Irradiance level and elevation shape the soil microbiome communities of *Coffea arabica* L.

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Abstract

Background The nexus plant-microbe-environment is essential to understand the ecosystem processes shaping plant health and fitness. Within this triangle, soils and associated microflora are among the key ecosystem's drivers, underpinning plant productivity and evolution. In this study, we conducted a comprehensive analysis (physicochemical properties, enzyme activities, and taxonomic diversity) of soils under the canopy projection of *Coffea arabica* trees along a gradient of elevation (600, 800, and 900 m) and shade (0, 50, 100%).

Results While shade had no influence on most parameters, altitude shaped the dynamics of microbial communities. Available phosphorus, soil organic carbon, and nitrate were significantly higher at 800 m, likely due to the higher activities of β-glucosidase and phosphatases at this altitude. Microbial biomass (carbon and nitrogen) and moisture were significantly higher at 600 and 900 m, which might be attributed to the abundance and richness of soil microorganisms. Indeed, metabarcoding analysis revealed a complex pattern of microbial consortia (bacteria, archaea, fungi) at the three altitudes, with the lowest index of richness recorded at 800 m. The highest number of Amplicon Sequence Variants was observed in bacteria, whose functional analysis revealed distinct metabolic adaptations across different altitudes. At 900 m, the main functional attributes favored the responses to environmental stimuli and microbial interactions; at 800 m, the predominant metabolic pathways were related to organic matter, fermentation, and bioremediation; and at the lower 600 m, the pathways shifted towards the breakdown of plant-derived compounds (e.g. geraniol, limonene, and pinene degradation).

Conclusion Overall, the results indicate a higher effectiveness of the microbial consortium at 800 m, which might result in better nutrient cycling. The study highlights the importance of canopy shade species and elevation for the composition of microbial consortia in *C. arabica*, unveiling ecological functions beyond plant health, with implications for bio-based solutions and biotechnology.

Keywords Agroforestry system, *Coffea arabica*, Enzymatic activities, Gorongosa mountain, Microbiome, Soil

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Background

The *Coffea* genus comprises 130 species, from which only *Coffea arabica* L. (Arabica coffee) and *Coffea canephora* Pierre ex A. Froehner (Robusta coffