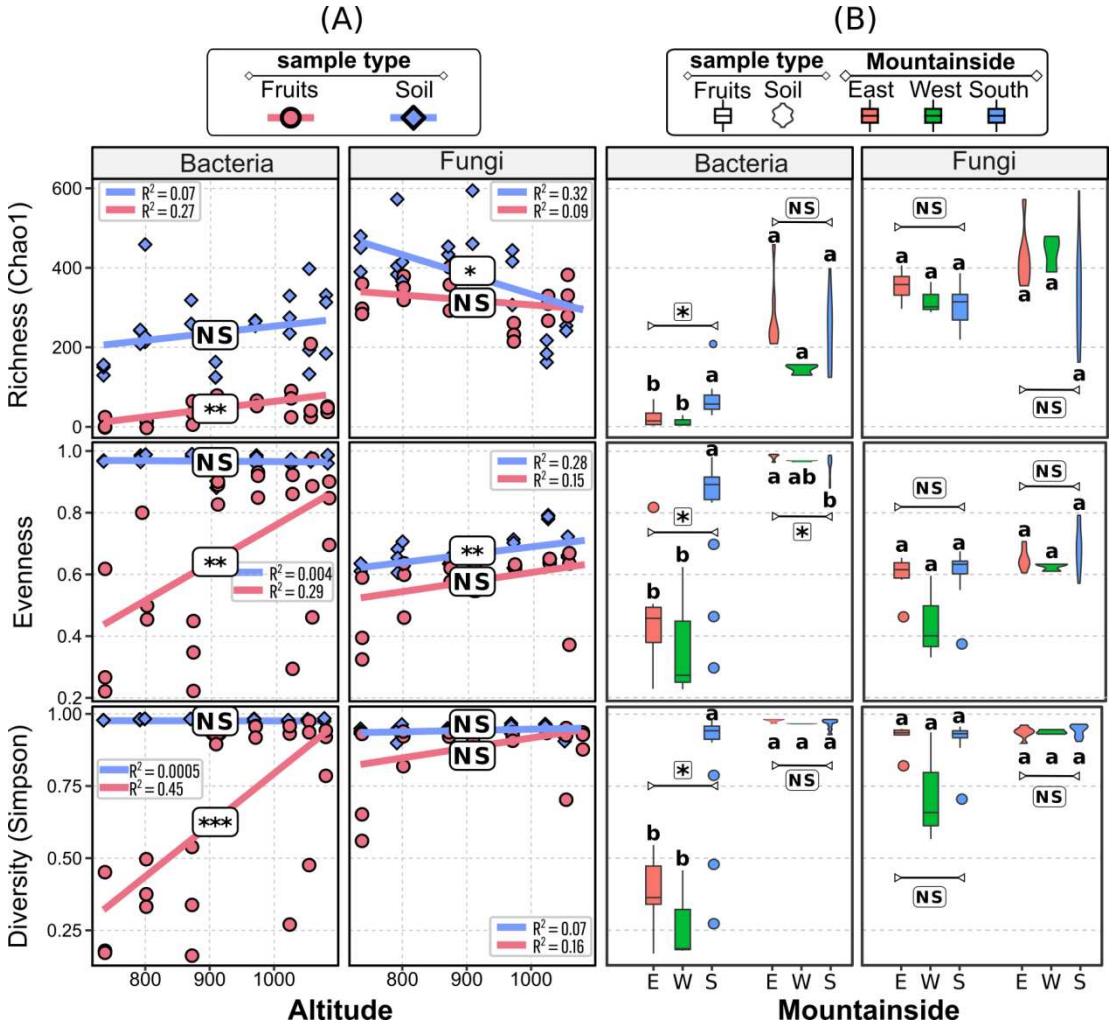


Figure 3: Estimated richness (Chao[1]), evenness and diversity (Simpson index) of the bacteriome and mycobiome in the fruits and soil of *Coffea arabica*, growth at (A) different altitudes and (B) sun-facing slopes. For altitude, NS, *, ** and *** stand for nonsignificant, significant by t-test at 0.05, 0.01 and 0.001, respectively, and for sun-face, NS, *, ** and *** indicate nonsignificant, significant at 0.05, 0.01 and 0.001, respectively, by Kruskal-Wallis test. E = east; W = west; S = south.

In the soil, these indices were not influenced by altitude (Fig. 3A). On the other hand, the richness and evenness of fungi were influenced in soil but not in fruits (p-value < 0.01; Fig. 3A). These results show that bacterial richness increased at higher altitudes while the fungal richness decreased. The slope exposure with respect to the Sun also influenced these three indexes of the bacteriome of fruits, but not the mycobiome (Fig. 3B). These three indexes were higher for the bacterial communities inhabiting fruits in south-facing slopes (p-value < 0.05)

than those in the east and west, except for the richness. In soil, only the evenness of bacterial community present in the south-facing slope were smaller (Fig. 3B).

Taxonomic composition and OTUs shared between fruits and soil in different altitudinal zones

We delimited three altitudinal zones: low altitudes (< 800 m), median altitudes (between 800 and 1000 m) and high altitudes (≥ 1000 m). For each zone, it were evaluated the taxonomic composition and bacteria shared between soil and fruits.

In all altitudinal zones, the most abundant bacterial phylum was Proteobacteria (see Supplementary Figs. S2 and S3). This group was more predominant in soil (ranging from 76 to 98 % of total number of reads) than in fruits (ranging from 35 to 98 %). The candidatus phyla Latescibacteria and WPS-2 were found exclusively in fruits. Regarding to composition of fungal community, Ascomycota was the most abundant phylum in both soil and fruits. We identified a total of five phyla in fruits (Ascomycota, Basidiomycota, Chytridiomycota, Mortierellomycota and Mucoromycota) and eleven in soil (Ascomycota, Basidiomycota, Blastocladiomycota, Calcarisporiellomycota, Chytridiomycota, Entomophthoromycota, Entorrhizomycota, Glomeromycota, Mortierellomycota, Mucoromycota and Rozellomycota). A large amount of sequences (24 to 36 % in fruits and 16 to 22 % in soil) could not be assigned to any known fungus from the UNITE database (see Supplementary Fig. S2).

Since the altitude was previously reported as an important factor to coffee's flavor[37] and we have found that both bacterial and fungal communities were affected by this variable (Fig. 2), we evaluated the percent of shared fungi and bacteria between soil and fruits among these three zones of altitude. We found that while altitude increased, the percent of shared bacterial OTUs decreased and fungal OTUs increased (Fig. 4). Additionally, we performed a functional profile prediction of these shared bacteria using FAPROTAX (Fig. 4) and found an increase of functional roles in in high altitudinal. Some shared functions were exclusively found in the

highest altitudinal zone (>1000 m) like aromatic compound degradation and nitrate reduction (Fig. 4).

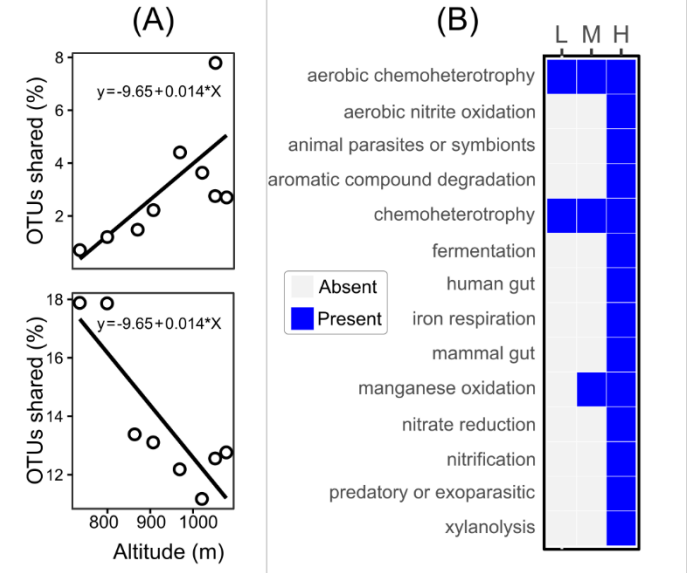


Figure 4: Shared OTUs between fruit and soil. (A) Linear regression of shared Operational Taxonomic units (OTUs) of bacteria and fungi, between fruits and soil of *Coffee arabica*, growing at different altitudes. *Linear coefficients followed by "*" indicate statistical significance at 0.05 of probability by Student's t-test. (B) Bacterial functional groups predicted using Functional Annotation of Prokaryotic Taxa (FAPROTAX) in each altitudinal zone. L = Low altitudes (≤ 800 m); M = Median altitudes (> 800 m and ≤ 1000 m); L = High altitudes (> 1000 m).

Complexity of co-occurrence networks among different zones of altitude

In addition to previous analysis we evaluated the co-occurrence and co-exclusion among OTUs in the three altitudinal zones. We built a co-occurrence network using only statistically significant SparCC correlations (p-value ≤ 0.01 and absolute correlation ≥ 0.7)[34]. The number of connections were higher in fruits at low than in high altitudes, however, the number of connections bacteria-bacteria increased in higher altitudes (Table 1 and Fig. 5A). The majority of connections took place among fungi, followed by bacteria-fungi and bacteria-bacteria (Fig. 5B and Table 1). While the majority of connections among OTUs from the same group (i.e. *bacteria-bacteria* or *fungi-fungi*) were positive (i.e. co-occurrences), the majority of connections among OTUs of different groups were negative (i.e co-exclusion). Using a community detection algorithm (*cluster_walktrap* function of igraph package[36]) , we found

eight, six and five communities at low, median and high altitudes, respectively (Fig. 5C and Table 1).

Table 1: Topological properties of microbial networks in fruits of *Coffea arabica*.

Altitude (m)	Diameter	number of nodes			edges							centrality[1]	modularity[2]	number of communities[3]
		all	bacteria	fungi	all	bacteria-bacteria			fungi-fungi					
						nº	+ (%)	- (%)	nº	+ (%)	- (%)			
≤ 800	5	235	9	226	407	7	85.7	14.3	400	62.3	37.8	0.42	0.49	9
> 800 & ≤ 1000	4	54	13	41	77	11	100.0	0.0	66	78.8	21.2	0.27	0.50	7
> 1000	7	45	13	32	62	16	100.0	0.0	46	91.3	8.7	0.41	0.59	5

[1] Calculated with the *centr_degree* function of the *igraph* package.

[2] Calculated with the modularity function of the *igraph* package.

[3] Each community corresponds a group of nodes that are more densely connected among them than the rest of network. Calculated witht the *cluster_walktrap* function of the *igraph* package.

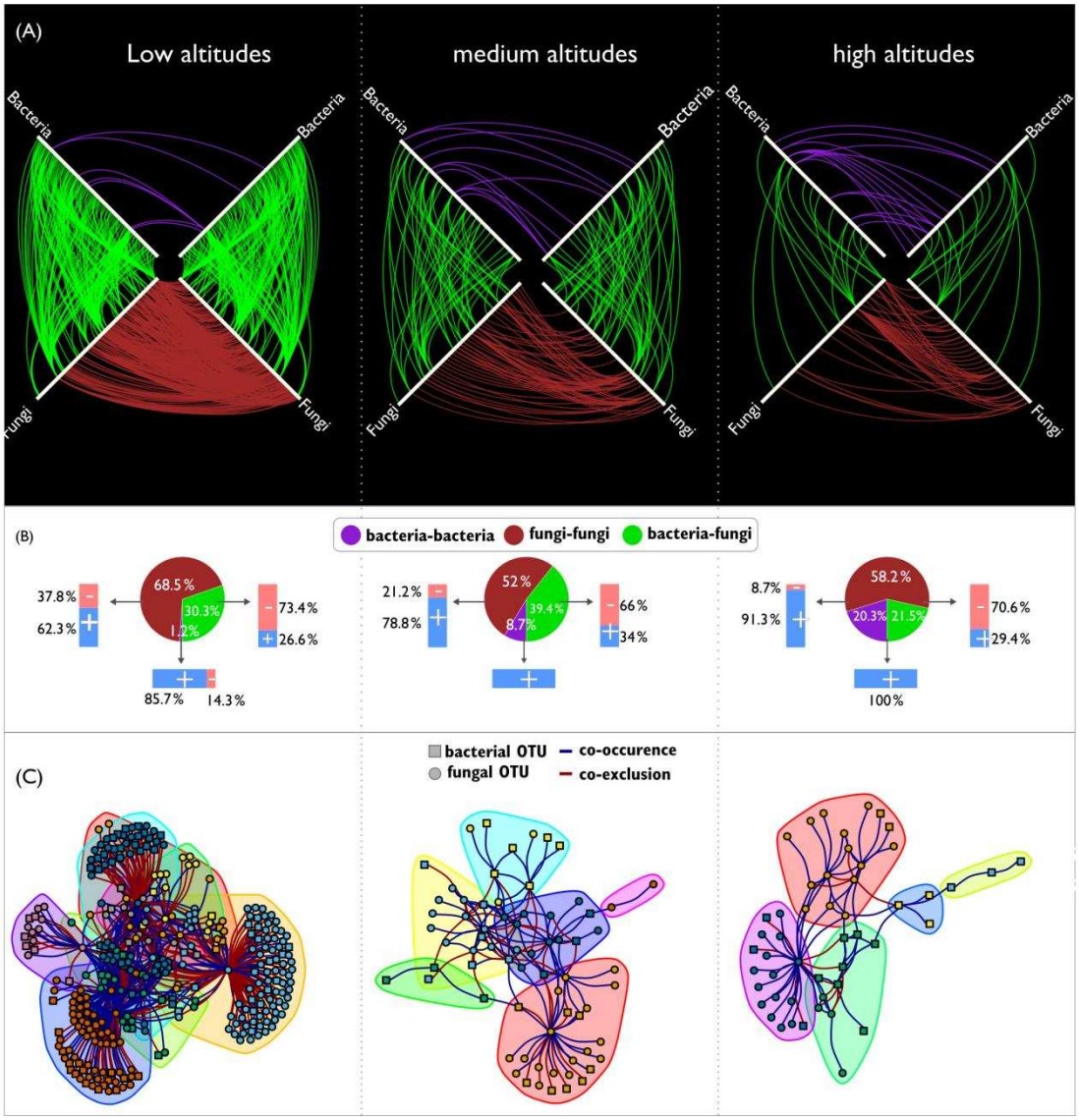


Figure 5: Co-occurrence/co-exclusion network of microbiome in fruits at three altitudinal levels. (A) Hive plot of co-occurrence/co-exclusion network of microbiome in fruits at three altitudinal zones: low altitudes (≤ 800 m), median altitudes (> 800 m and ≤ 1000 m), high altitudes (> 1000 m) based on significant SparCC correlations (p-value < 0.01 and absolute correlations > 0.7). Bacterial and fungal OTUs were displaced along each one of the white axes. Edges colored with blue, red and green represents, respectively, co-occurrence or co-exclusion of type bacteria-bacteria, fungi-fungi and bacteria-fungi. (B) Pie charts shows the percent of each type of interaction (i.e. bacteria-bacteria, fungi-fungi and bacteria-fungi) and bar plots the percentage of positive (co-occurrence) and negative (co-exclusion) connections of each type. (C) Microbial communities at each altitudinal zone. Each cluster in C represents a community detected by the function *make_clusters* function in the igraph package. Each node represents a 97 % identity operational taxonomy unit (OTU). Blue and red edges represent positive and negative cooccurrence of OTUs linked by them. Each node was labeled at the class level.

Discussion

This was the first study to evaluate in depth the influence of environmental factors on the microbiota inhabiting coffee fruits and soil; other studies have described the microbiota in coffee fruits or in soil, without correlating them[8,9,11]. Furthermore, other studies have focused on the microbiota during the fermentation of coffee[6,12,38], not in the microbiota present *in situ*.

The differences found in structure (Fig. 2) and diversity (Fig. 3) of microbiota in fruits and soil indicate that climatic and microclimatic alterations can directly influence the microbiota associated with coffee, as reported by other authors[39]. For example, the structure of the bacterial community can vary as a function of slope exposure, since topographic factors cause differences in the microclimate, especially in soil temperature, which correlates with the carbon and nitrogen content of the soil[40]. Additionally, the intensity of the light, the temperature and the electrical conductivity of the soil are influenced by the elevation of the mountain slope, cultivation system and/or an interaction of the two, and these factors affect the soil macrofauna in coffee plantations[41].

The richness and diversity (Fig. 3) observed here were higher than those in previous studies[9,11]. The condition of the environment, like the incidence of solar radiation, can lead to changes in the internal metabolites, creating a stress conditions and consequently different conditions that may affect the development of microorganisms[2], and the position and altitude of the fields were the main variables that influence coffee quality[42]. Here we did not find change in richness, evenness or diversity of bacteria in soil along the altitude (Fig. 3). However, we found an increase of bacterial diversity in fruits at high altitudes. The bacterial predominance has been observed in soil of organic coffee systems in India[43], and the relative bacterial abundance increased at higher altitudes, which was related to increasing levels of soil organic matter and nutrients with altitude[44]. The boost of this diversity at high altitudes might be due to the greater contribution of soil as a source of bacteria than the soil at low altitudes. Soil can act

as a source of bacteria for bacteria endophytes[45] and the functional prediction of these shared bacteria (Fig. 4) reveals that soil in high altitudes furnishes bacteria with functional properties that can contribute to the fermentation of fruits in the postharvest processing. Once coffee beans at high altitudes presents more mucilage/water ratio[46] and more fat content[47], the presence of an bacterial community with a high functional diversity can improve the processing of compounds by providing enzymes and compounds that can be useful for the fermentation process of coffee mucilage[46].

For the first time, the co-occurrence/co-exclusion network of microbiota in coffee fruits was investigated (Fig. 5). Most connections between fungi- fungi and bacteria-fungi were found in low altitudes while bacteria-bacteria connections were greater in high altitudes (Fig. 5). Inoculation of coffee at higher altitudes is not as effective as that performed at lower altitudes[2,48]. Since the fermentation process might be affected by the indigenous microbiota[49], the microbial networks connections in each altitudinal zone might affect the growth of the inoculum. Thus, the study of microbial ecology of coffee is essential to fully understand the production and conversion of beneficial metabolic precursors that provide high-quality brewed coffees and their unique flavors[11]. This information can be related to the sensorial quality of coffee from those trees growing at high altitudes, with an annual rainfall less than 1500 mm[47] and with open or medium shading[50].

Two OTUs closely related to *Colletotrichum kahawae*, which causes coffee berry disease, were not found in higher altitudes, although this disease be often in this condition[51]. Bacterial networks with high connectance can inhibit the attack of pathogens due to increase of niche overlap between indigenous microbiota and pathogen, which increases the resources competition[52]. The increase of bacteria-bacteria connections in higher altitudes might be due to reduction of the connections fungi-bacteria which the most were negative, so that fungi could be inhibiting bacteria to interact to each other (Fig. 5B).

These results allow us to understand the influence of the environment on both bacteriome and mycobiome. This understanding suggests that the final quality of coffee beverages might be determined by the microbial diversity/interaction, which is influenced by the environmental condition where the crops are being grown, which may affect the coffee beverage quality.

Competing Interests

The authors declare no conflict of interest.

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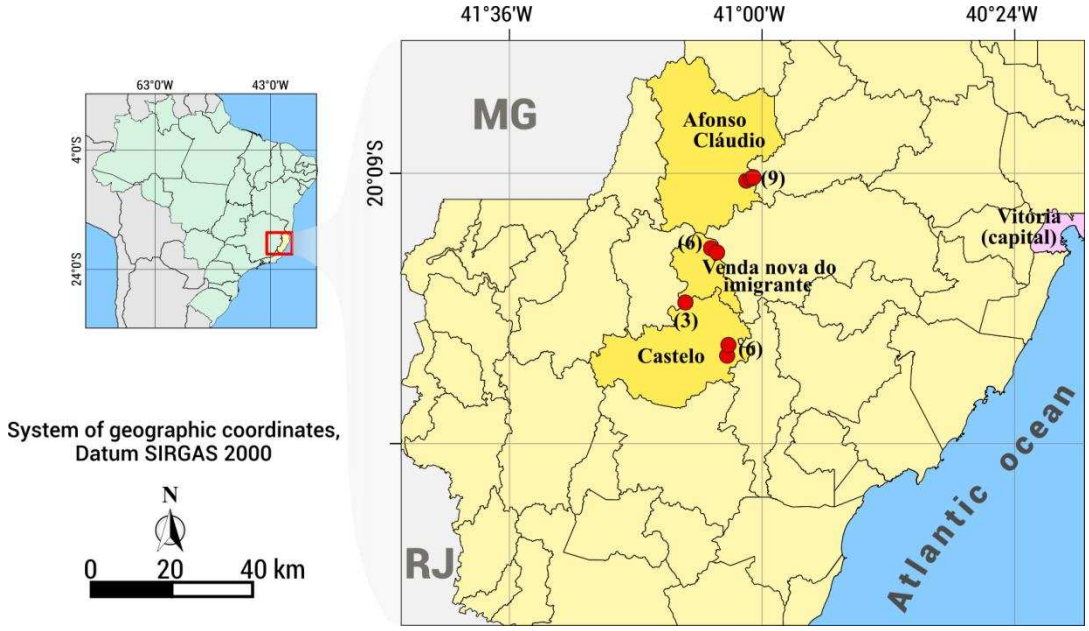
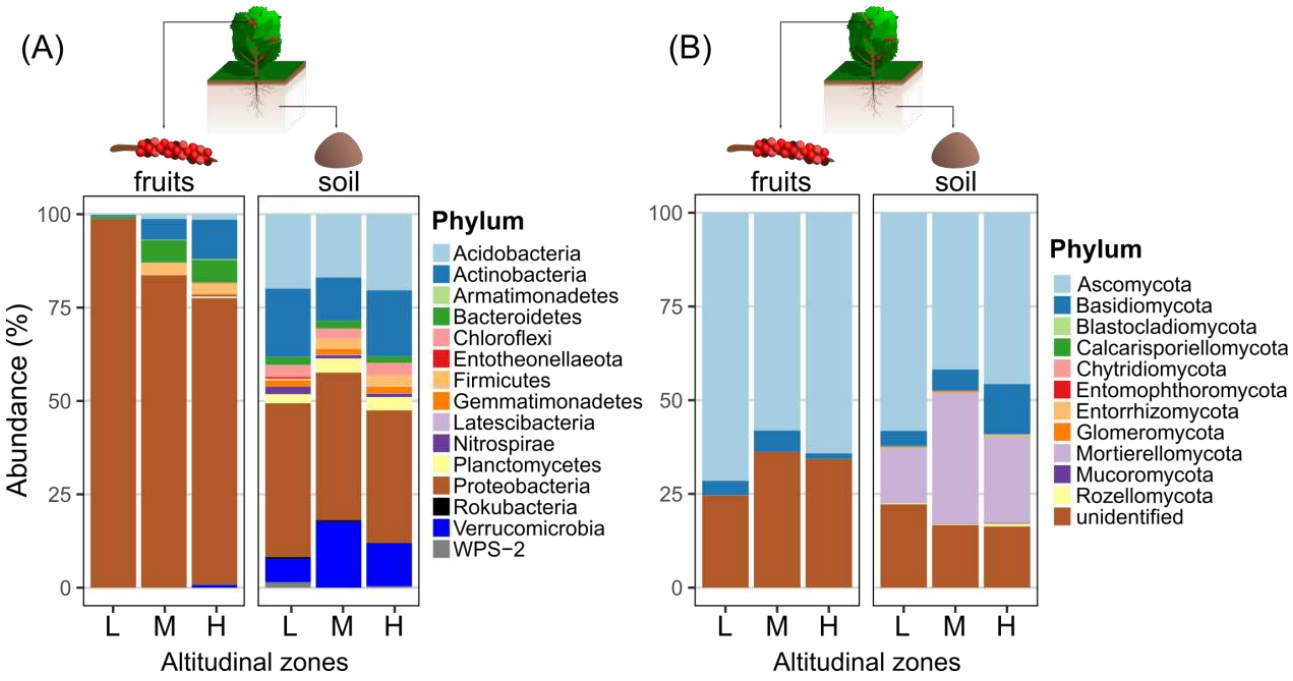
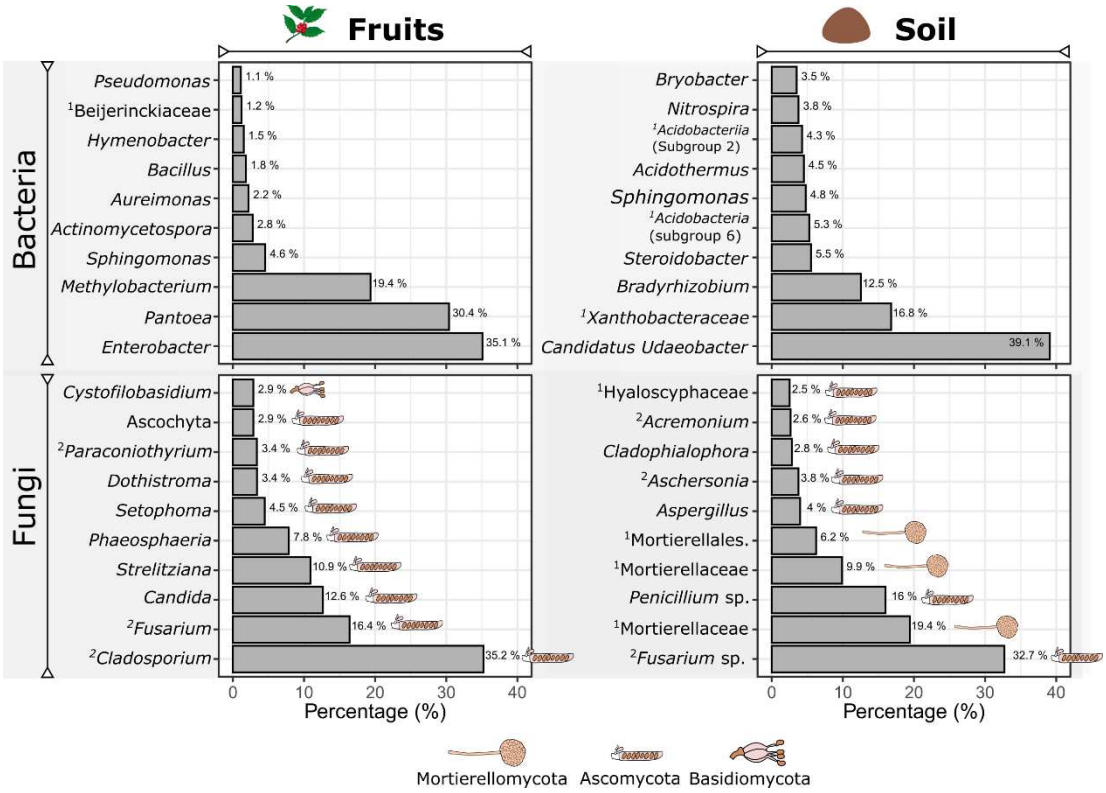
Supplementary material



Supplementary Figure S1: Sampling sites in the state of Espírito Santo, Brazil. A total of 24 samples were collected. *The number in parentheses show the number of samples in each region because some points overlap. RJ = Rio de Janeiro state. The map was generated by the software QGIS version 3.4.11 (https://qgis.org/en/site).



Supplementary Figure S2: Percentage of phyla of the (A) bacterial and (B) fungal OTUs found in the fruits and soil of coffee crops along a wide range of altitudes in Espírito Santo, Brazil. L = Low altitudes (≤ 800 m); M = Mediam altitudes (> 800 m and ≤ 1000 m); L = High altitudes (> 1000 m).



Supplementary Figure S3: Ten most frequent OTUs of the bacteria and fungi found in fruits and soil of coffee crops. [1] Identification was not performed at genus level due the absence of a suitable similar sequence in the databases (UNITE and GenBank).[2]Sequences annotated with

the GenBank database because the annotation with UNITE did not provide resolution at the genus level.

CAPÍTULO 2 : The microbiota playing “musical chairs” in a glacier retreat zone of Maritime Antarctica

The microbiota playing “musical chairs” in a glacier retreat zone of Maritime Antarctica

The microbiota playing “musical chairs” in a glacier retreat zone of Maritime Antarctica

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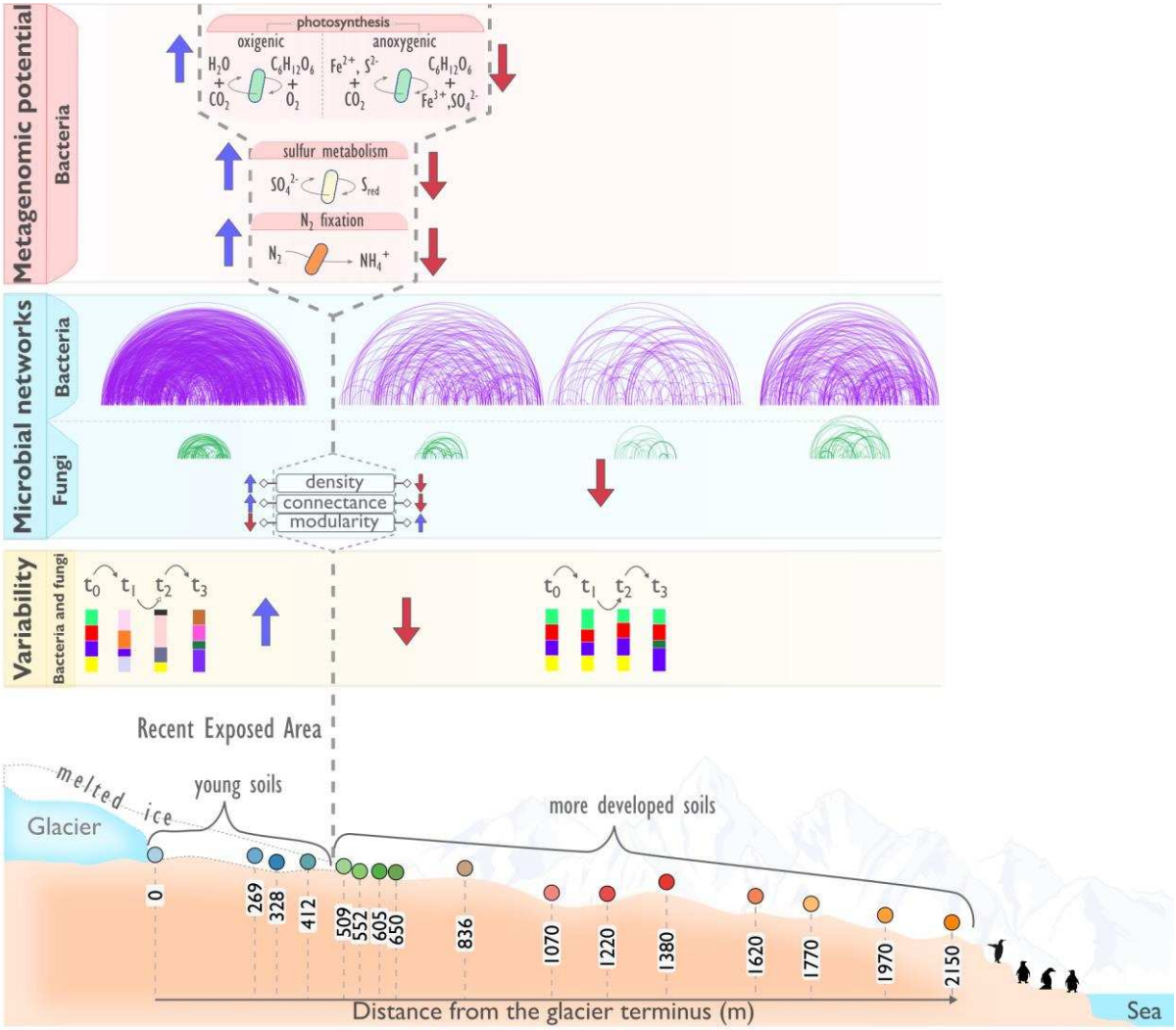
Conflit of interest

We have no conflict of interest to declare.

Graphical Abstract



Abstract

Most of the Antarctic continent and surrounding islands are permanently covered by ice. However, due to long-term natural and short term human-induced climate changes, glaciers in the maritime Antarctic islands are currently retreating, exposing new substrates for colonization by microorganisms and plants. Therefore, the aim of this study was to characterize the microbial communities along a transect comprised of soils with different exposition times since the glacier retreat in Barton Peninsula (King George Island). We found a intense degree of microbial succession in young soils located in the Recently exposed Area (REA), than in more developed soils farther away. The co-occurrence networks of both bacteria and fungi presented a less modular structure and a higher connectance in the REA, whereas a more modular structure was found in the three networks of the more developed soils. This suggests that these communities are more susceptible to external perturbations and microbial succession. Furthermore, the functional prediction demonstrated that the functional redundancy is lower in the REA than in more developed soils. The surprising high diversity of microbial communities adjacent to the glacier front deserves further studies to compare with different areas (in terms of substrate, climate) under a common present-day warming scenario.

Introduction

In the Antarctic continent the microbial growth rate is driven by extreme low temperatures, low water availability, nutrient scarcity, freeze-thaw cycles, low winter radiation and high UV radiation in the summer[1]. However, even in these conditions, many studies showed that the Antarctic harbors a high microbial diversity2[2,3]. In the last 15 years, with the discovery of new DNA sequencing techniques, microbial diversity has revealed to be an even greater than expected[3].

The majority of the Antarctic continent is permanently covered by glaciers, however, due to climate change a significant loss of ice occurs every year[4]. The retreat of the glacier exposes a new area called forefield (or foreland) for the process of pedogenesis. Microorganisms play a key role in this process by performing the primary colonization of this harsh environment due to their abilities of carbon and nitrogen fixations from the atmosphere and solubilization of minerals and rocks[5,6]. Previous research have tried to elucidate the structure and the ecological succession in recently exposed areas, but most only study one group [7–9] or a few groups of microorganisms[10,11]. Studies focusing the initial colonization in ice-free areas (forefields)[11,12], are still scarce compared with studies related primary plant colonization [13–17].

King George Island (KGI) is the largest island of the South Shetland Island archipelago and is covered by more than 90 % ice[18]. The Barton peninsula, which is located at the southwest of the KGI, has one of the largest ice-free areas and presents contrasting soil types, including basaltic, ornithogenic and thiomorphic[19]. The thiomorphic soils occur due to the presence of iron pyrite (FeS_2) which, which is oxidized to sulfates when exposed to oxygen. The iron sulfide compounds can act as source of electrons and energy to the microbial communities that inhabits these soils. Therefore, the role of the microbiota in the formation of these soils may be crucial, although it is still poorly investigated[20]. In addition, the south-western front of the Collins

glacier has undergone a retreat in the last decades[21], exposing new soils to microbial colonization.

As far as we know, no study has investigated the microbial succession of both bacteria and fungi in ice-free areas recently exposed by glacier retreat, espeacially those with thiomorphic soils. Our hypothesis is that the recent exposed area (REA) by the glacier retreat are mainly colonized by phototrophic and lithotrophic microorganisms due to the harsh edaphic conditions. The aim of this study was to investigate the profile of bacterial and fungal communities along a transect ranging from the REA with younger soils to more developed soils farther away from the glacier, in Barton Peninsula (KGI).

Material and methods

Study area and soil sampling

This study was conducted in the foreland of the Collins glacier in Barton Peninsula, King George Island, representing one of the largest ice-free areas in the King George Island. This area presents contrasting soil types, including basaltic, ornithogenic and thiomorphic. The latter occurs due to the presence of the mineral pyrite (FeS_2). It is also known that the south-western front of the Collins glacier at the Barton Peninsula (King George Island, Figure 1) has retreated at least 180 meters since 2005[21].

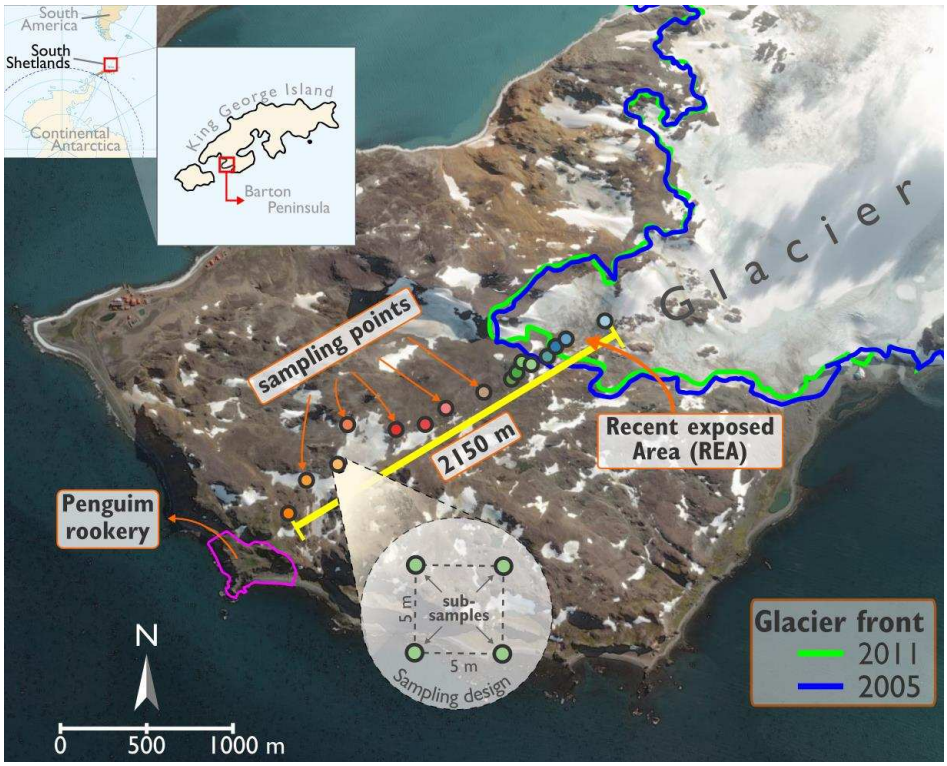


Figure 1: Study area in Barton peninsula. For each one of the 16 sampling points, four samples were collected.

The evaluation of the bacterial and fungal communities was carried out using 64 samples from 16 sampling points of active layers of soil (which overlays permafrost) along a transect line of 2,150 meters (see Figure 1). Before sampling, we hypothesized that the variance of the microbial community in the recent exposed area (REA) could be greater than in the area far from it. Therefore, in order to determine the microbial variability, we sampled the eight first points using a distance of approximately 30 meters between them, and for the remaining ones the distance between the sampling points was approximately 200 meters. The transect starts at the edge of the glacier and ends near to a penguin rookery. Within each sampling point we established a 5 m square and collected one soil sample from each vertex, totaling 64 soil samples (Figure 1). The samples were stored on ice and transported to the Laboratory of Mycorrhizal Association at the Universidade Federal de Viçosa, where they were stored at - 20 °C.

DNA extraction, PCR and sequencing

Eight grams of soil were used for DNA extraction. The intracellular DNA from living cells was separated from the extracellular DNA from dead cells to avoid an overestimation of diversity [22]. The cell lysis was performed in a Precellys 24 High-Powered Bead Mill Homogenizer (Bertin technologies) for 50 s at 4000 rpm. The following steps of DNA extraction were performed using Nucleospin Soil® (Machanarey-Nagel) according to the manufacturer's protocol. The quality of DNA extraction was evaluated by electrophoresis in 0.8 % agarose gel stained with Ethidium Bromide under UV light.

To access the bacterial and fungal communities, the sequences of the gene coding for the V4 region of 16S rRNA were amplified using the primers 515F (5'-GTGYCAGCMGCCGCGGTAA-3') [23] and 806R (5'-GGACTACNVGGGTWTCTAAT-3')[24]. The pair ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS2 (5'-GCTGCGTTCTTCATCGATGC-3') was used to amplify the fungal ITS1 region. The PCR libraries were quantified and pooled according to ref[25]. The sequencing of the 16S and ITS1 libraries were performed using the platforms Illumina MiSeq 2 × 151 bp and Illumina MiSeq 2 × 250 bp, respectively.

Analyses of soil

About 500 g of soil was used to perform all chemical analyses. The soil pH was measured using a soil:water ratio of 1:2.5. P, Na, K, Fe, Zn, Mn and Cu were extracted with the Mehlich-1, whereas Ca, Mg and Al were extracted using a 1 M KCl solution. The potential acidity (H + Al) was evaluated by performing an extraction with 0.5 M calcium acetate at pH 7.0. The B content were determined in hot water. The quantification of Sulfur content was carried out with an extraction using monocalcium phosphate in acetic acid. The quantification of soil organic matter was measured according to the method of Walkley-Black [26].

Bioinformatic analyses of bacterial V4 region

The raw reads were demultiplexed and trimmed to remove primers and adapters. All those with a maximum number of expected error (*maxee*) equal or greater than one, as well as those identified as chimeras or singletons were removed. The remaining sequences were clustered in Amplicon Sequences Variants (ASVs) using the Divisive Amplicon Denoising Algorithm (DADA2) [27]. Finally, the SILVA 138 database was used to annotate the ASVs. All the ASVs classified as organellar DNA (mitochondrial, chloroplast) were removed from the downstream analyses. All the steps described above were performed using QIIME2 2019.10b[28].

Bioinformatic analyses of fungal ITS1 region

Due to the variability in length, only the forward reads from the ITS region were analysed. After demultiplexing, the adapters and barcodes bounded to the 5' end of each primer were removed. The ITSx was used to remove the flanking regions 18S and 5.8S from fungal reads. All the remaining reads that were identified as singleton, chimera or had a low quality (maximum expected error equal or above 1) were also removed. The high quality reads obtained from the steps above were then clustered into ASVs using the DADA2 algorithm [27] and annotated using scikit-learn classifier and the UNITE database (version 18.11.2018)[29].

Statistical analyses

The Principal Coordinates Analysis (PCoA) based on Bray-Curtis distance matrices were calculated to evaluate the magnitude of change in the fungal and bacterial beta diversities along the transect. All alpha diversity, including the Chao1, Pielou's evenness, Simpson's diversity and Phylogenetic Diversity (PD) were calculated using vegan v.2.5.3[30] The correlation between the microbial community matrix and the chemical variables of soil was evaluated by canonical redundancy analysis (RDA) and the significance of each variable was evaluated using the function *ordistep* available in vegan v.2.5.3.

The possible interactions patterns of each fungal and bacterial community along the transect were determined by building four co-occurrence networks using significative SparCC correlations [31]. A total of 1000 permutations of the dataset were performed to estimate the p-values using *FastSpar*[32]. Only the correlations with absolute value greater than 0.7 and p-value < 0.01 were used to build the network. The R's package *igraph* was used to plot the network and to calculate the topological properties of each individual network.

In order to gain more information about the potential genes present in the bacterial community, we performed a phylogenetic investigation of the bacterial communities by reconstructing the unobserved states using PICRUSt 2.1.2[33]. All sequences with a Nearest Sequenced Taxon Index (NSTI) score above two, which indicates a low reliability prediction, were removed from the analysis before the metagenome prediction. From the predicted metagenome, we evaluated the abundance of genes found in each sampling point. We did not perform this analysis for fungi because of the low accurate predictions associated to the ITS1 marker[33]. To get insights into the amount of functional redundancy present in the REA when compared to more developed soils, we calculated the Functional Redundancy Index (FRI) using the Tax4Fun2[34] and used the log ratio of the mean values obtained in the soils of the REA and outside, i.e. log ($FRI_{insideREA}/FRI_{outsideREA}$).

Results

Alpha and Beta diversities

A total of 4,431,104 sequences of 16S and 2,714,968 of ITS were obtained. After filtering, denoising and removal of the chimeric sequences, 1,477,234 16S and 814,445 ITS sequences were retained. The sampling effort was measured by the Zhang-Huang's estimator (Figure S1), which showed that it was sufficient to carry on the downstream analysis. The sequences were clustered into 4791 and 997 ASVs of bacteria and fungi, respectively.

The difference in the microbial community diversity (beta diversity) was calculated as the Principal coordinate analyses (PCoA). These analyses showed clear dynamics of microbial communities: the further the soil is from the glacier edge; the more diverse its bacterial and fungal communities are from those close to the glacier (PCoA; Figure 2). Furthermore, the bacterial community of soils in the REA showed a greater variation of microbial community when compared to soils in later stages of development. This is demonstrated by the high variation of the CP_1 scores (see ΔCP_1 in Figure 2B). The bacterial community, and the centroids of CP_1 scores changed more than 0.4 in the first 509 m (less than 1/4 of the transect) and only 0.3 in the remaining distance. On the other hand, the fungal community showed lower variability among the samples collected in the REA (see ΔCP_1 in Figure 2C). However, a major change in community composition was observed between the samples collected at the beginning of the REA (412 m from the glacier edge) and those collected 509 m from the glacier edge (Figure 2C).

The richness (Chao1 estimator), evenness (Pielou's evenness), Simpson diversity index and phylogenetic diversity (PD) of the bacterial communities were higher than in fungal communities in all the sampling points (Figure 2B and 2C). A greater increase in bacterial richness, evenness, Simpson Diversity and Faith's PD was observed in the samples collected in the REA. After 509 meters, these indicators continued to be higher when compared to those close to the glacier snout. The furthest point (at 2150 m) from the glacier terminus, which is located close to ornithogenic soils of penguin rookeries, showed the highest diversity in the whole transect (Figure 2B). The fungal

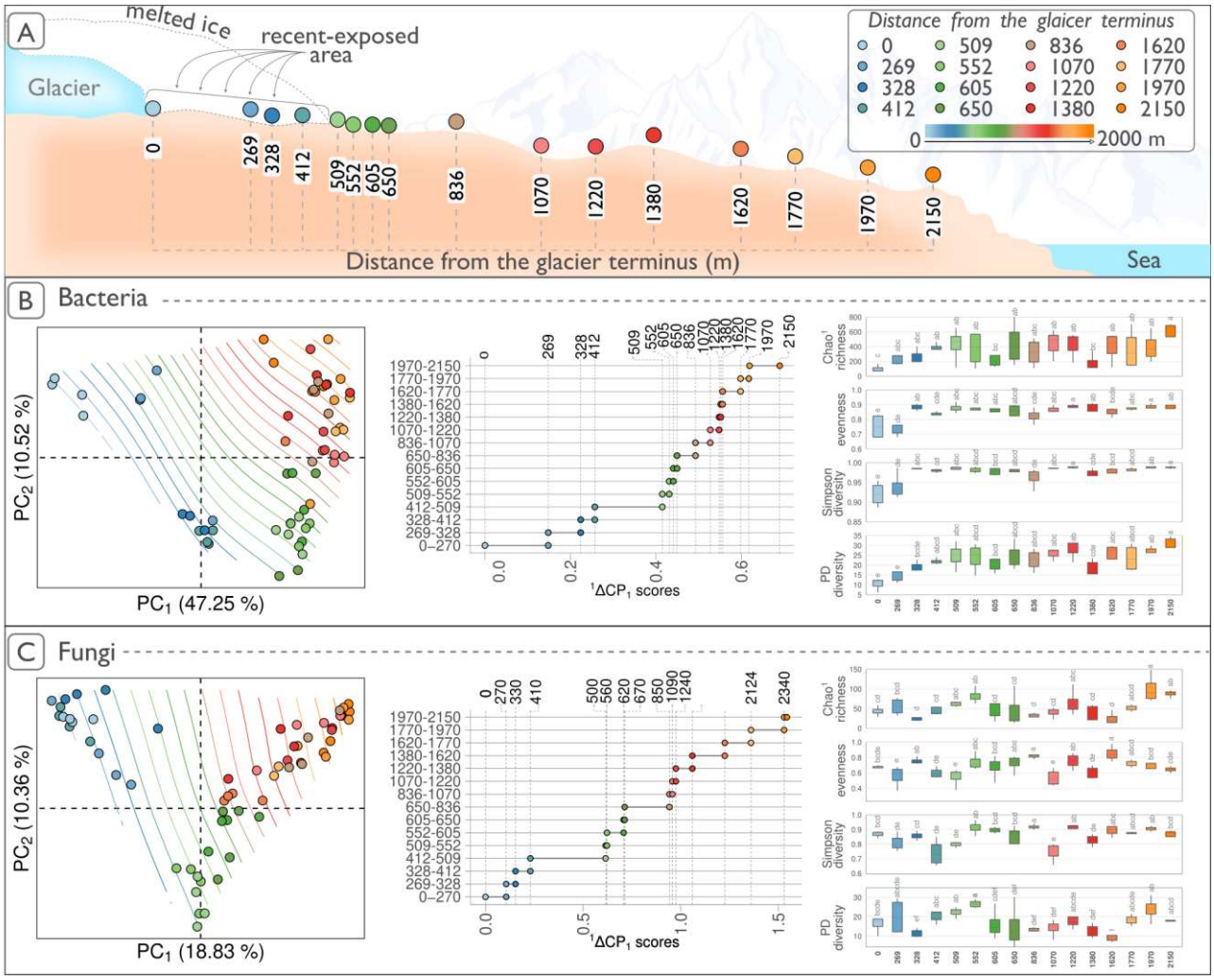


Figure 2: (A) Location of each sampling point in the transect. The samples were collected up to the foot of cap until areas close to the sea. (B, C) The left plots show a Principal Coordinate Analysis (PCoA) based on Bray-Curtis dissimilarity of the (B) bacterial and (C) fungal communities in each sample. The middle plots represent the variation in pairwise distances between the PC1 (Principal Component 1) and the centroids of adjacent sampling points. In summary, they show the magnitude of the microbiota changes from one sampling point to the next closest point. The right plots illustrate the estimate of richness (Chao1 index), evenness (Pielou) and diversity indices (Simpson and Phylogenetic Diversity). Each point represents the median levels of the alpha diversity measured in the four samples from the same sampling point. Contour lines show a smooth general additive model surface reflecting the turbidity gradient among the samples.

community showed an unclear pattern of these indicators along the whole transect. However, the two furthest points located close to ornithogenic soils were the only sampling points that showed values of richness above 80.

Edaphic factors and microbial community

The soils near the glacier terminus presented high contents of Ca, Fe, Mn, S and Zn and very low amounts of organic matter (OM) (Figure S2). In general, the pH was close to neutrality in almost all the sampling points. Redundant analysis (RDA) indicated that the sampling points close to the glacier terminus presented a high content of exchangeable bases (Mg, K, Ca, Fe, Zn, Mg), as well as Sulfur (Figure 3). The five edaphic variables that most influenced the bacterial community were the distance from glacier terminus and the contents of Zn, Mg, K and Fe, while for fungi distance and organic matter content, Mg, P and S were the most influencing variables.

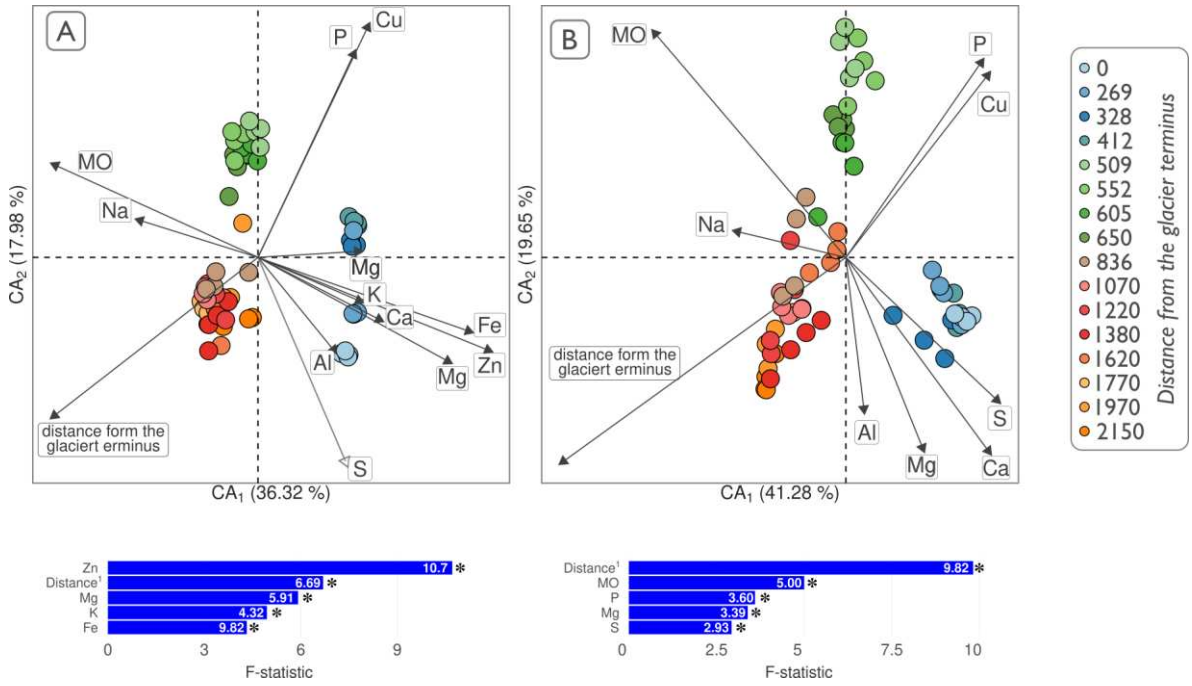


Figure 3: Redundancy analysis of (A) bacterial and (B) fungal communities along different distances from the glacier edge. Only the two first dimensions and the significant environmental variables were plotted. Values in parenthesis indicate the percent of explanation of each axis. Bar charts show the five most important environmental variables that shaped the bacterial and fungal communities. The values within the bars are the F values from the ordistep procedure in vegan.

[1] Distance from the edge of the glacier.

* Significant at 0.01 by F-test using ordistep function in vegan.

Community composition

The 4791 bacterial ASVs were distributed in 33 phyla, 102 orders, 270 classes, 456 families and 686 genera. The most abundant were Proteobacteria and Acidobacteria, followed by Bacteroidetes, Actinobacteria and Gemmatimonadetes (Figure 4). Some bacteria were detected in a low percentage, such as Nitrospireae, Rokubacteria, Elusimicrobia and Fusobacteria. Bacteroidetes and Proteobacteria combined represented more than 50 % of the bacterial community in the area close to the glacier. The relative abundance of Bacteroidetes, Proteobacteria, Firmicutes and Actinobacteria decreased along the chronosequence. An opposite pattern was found for Chloroflexi, Acidobacteria, Verrucomicrobia and Nitrospireae. No Nitrospireae, Dependentiae or Rokubacteria were detected in the REA. The phyla Rokubacteria, FCPU426, Latescibacteria, Omnitrophicaeota, Nitrospirae, Thaumarchaeota, Dependentiae, WS2, Kiritimatiellaeota and BRC1 were not present in the REA. Caldiserica and Epsilonbacteraeota were found exclusively in the REA, however ,they represented a very small fraction of the bacterial community (below 2 %). Among genera, *Rhodoferax* (Spearman's rho = -0.58, $P < 0.001$), *Ferruginibacter* (Spearman's rho = -0.60, $P < 0.001$), *Polaromonas* (Spearman's rho = -0.39 $P < 0.01$), *Flavobacterium* (Spearman's rho = -0.27, $P < 0.05$), *Pedobacter* (Spearman's rho = -0.61, $P < 0.001$), *Hymenobacter* (Spearman's rho = -0.40, $P < 0.01$) and *Brevundimonas* (Spearman's rho = -0.30, $P < 0.05$) were more abundant close to the glacier (Figure 5A).

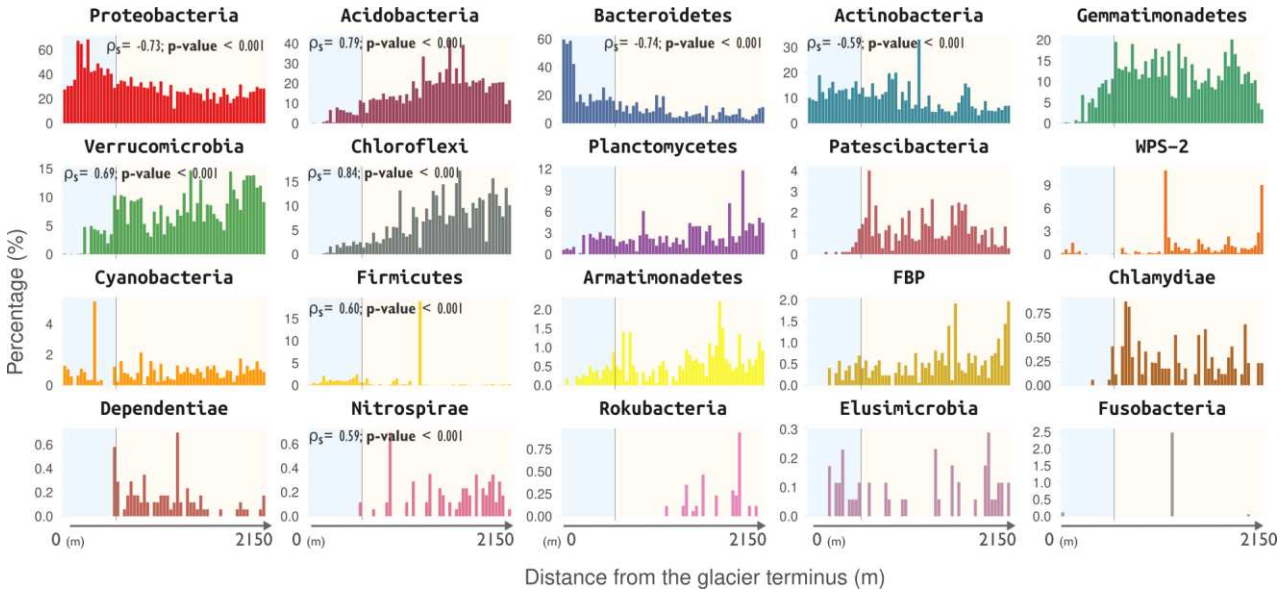


Figure 4: (A) Percentage of each bacterial phylum in the soils sampled at different distances from the glacier front. The plot area highlighted in blue represents the recent exposed area (REA) after the glacier retreat. The plots are ordered from the most abundant taxa in all samples (Proteobacteria, Acidobacteria, Bacteroidetes, etc) to the lowest abundant (Rokubacteria, Elusimicrobia, Fusobacteria). The Spearman's rank correlation coefficient (ρs) is showed in the plots of all phyla that displayed a statistically significant linear relationship between its relative abundance and the distance from the glacier terminus. Only phyla with abundance of two or more are shown.

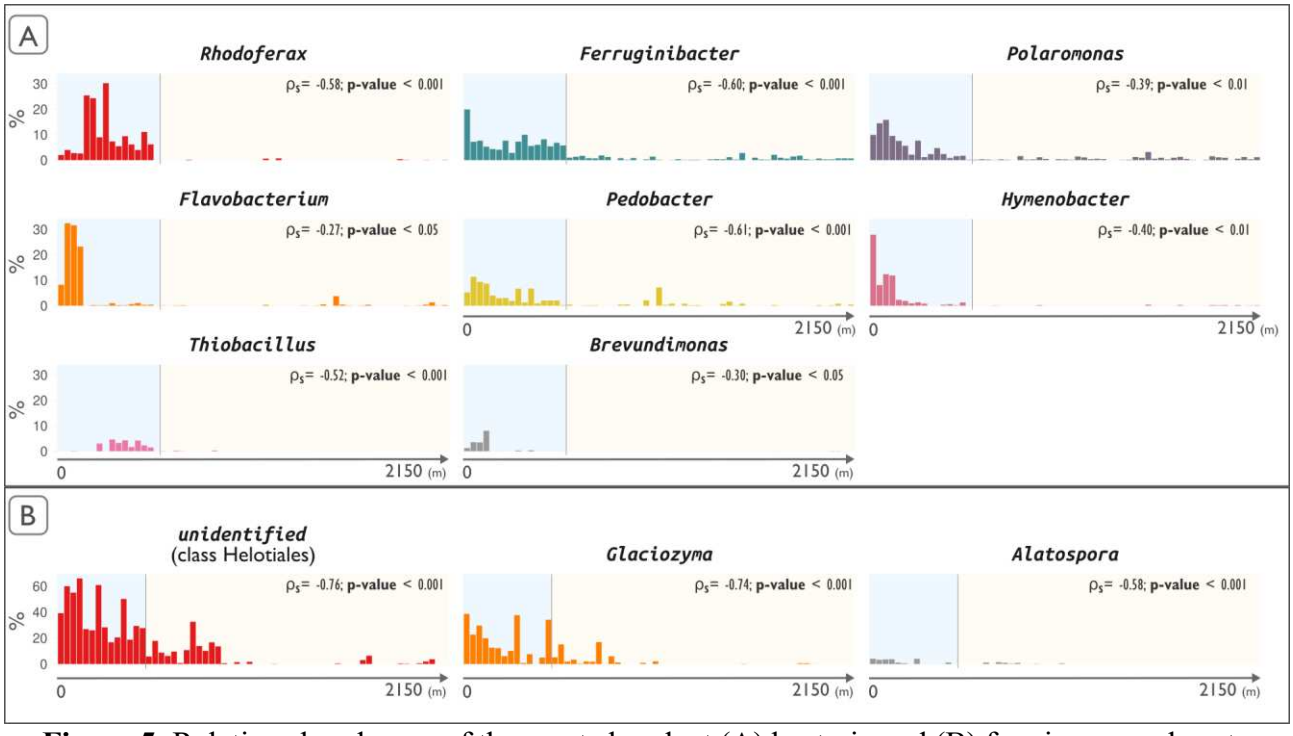


Figure 5: Relative abundances of the most abundant (A) bacteria and (B) fungi genera close to the glacier terminus. Blue area represents the recent exposed area (REA) after the glacier retreat. ρs= Spearman's rank correlation coefficient.

Regarding the fungal community, the 997 ASVs were assigned to eight phyla, 21 orders, 59 classes, 109 families and 164 genera. More than 70 % of the sequences were assigned to phyla Leotiomycetes (Figure 5), Mortierellomycetes and Tremellomycetes. The relative abundance of Microbotryomycetes, and Leotiomycetes decreased along the chronosequence., whereas Mortierellomycetes and Tremellomycetes increased. The four classes: Basidiobolomycetes, Taphrinomycetes, Arthoniomycetes and an unidentified one belonging to the Chytridiomycota phylum were not found in samples collected in the REA. The genera Glacyozima, Alatospora and an unidentified Helotiales displayed a significative increase close to the glacier (Figure 6).

Co-occurrence networks of bacterial and fungal communities

In general, the bacterial networks were larger than the fungal networks along the transect (Figure 7). The complexity of the bacterial network peaked in the REA, where the highest number of edges (1190) were present, totaling four times higher when compared to the second largest bacterial network. The highest percentage of the bacterial community connected in the network where 79.4 %

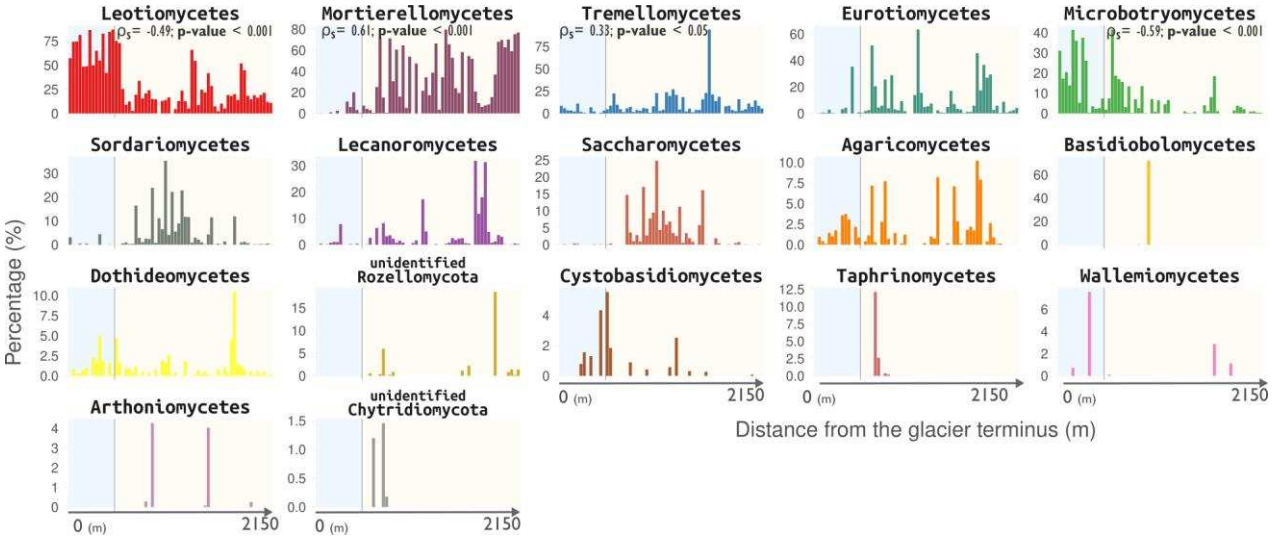


Figure 6: (A) Percentage of each fungal class along soils sampled at different distances from the glacier terminus. Plot area highlighted in blue represents the recent exposed area (REA) after the glacier retreat. The plots are ordered from the most abundant class in all samples (Leotiomycetes, Mortierellomycetes, etc) to the lowest (Arthoniomycetes, unidentified Chytridiomycota). Rozellomycota and Chytridiomycota have no close-related class in SILVA

database, therefore we identified them by the Phylum name. The Spearman's rank correlation coefficient (ρs) is showed in the plots of all phyla that displayed a statistically significant linear relationship between its relative abundance and the distance from the glacier terminus. Only phyla with abundance of two or more are shown.

of all the ASVs present in the REA showed at least one significative connection with another ASV. We also found the lowest modularity (i.e. the tendency of a network to contain sets of nodes in sub-clusters) in the REA in comparison with more modular networks found in the remaining areas. Similar to bacteria, the topological properties of the fungal networks showed that the two greater fungal networks were those close to the glacier terminus and penguin rookeries (Figure 7).

Prediction of the functional potential from 16S sequences

In order to evaluate the functional pathways present in the samples, we predicted the functional profiles of the microbial community using PICRUSt2 (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States). The values of NTSI (Nearest sequence taxon index) ranged from 0.02 to 0.18 (mean = 0.11, sd = 0.041) (Figure S3). Generally, NSTI values below 0.06 and above 0.15 can indicate low and high qualities of prediction, respectively, therefore, the results must be interpreted with caution.

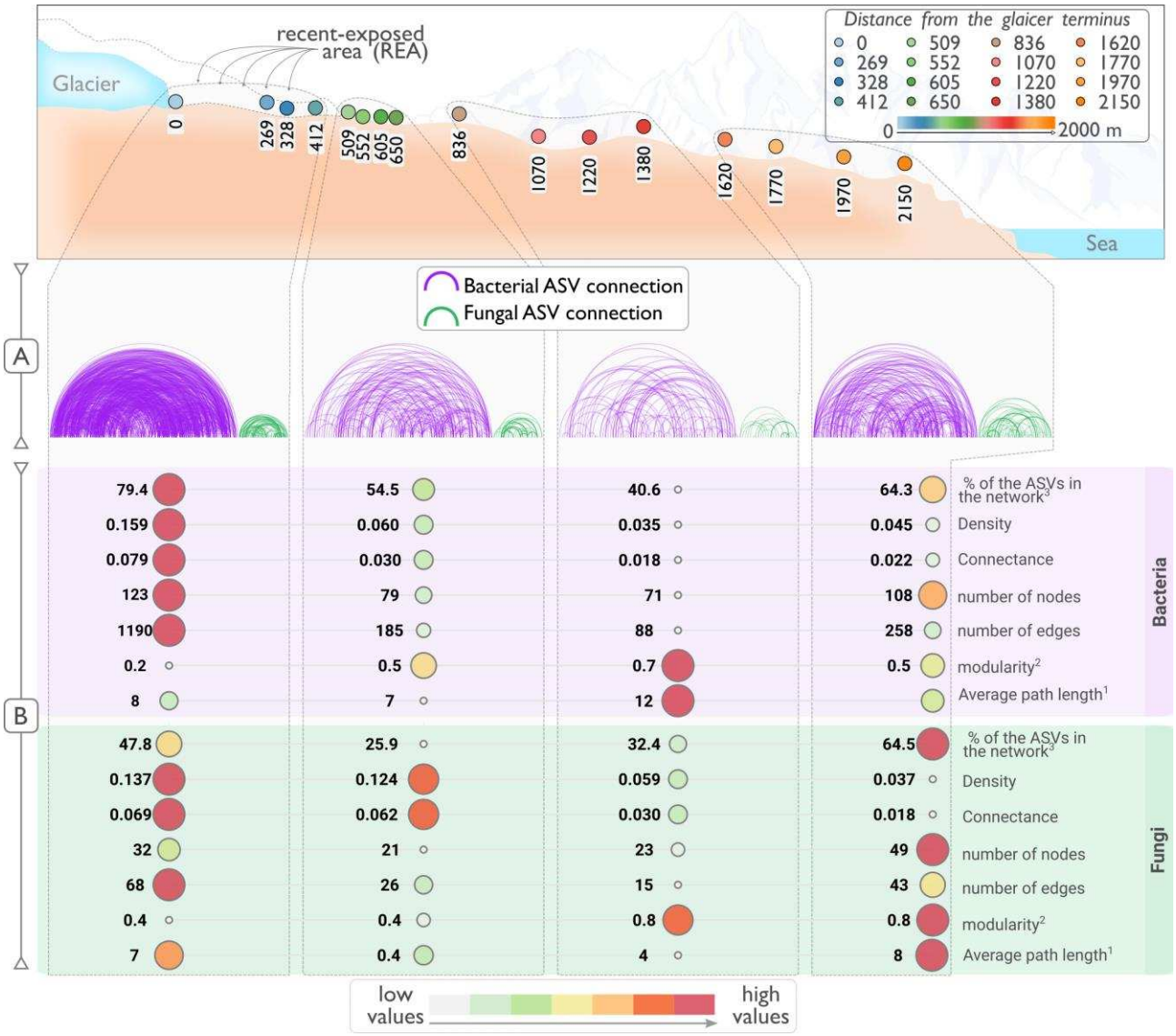


Figure 7: Co-occurrence/co-exclusion patterns of bacterial (purple arcs) and fungal (green arcs) along different distances of the glacier front. (A) Arc diagram of bacterial (purple arcs) and fungal (green arcs) along different distances from the glacier. Only significant SparCC correlations (p-value < 0.01 and absolute correlations > 0.7) are shown. Bacterial and fungal OTUs were displaced along each one of the purple and green axes. (B) Network properties were calculated using igraph package 1.2.4.2 package in R 3.5.3.

[1] Calculated with the centr_degree function in the igraph package.

[2] Calculated with the modularity function in the igraph package.

[3] Each community corresponds a group of nodes that are more densely connected among them than the rest of network.

[4] Percentage of ASVs with significative co-occurrence or co-exclusion related to all ASVs in the community.

The REA had the highest abundance of predicted genes relevant to sulfur metabolism, including sulfate reduction (assimilatory or dissimilatory) and thiosulfate oxidation. (Figure 8). The soils of this area also showed a higher abundance of predicted genes related to

photosynthesis activity (oxygenic and anoxygenic) than in the areas far from the glacier terminus. This potential of anoxygenic photosynthesis was divided among three groups of bacteria: green sulfur (KEGG module M00614), green nonsulfur (KEGG module M00613) and purple bacteria (KEGG module M00612). In addition, nitrogen fixation (KEGG module M00175) and denitrification (KEGG module M00529) were more pronounced in areas close to the glacier terminus (until 650 meters from it).

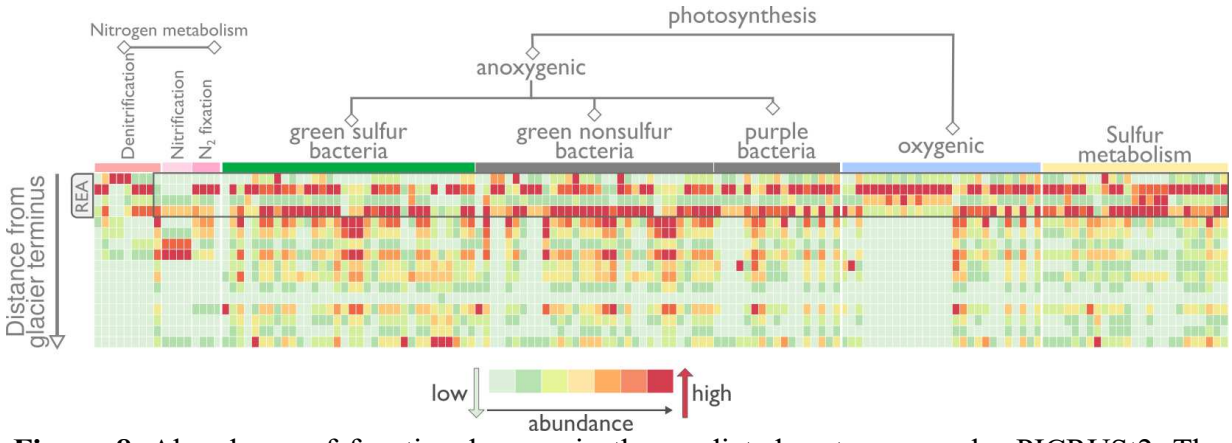


Figure 8: Abundance of functional genes in the predicted metagenome by PICRUSt2. The colour intensity of each cell represents the abundance of each gene. The distance along the transect is represented in rows while each column represents a gene related to its respectively metabolism (sulfur metabolism, oxygenic photosynthesis, etc.).

Discussion

This study evaluated the fungal and bacterial communities of soil along a transect of more than 2 km, departing from areas adjacent to the glacier terminus down to coastal areas farther away, at Barton Peninsula (KGI). Although other studies on the bacterial community have been conducted in this area[35], our study is the first one to evaluate both bacterial and fungal communities using the next generation sequencing techniques in part of Antarctica.

The highest variability in both bacterial and fungal communities were found in younger soils (within the REA) when compared to the more developed ones, more distant. More than half (60 %) of the total variance in the first component of the PCoA ordination of bacterial community occurred in the first 509 meters of the transect (Figure 2A), suggesting an intense

microbial succession in young soils. The same pattern was observed for the fungal community, but with a smaller proportion (40 %). Similar to the musical chair game, where an individual that do not fit to a specific place (chair) are eliminated, in the present microbial community the specific places are the young and organic matter poor soils.

Our study agrees with others when they reported that the greatest fluctuations in the microbial community occurred in soils close to the glacier edge[8,12,36]. This is reported even in studies that evaluate the change in microbial communities in soil with more than 50 thousand years[37,38].

In general, the number of bacteria detected in this study was lower than in others[11,35,36]. A previous study in this same area found a diversity of more than 24.000 OTUs[35]. However, besides the differences in the 16S region and the sequencing technologies used, this can be explained by the DNA extraction method applied, which allows the recovery of DNA only from the intact cells. Our approach avoids the overestimate of diversity due to the presence of extracellular DNA from the dead cells[39] that can remain in soil for many years. The diversity of bacteria was higher than the fungi in all the sampled areas, which is consistent with other studies on glacier retreat zones[36,40]. The steeper increase in the bacterial phylogenetic diversity (Figure 2B) may be related to the low levels of organic carbon in the young soils of REA, which leads to the dominance of some specialized bacterial groups, mainly lithotrophic bacteria. This bacterial group use reduced inorganic compounds as a source of electrons and energy and can fix carbon and nitrogen from the atmosphere.

The main bacteria found in the REA were Bacteroidetes and Proteobacteria[41]. The class AD3 (phylum Chloroflexi) is considered a primary producer in Antarctic soils[42] because of its ability to produce enzymes to fix carbon dioxide, for example ribulose-1,5-bisphosphate carboxylase (RuBisCO). Although this class is present at higher abundance in more developed soils, it was not detected in the young soils, suggesting that other microorganisms are

responsible for primary production in the REA. Moreover, the presence of pyrite (FeS_2) in this area can contribute to the development of primary producers, which uses sulfides as electron donors such as *Thiobacillus*[43], and the high levels of cations can favour the growth of *Rhodoferax*[44]. This psychrotolerant genus was the most abundant genus found close to the glacier edge, which exhibits high metabolic flexibility. Some members can perform photosynthesis [44], while others can grow facultatively using acetate as the sole electron donor and Fe(II) as the unique terminal electron aceptor [45]. Genera such as *Polaromonas* (Proteobacteria) and Ferruginibacter (Bacteroidetes) are widespread in many glaciers around the world, but the role of these bacteria in the biogeochemical cycle needs more investigation. These genera probably come from the glacier runoff [46].

The main environmental variables shaping fungal communities were the distance from the glaicer edge and the content of organic matter (Figure 3). Both factors are correlated because soils located adjacent to the glacier are younger than those at longer distances, which leads to an increase in organic matter content with soil age (Figure S2). The organic matter is crucial for the development of fungi because this group of microorganisms depend on a reduced carbon source fixed by other primary producers[47]. The most abundant fungi in the REA belong to the order Helotiales (Class Leotiomycetes), which comprised of more than 50 % of the community in some samples. Due to the lack of a close sequence in the database, it was only possible to be identified these sequences until the class level. This shows the importance of isolation studies in this soil, in order to elucidate the role of this unknown fungus in the environment and also to update online databases allowing comparative studies. On the other hand, although recently described[48], the second most frequent genus close to the glacier (*Glaciozyma*) has already four species described and one sequenced genome[49]. This fungus has the potential to develop genetic responses to temperatures lower than -10 ºC[50].

Although the alpha bacterial phylogenetic diversity (Figure 2B) is lower in areas close to the glacier, the amount of predicted connections between the members of the community is the highest one, showing 1190 connections (Figure 7). The bacterial and fungal networks in the REA showed the smallest modularity scores and the highest connectance and densities. These networks tend to have nodes with a high number of connections, so that the perturbation in these nodes can provide a great impact in the microbial network[51,52]. *Thiobacillus*, for example, presented 45 connections with other ASVs. A perturbation to this node can lead to serious impact to the structure of the network because it has the potential to affect all the ASVs connected to *Thiobacillus*., The microbial communities in more developed soils exhibited more modular networks, with lower connectance and density. This indicates that the microbial community in the REA may be more susceptible to changes in the community structure, as shown by the PCoA (Figure 2B).

When compared to more developed soils, the young soils close to the glacier terminus presented a bacterial community with greater metagenomic potential for genes involved in oxygenic and anoxygenic photosynthesis, sulfur metabolism and N_2 fixation (Figure 8). Although this study did not evaluate the eukaryotic algae [36,53], the results of the functional prediction reveals that, at least in part, the bacteria increases input of carbon by photosynthesis in these areas. The presence of genes involved in anoxygenic photosynthesis enables the use of reduced compounds such as sulfides, and Fe(II) as electron donors, which can be derived from the pyrite. In more developed soils, a decrease in the number of genes associated with photosynthesis (Figure 7) takes place. This may be related to the presence of plants, which can act as the main source of carbon fixation.

The alpha diversity metrics showed that the lowest bacterial phylogenetic diversity (PD) present in the young soils of REA was lower than the more developed soil from this area (Figures 2 and S4). At the same time, the functional redundancy of the bacterial community in

the REA was smaller than outside of it. This suggests that the community of the more developed soils (outside the REA) hosts bacterial communities with more functionally redundant members, which may provide more stability to the community in the face of perturbations. This observation corroborates with the changes observed in the beta diversity (Figure 2B), where the greatest changes observed were in the soils located in the REA.

We cannot confirm the source of microorganisms colonizing the REA, as the result is highly differentiated microbial composition when compared to more developed soils (Figure 2A). However, the REA microbiome may represent a mixture of ancient microorganisms, which were already present before the glacier advance, and microorganisms from allochthonous sources, transported by wind and ice melting from the glacier surface upslope, as shown by previous studies[11,54]. This area seems to be mainly composed of photosynthetic and lithotrophic bacteria, which are able to colonize young soils that have a low content of organic matter and a high availability of inorganic compounds.

Conclusions

The bacterial diversity and functional redundancy in young soils are smaller than developed soils. Therefore, the microbial communities inhabiting recent deglaciated areas are more susceptible to external disturbances and display a greater microbial succession than those living in older. Moreover, in REA, the bacterial community displays a high potential to perform lithotroph and oxygenic and axonygenic photosyntesis, which is crucial due to the low availability of organic matter in younger soils of recent ice-free areas. However, many bacterial and fungal taxa found did not have their physiologic traits described. So, future studies are need for elucidate the role of these microorganisms in recent deglaciated zones of Antarctica, under increasing warming scenario.

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Supplementary material

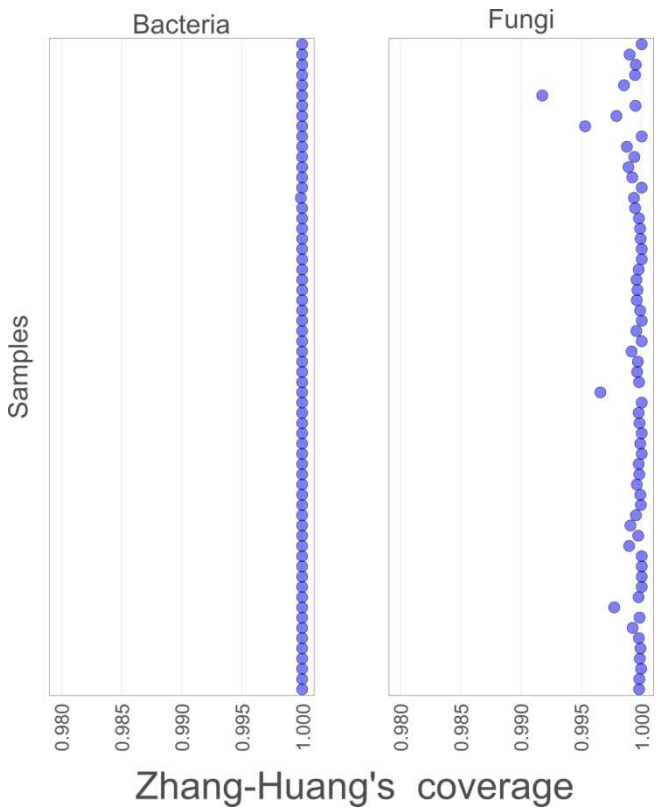

Figure S1: Sampling effort measured by the Zhang-Huang's coverage. This index considers how many doubletons (i.e. reads appear only twice) in a sample in comparison to the total number or reads in the same sample. High values (i.e. close to one) reflects enough sampling effort.

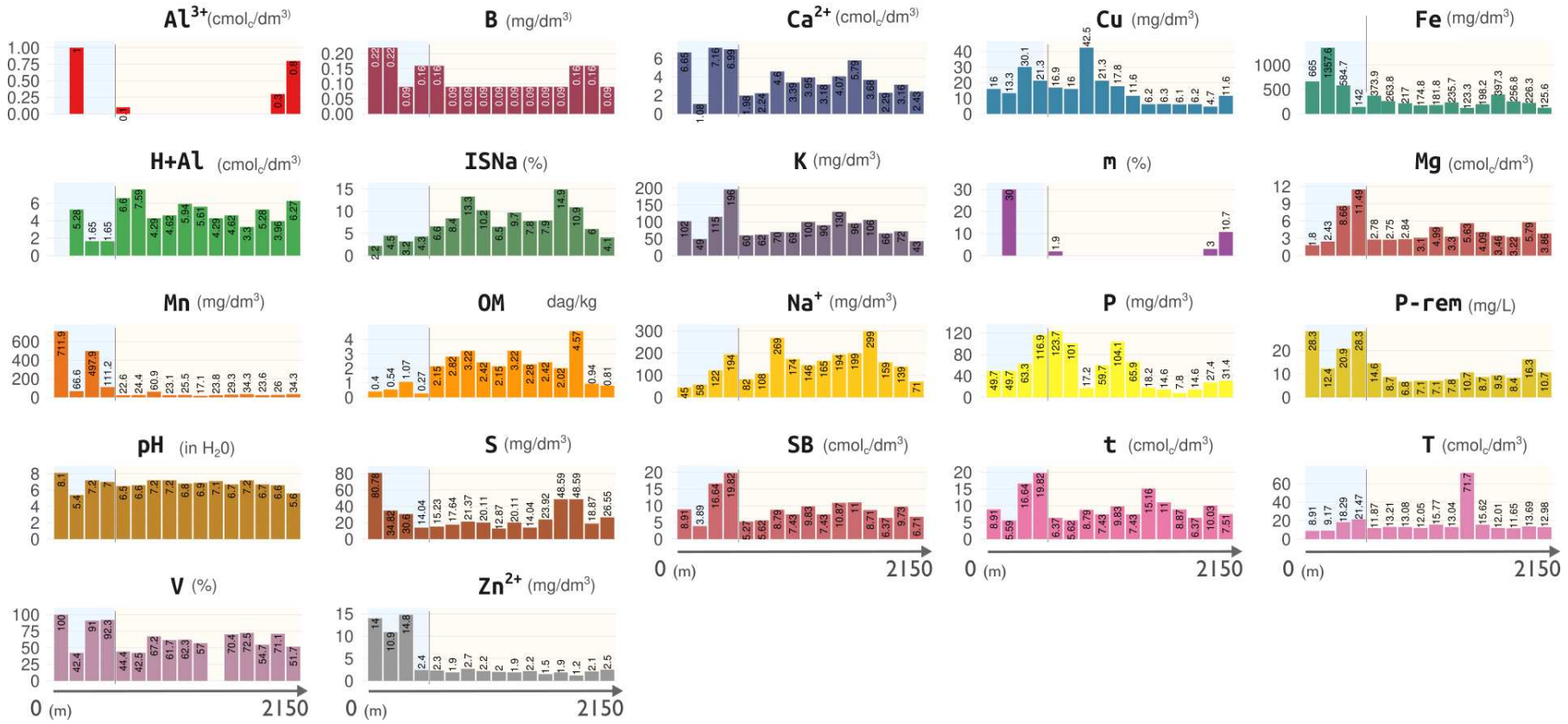

Figure S2: Chemical properties of soils along a chronosequence of different times of exposition after glacier retreat. Values inside or above the bars indicate the original value. ISNa = sodium saturation index; m = aluminum saturation; P-rem = remaining P; SB = sum of bases; t = effective cation-exchange capacity; T = potential cation-exchange capacity at pH 7.0.

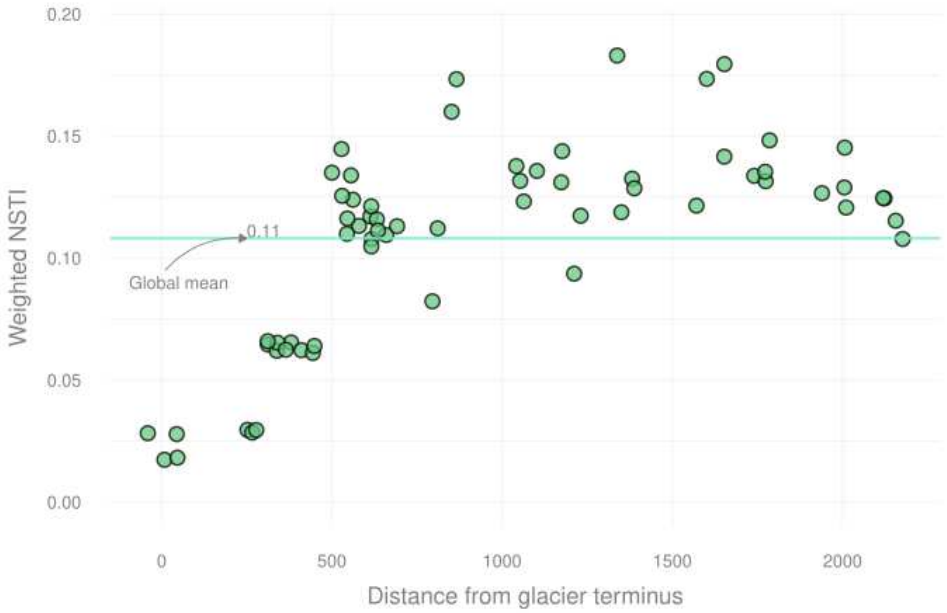

Figure S3: Weighted NSTI (Nearest Sequence Taxon Index) values for each sample. This index summarizes how distant are the ASVs found in this study to their closest related sequenced genomes in the database during functional prediction. Low values indicate good predictions.

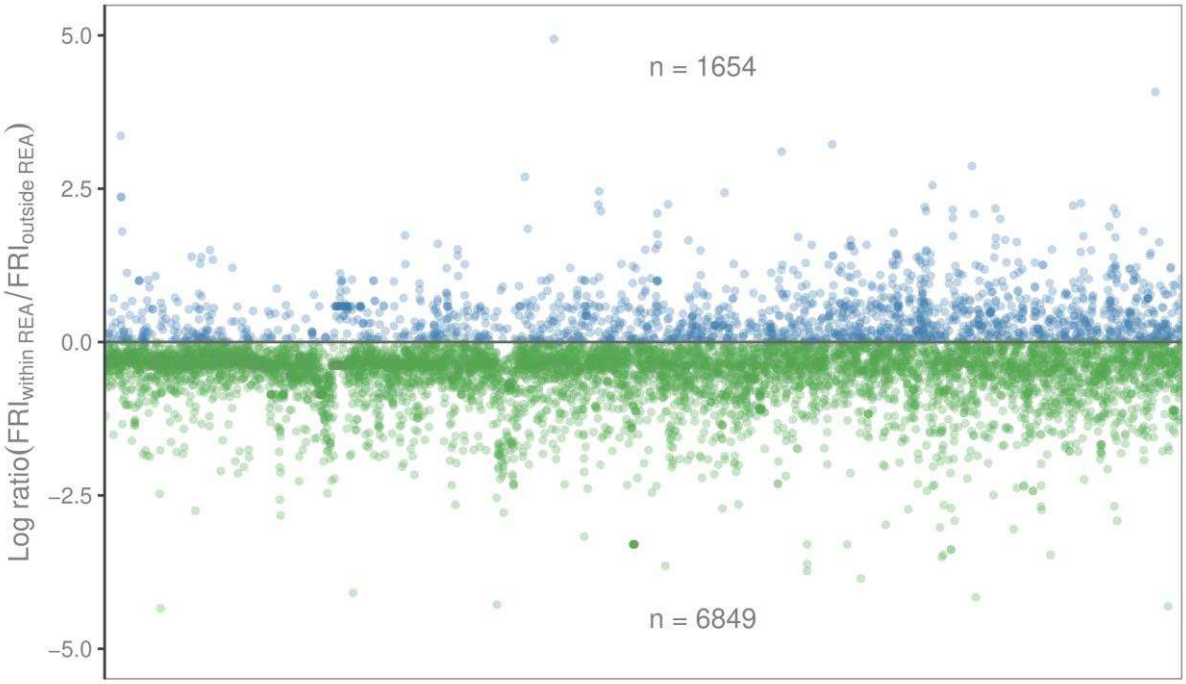

Figure S4: Comparison of the functional redundancy present in the community within and outside the REA. Points with log ratio greater than 0 (blue) represents a function more redundant in the REA. These predictions were calculated using TaxFun2.

CAPÍTULO 3: Bacterial and fungal diversities in actual and abandoned ornithogenic soils from Maritime Antarctic

Bacterial and fungal diversities in actual and abandoned ornithogenic soils from Maritime Antarctic

Bacterial and fungal diversities in actual and abandoned ornithogenic soils from Maritime Antarctic

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Conflit of interest

We have no conflict of interest to declare.

Abstract

Ornithogenic soils in coastal areas are the main source of organic matter in Antartic soils. These soils are formed by the activity of penguins during their nesting period. The less harsh conditions of Maritime Antartic enables a great development of these soils compared to the soils at high latitudes. The Narebski Point, located at the southest of Barton Peninsula (King George Island), is a breeding site for two penguin species: gentoo (*Pygoscelis papua*) and chinstrap (*Pygoscelis adelie*), and present actual and abandoned penguin rookeries due to the glacio-isostatic uplift, being an ideal place to study the microbiota in ornithogenic soils. Therefore, the aim of this study was to characterize the profiles bacterial and fungal communities inhabiting actual and abandoned ornithogenic soils. The results of our study show that evenness and Simpson diversity of the fungi is reduced in active ornithogenic sites, whereas bacterial did not change among evaluated soils. On another hand, the beta diversity of both bacteria and fungi are widely different. Functional prediction analysis revealed that the abandoned ornithogenic sites are rich in chitinolygic bacteria, belonging mainly to the family Chitinophagaceae and genus *Granuciella*, which can metabolize chitin present in guano. Furthermore, these soils have also a higher microbial potential to act in the nitrogen and sulfur cycles than actual ornithogenic soils.

Introdução

The Antartic continent display one of the harshest conditions for plant and microbial growth due to extreme climate conditions (Cary et al. 2010). As a result of that, the input of organic matter in the soils of ice-free areas are limited. In constrast, ornithogenic soils in coastal areas are rich in organic matter due the deposit a colossal amount of guano by penguins from September to February, during the breeding season (Ancel et al. 2013). This activity leads the formation of ornithogenic soils, which are the the main source of organic matter in Antarctica (Ugolini 1972; Simas et al. 2007a).

As a result of glacio-isostatic uplift in the Holocene (Fretwell et al. 2010), populations of penguins start to nest in the newly emerged beaches, leaving sites with ornithogenic soils without new inputs of guano. In active penguin rockery, vegetation growth is inauspicious for plant growth because of the presence of toxic elements and the intense trampling by penguins (Santamans et al. 2017). On other hand, the abandoned ornithogenic soils display great potential for biological development due to the less severe conditions. These soils are commonly found in the coastal regions of Maritime Antarctic, where pinguins nest (Simas et al. 2007b). The warmers temperatures and higher water availability in this region enables a greater microbial activity that mineralize the guano deposited by penguins, playing a key role in the pedogenesis of these soils, acting directly in the cycles of carbon, nitrogen, phosphorus, sulfur and others elements by the processing of guano (Grzesiak et al. 2020).

The Narebski Point, located at the southest of Barton Peninsula (King George Island), is a breeding site for two penguin species: gentoo (*Pygoscelis papua*) and chinstrap (*Pygoscelis*

adelie). This area present actual and abandoned penguin rookeries due to the glacio-isostatic uplift and present an ideal place to study the microbiota in ornithogenic soils, which were the objects of this study, aiming to characterize the profiles of bacterial and fungal communities inhabiting of these areas.

Material and methods

Sampling Area

The sampling of materials was done in the southeast cost of the Barton Peninsula (Narebski Point), King George Island. A total of 16 samples were collected from four sites (4 samples per site): two from actual penguin rockeries (P1 and P2), one from an abandoned penguin rockery (P3) and one represent a mineral soil (P4). Within each site we established a 5 m square and collected one soil sample from each vertex, totaling 16 soil samples (Figure 1). The samples were stored on ice and transported to the Laboratory of Mycorrhizal Association at the Universidade Federal de Viçosa, where they were stored at - 20 °C.

DNA extraction, PCR and sequencing

Two hundred and fifty milligrams of soil were used for DNA extraction. The cell lysis was performed in a Precellys 24 High-Powered Bead Mill Homogenizer (Bertin technologies) for 50 s at 4000 rpm. The remaining steps of DNA extraction were performed using Nucleospin Soil® (Machanarey-Nagel) according to the manufacturer's protocol. The quality of DNA extraction was evaluated by electrophoresis in 0.8 % agarose gel stained with Ethidium Bromide under UV light.

The V4 region of the bacterial 16S rDNA and the fungal ITS1 were amplified using the primer pairs 515F/806R(Parada et al. 2016) and ITS1F/ITS2(Veloso et al. 2020). The PCR amplicons were sequenced on an Illumina MiSeq in paired-end mode: 2 x 151 bp for V4 and 2 x 251 bp for ITS1 products.

Data analyses

All the sequences were processed using Qiime2 v. 2020.8 (Bolyen et al. 2019). Initially, adapters and primers attached to the 5' of the reads were discarded and the sequences identified as chimera, singletons or with low quality (maximum expected error equal or above 1) were also removed. The remaining sequences were clustered into Amplicon Sequence Variants (ASVs) using DADA2 algorithm (Callahan et al. 2016) and each one was taxonomically affiliated to taxa of the UNITE database (version) (Nilsson et al. 2019).

After demultiplexing, the adapters and barcodes bounded to the 5' end of each primer were removed. The ITSx was used to remove the flanking regions 18S and 5.8S from fungal reads. All the remaining reads that were identified as singleton, chimera or had a low quality (maximum expected error equal or above 1) were also removed. The high-quality reads obtained from the steps above were then clustered into ASVs using the DADA2 algorithm (Callahan et al. 2016) and annotated using scikit-learn classifier and the UNITE database (version 4.02.2020) (Nilsson et al. 2019).

Alpha diversity metrics were measure using three indices available in Vegan v.2.5.3 (Oksanen et al. 2019): Chao1, Pieou's evenness and Simpson diversity. Beta diversity, on the other hand, was measured using Principal Coordinates Analysis (PCoA) based on Bray-Curtis distance matrices calculated in Phyloseq v.1.34.0 (McMurdie and Holmes 2013). The distances of the Bray-Curtis matrices were used

to quanfiy the magnitude of change in the bacterial and fungal beta diversities. To evaluate which taxa are statistically more abundant in some sites, we performed a Linear Discriminant Analysis (LDA) Effect Size and due to the non-parametric nature of the variables in this study, we performed Kruskal-Wallis to perform statistical comparisons among samples. The correlation between the microbial community matrix and the chemical variables of soil was evaluated by canonical redundancy analysis (RDA) available in vegan v.2.5.3 (Oksanen et al. 2019).

The possible interactions patterns of each fungal and bacterial community along the transect were determined by building four co-occurrence networks using significative SparCC correlations (Friedman and Alm 2012). A total of 1000 permutations of the dataset were performed to estimate the p-values using *FastSpar(Watts et al. 2019)*. Only the correlations with absolute value greater than 0.7 and p-value < 0.01 were used to build the network. The R's package *igraph* was used to plot the network and to calculate the topological properties of each individual network.

The functional potential of the bacterial community was investigated using the Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUST) version 2.3.0 (Douglas et al. 2019). All sequences with a Nearest Sequenced Taxon Index (NSTI) score above two were removed from the analysis before the metagenome prediction to increase the reliability of the prediction.

Results

A total of 1,065,288 16S sequences and 768,232 ITS sequences were obtained. After denoising procedure, 226,028 16S and 373,488 ITS sequences were retained. The 16S

sequences were clustered into 2556 bacterial ASVs distributed in 39 phyla, 98 classes, 213 orders, 325 families and 469 genera. The ITS sequences, on the other hand, were distributed in 8 phyla, 20 classes, 54 orders, 90 families and 139 genera. According the ZhangHuang's coverage index the sampling effort was enough to access the bacterial and fungal diversity present in the samples (Figure S1).

All alpha indices of the bacterial communities (richness, evenness and diversity) did not differ among the soils (Figure 1A). On the other hand, the evenness and diversity of fungi were higher in the abandoned ornithogenic soils (P3) and mineral soils (Figure 1B). Although

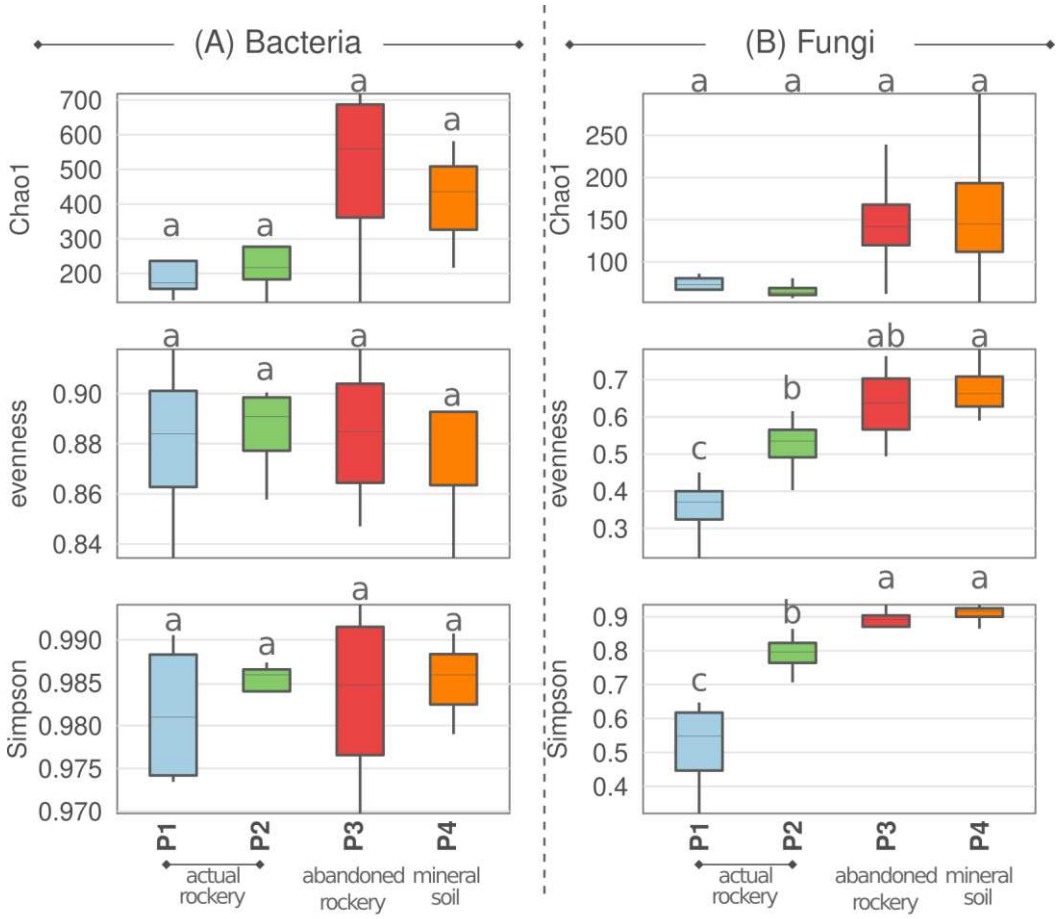

Figure 1: Estimate richness (Chao1 index), evenness and diversity (Simpson index). Boxplots with the same letter does not differ at 0.05 of probability by the Kruskal-Wallis test.

no difference was found in the alpha bacterial diversity indices among the soils, the Principal component Analyses (PCoA) revealed a clear difference in the beta diversity among them (Figure 2A). The number of ASVs shared between the two sites of actual rookeries (P1 and P2) were almost 10 times higher (188 ASVs) than those shared between abandoned and actual (P2 and P3; 20 ASVs) (Figure 2A). The number of bacterial ASVs shared between the mineral (P4) and abandoned ornithogenic soils (P4) was also high (168 ASVs). The same pattern was observed for the fungal community (Figure 2B): whereas 86 ASVs were shared between the two actively colonized ornithogenic soils, only 33 were shared between the actual and abandoned nesting sites. It reveals that a new microbial community emerges in abandoned ornithogenic soils after penguins have abandoned the site. Eighty eight percent of the fungal individuals were condensed in only two phyla: Ascomycota (46.4 %) and Basidiomycota (41.9 %). The remaining fungal sequences were from Mortierellomycota (8.7 %), Rozellomycota (2.7 %), Monoblepharomycota (< 1 %), Chytridiomycota (< 1 %), Zoopagomycota (< 1 %).

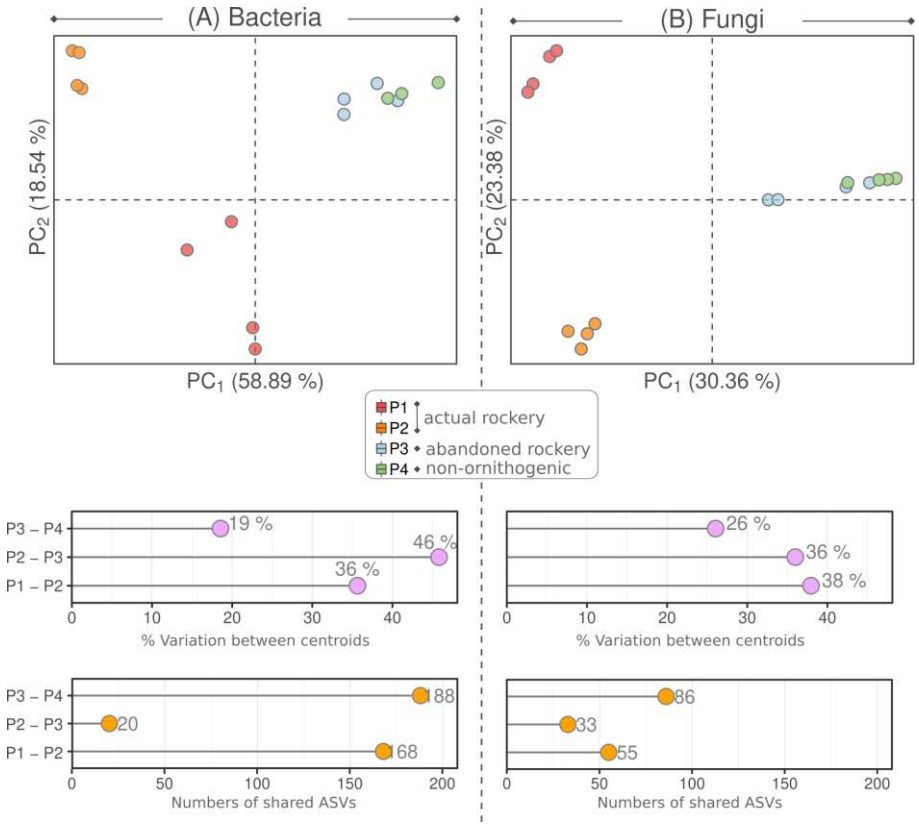

Figure 2: Principal Coordinate Analyses (PCoA) based on Bray-Curtis dissimilarity of the (A) bacterial and (B) fungal communities in actual (P1 and P2) and abandoned (P3 and P4) breeding sites. The upper and lower lollipops display, respectively the percentage variance between the centroids of consecutive sampling points and the number of shared ASVs.

The Linear Discriminant Analysis showed that some phyla are associated with specific sites. The candidate phylum WPS-2, Abdibacteriota, Proteobacteria, Bacteroidota, Firmicutes, Cyanobacteria and Desulfobacterota were more abundant in actual soils, whereas Gemmatimonadota, Plactomycetota, Nitrospirota and Patescibacteria were abundant in abandoned ornithogenic soils (Figure 3). Two fungal classes: Tremellomycetes and Rozellomycotina were more present in the actual breeding sites, and only the class Microbotryomycetes was differently more abundant in the abandoned ornithogenic soils. The mineral soils were more enriched with Mortierellomycetes, Eurotiomycetes and Agaricomycetes.

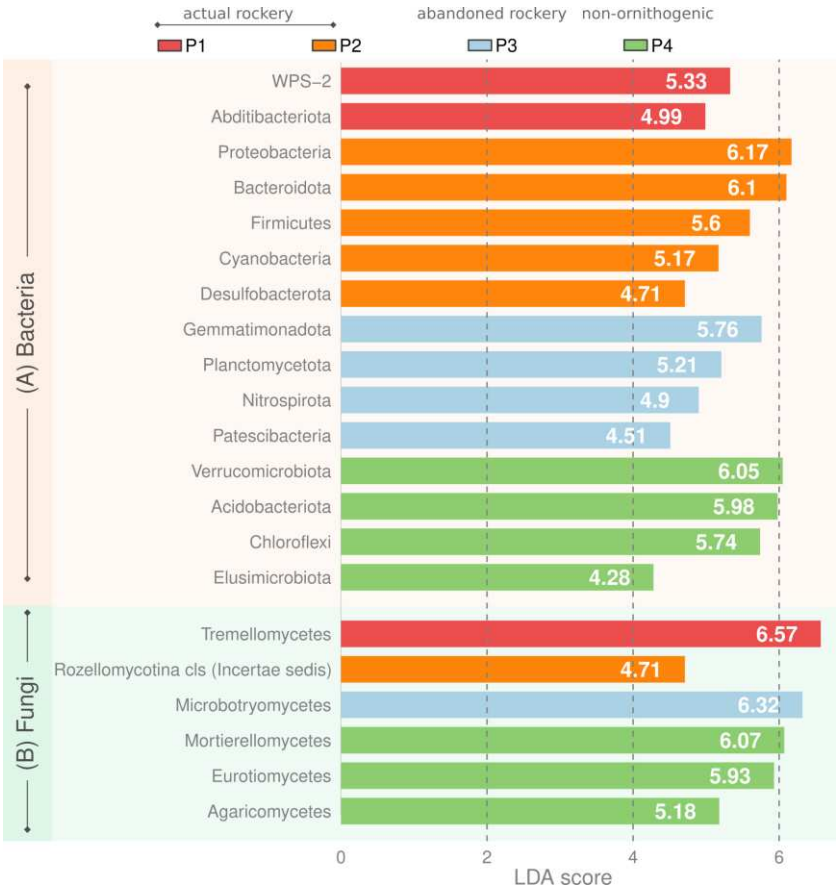

Figure 3: Score of Linear Redundancy analysis' score Redundancy of (A) bacterial phyla and (B) fungal classes which are statistically different among the sites. Only LDA scores higher than four are shown.

In order to gain information about the association between the soil chemical composition and the microbiota we fitted the soil chemical variables to the PCoA ordination. The soils in the actual penguin rookeries displayed the lowest pH and the highest concentrations of Al^{3+}, Ca, Mg, K and organic carbon (Figure 4). The mineral and abandoned ornithogenic soils tended to have more similar chemical attributes, as well as the microbiota community. According to the variable selection performed by *ordistep* function in R, the variables K, Al^{3+} and pH were the main variables that shaped the bacterial community (p-value < 0.05), whereas the fungal community cation exchange capacity (T), Ca and Phosphorus were the most important.

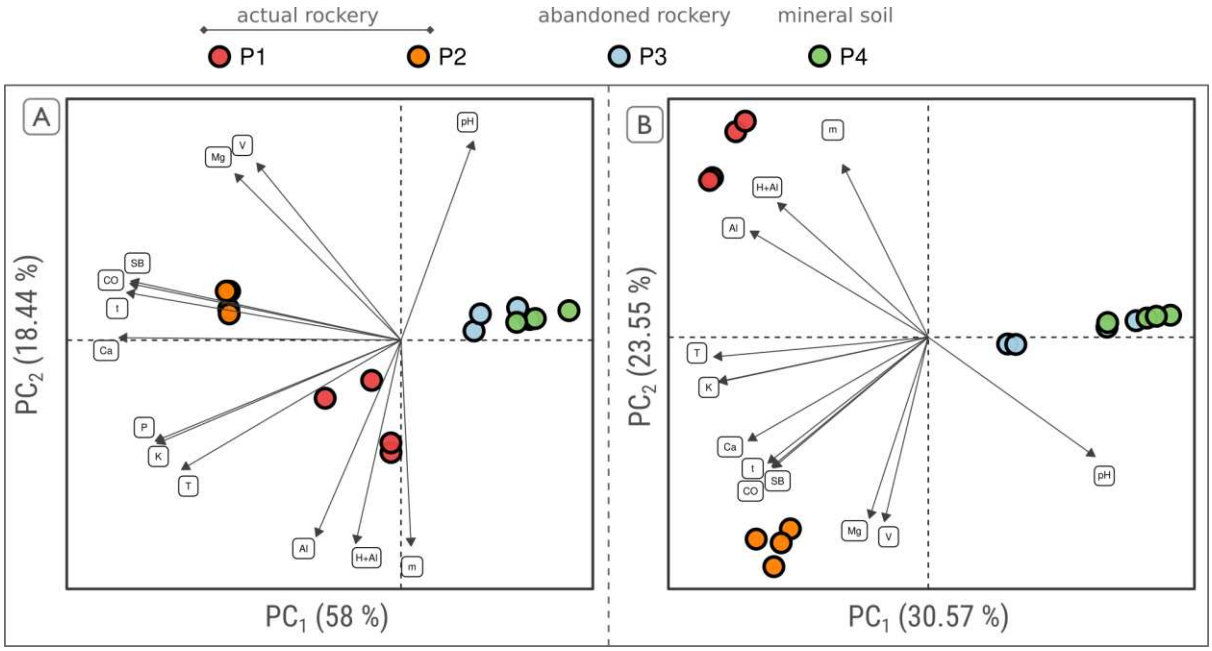

Figure 4: Redundancy analysis of (A) bacterial and (B) fungal communities in actual (P1 and P2) and abandoned (P3 and P4) breeding sites. The arrows display the distribution of the chemical variables in the samples. CO = Organic Carbon; Ca = Calcium; m = Alminium saturation; T = Cation Exchange Capacity at pH 7.0, SB = sum of bases.

The co-occurrence networks of fungi displayed the highest number of connections in abandoned ornithogenic soils (226) and in the mineral soils (64) (Figure 5). Bacteria, on other hand, has a high number of connections in both actual and abandoned breeding sites. It shows that fungal communities are less structured than bacteria in soils of actual penguin rookeries, which is maybe a reflect of the reduced evenness and diversity of fungi in the conditions of the actual penguin rockeries (Figure 5B).

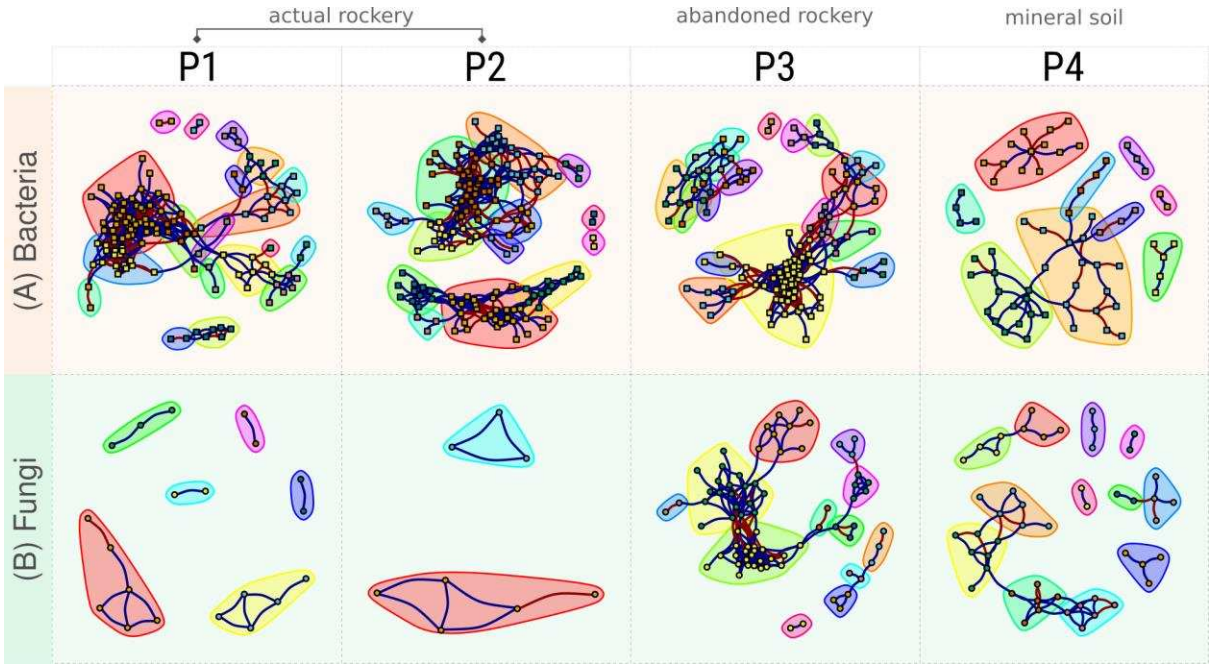

Figure 5: Co-occurrence/co-exclusion networks of (A) bacterial and (B) fungal communities in actual and actual (P1 and P2) PRE, and abandoned (P3) PRE and non-ornithogenic soils. Each node represents a bacterial or fungal ASV. Positive and negative relations between ASVs are represented, respectively, by red and blue edges. Only significant SparCC correlations (p-value < 0.01 and absolute correlations > 0.7) are shown.

It is known that abandoned ornithogenic soils (non-published data) show a high potential for nitrification. Then, we decided to investigate how is the metagenomic potential for nitrogen metabolism-related genes (Nitrification, denitrification, N_2 fixation, nitrate reduction) in these soils. Moreover, we also investigate the metagenomic potential for sulfur metabolism-related genes due the area harbors ornithogenic soils, enriched in pyrite (FeS_2). The functional predicted profiles of the bacterial community by PICRUSt2 (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) showed that the mineral and abandoned ornithogenic soils (Figure 6) harbor bacterial communities with greater metagenomic potential to perform these two types of energetic metabolism than actively penguin-colonized ornithogenic soil. Furthermore, the later harbors more bacteria chitinolytic potential (Figure 6).

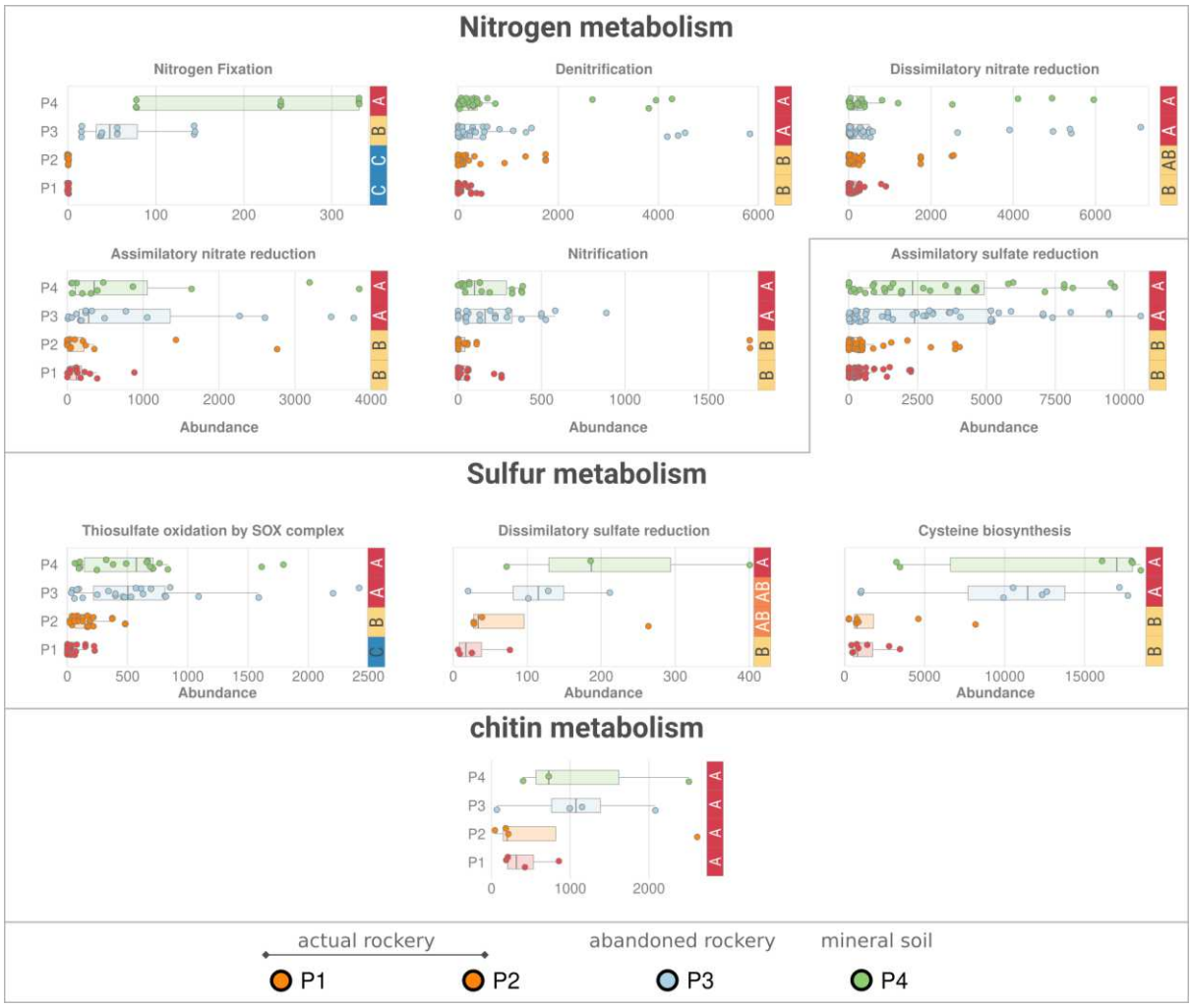

Figure 6: Abun dance of functional genes related to nitrogen, sulfur and chitin metabolisms in the predicted metagenome by PICRUSt2. Different letters in the left side of each plot represents differences by the Kruskal-Wallis' test at 0.05 of probability.

Discussion

The ornithogenic soils are a very important source of carbon and nutrients for the microbial and vegetal growth in these areas. The microbial communities play a key role in the development of these soils (Guo et al. 2018; Grzesiak et al. 2020), however, the microbial composition in these soils is still poorly understood and few studies investigated at the same time both bacterial and fungal communities in actual and abandoned ornithogenic soils (Zhang et al. 2018).

The actual and abandoned ornithogenic soils did not differ in bacterial richness, evenness and diversity from the mineral soils, even with higher contents of Organic carbon. This pattern for bacteria was previously reported (Guo et al. 2018). On other hand, the eveness and diversity of the fungi were smaller in the actual nesting sites than abandoned and mineral soils (Figure 1). It occurs due to the dominance of few taxa over the microbial community. For example, the genus *Filobasidiella* (teleomorph of *Cryptococcus*)., order Tremellomycetes, represented from 64 to 86 % of all fungal sequences in the samples of active ornithogenic soils. This fungus was previously isolated from penguin guano (Vaz et al. 2011) and is usually present in bird excrement, therefore, the microbial composition in soils of actual penguin rockeries is tightly influenced by the penguins' excrements that leads the presence of many enteric bacteria in these soils (Guo et al. 2018). These finding reveals that harsh conditions of actual penguin rockeries affect more the fungal alpha diversity than the bacterial one. Due to the heterotrophic nutrition mode of fungi, they rely on carbon fixed by plants or autotrophic microorganisms. The absence of penguin trampling in the abandoned rookery areas enables the growth of plants that releases exudates with reduced carbon that can be used as a source of carbon and energy by fungi (Eisenhauer et al. 2017).

In contrast to observed for alpha diversity index, the beta diversity of both bacteria and fungi are strongly different in all soils evaluated (Figure 2). The few ASVs of bacteria and fungi shared between actual and abandoned breeding sites display a transition of the microbial composition from the active to the abandoned and ornithogenic soils. This transition is related to the changes in the geochemical composition between actual and abandoned sites and the

absence of direct input of penguin feces and trampling (Zhang et al. 2018; Grzesiak et al. 2020) These conditions allow the growth of a vegetation, which produces root exudates, that together with the soil chemical features, alters strongly the taxonomic composition (Hermans et al. 2020).

The main source of nitrogen are the urates of the white fraction of guano (Myrcha and Tatur, 1991). It is known that abandoned ornithogenic soils has a great potential for nitrification (unpublished data), and, interestingly, the abandoned penguin rockeries displayed a greater potential for genes related to nitrogen metabolism, including nitrification, denitrification and N_2 fixation than the active ones (Figure 6). The results from functional prediction analysis shows that these soils harbor more bacteria with nitrification capability than soils of active penguin rookeries and they may be driving the nitrification process. The main genus responsible for the nitrification seems to be is *Nitrospira* from the phylum Nitrospirota, which was more abundant in this site. *Nitrospira* species play a key role in the final step of nitrification, converting nitrite to nitrate. However, studies have also shown that this genus have the metabolic potential to perform the whole nitrification process, i.e, convert ammonia to nitrite and then oxidize the latter to nitrate (Van Kessel et al. 2015; Koch et al. 2019).

More than half of the red fraction of guano is composed of chitin, which is derived from the exoskeletons of krill ingested by penguins (Myrcha and Tatur 1991; Cristancho et al. 2014). The bacteria with chitinolytic capability found here are mainly Bacteroidetes of the Chitinophagaceae family (Rosenberg 2014) and Acidobacteria of the genus *Granuciella*. These bacteria are commonly found described in studies of ornithogenic soils (Guo et al. 2018; Zhang

et al. 2018), however, none of these studies have tried to predict their functional potential. However, it worth highlight that this is just a prediction based on 16S sequences, therefore, additional studies based on shotgun sequencing and culture-dependent methods must be applied to support these findings.

Our study shows a clear variation in the community composition (beta diversity) from actively to abandoned penguin-colonized ornithogenic soils. However, it is worth remembering that fluctuations in the microbial composition over time can occurs in these soils, even in a small timescales (Grzesiak et al. 2020), however, the temporal dynamics is still poorly explored. Therefore, future studies resampling the same points are necessary for the full overview of these communities over time. We also found microorganisms able to perform metabolic compounds, like ammonia, chitin and sulfur. Therefore, the functional potential of the microbiota seems to be related with physico-chemical traits of these soils, changed by the guano deposited in the rock.

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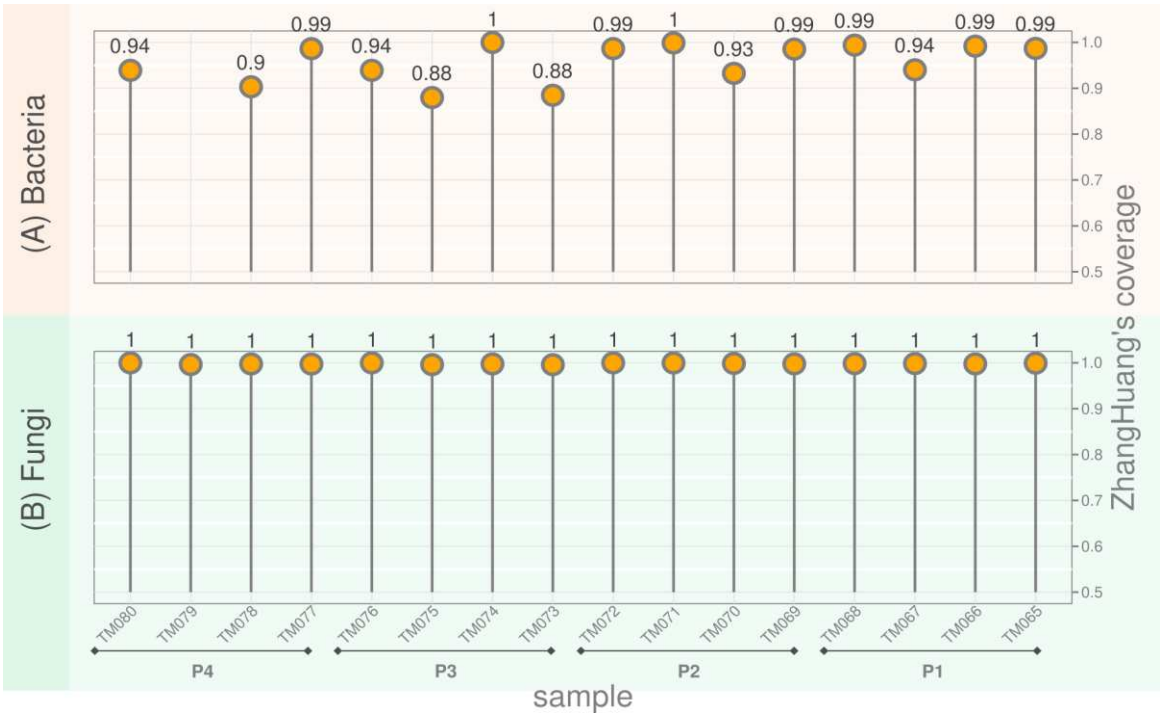
Supplementary material



Supplementary Figure 1: Zhang-Huang's coverage scores. This index allows to evaluate if the sampling effort measured of the samples are enough to capture the microbial diversity of samples. It considers how many doubletons (i.e. reads appear only twice) in a sample in comparison to the total number or reads in the same sample. High values (i.e. close to one) reflects enough sampling effort.

CONSIDERAÇÕES FINAIS

Nos solos tropicais, as comunidade fúngicas e bacterianas em frutos e solo de cafeeiro (*Coffea arabica*) são afetadas tanto por fatores químicos do solo, como por fatores relacionados ao relevo, como a face de exposição da lavoura ao sol e à altitude. Estes dois fatores, altitude e face de exposição ao sol, foram os atributos que mais contribuíram para determinação da composição bacteriana e fúngica em solos e frutos de cafeeiro. Também observou-se que em altitudes maiores, mais bactérias são compartilhadas entre o solo e frutos de cafeeiro. Isso mostra que a escolha da área para o cultivo do cafeeiro terá um grande impacto nas comunidades microbianas do local, o que poderá refletir na qualidade final do café.

Em relação aos solos antárticos, aqueles localizados em áreas recém-expostas pela deglaciação, apresentaram as comunidades bacterianas e fúngicas mais distintas de todo o transecto estudado, mostrando que a idade do solo e sua localização na base da geleira tem papel determinante na composição microbiana. Pela análise de predição funcional, a comunidade bacteriana nestes destes solos jovens das áreas recém-expostas apresentaram maior potencial genético para realização de metabolismo litotrófico, do que solos mais desenvolvidos, o que pode estar relacionado à menor presença de matéria orgânica, o que exige que estes microrganismos obtenham suas fontes de energia, elétrons e carbono a partir de compostos inorgânicos.

Em suma, tanto em solos tropicais como em solos antárticos, os fatores edafoclimáticos e temporais afetam a estrutura da comunidade microbiana. Portanto, estudos dessa natureza são de grande importância para o melhor entendimento da dinâmica microbiana e biogeoquímica nos diferentes ambientes, uma vez que esses processos estão intimamente interligados.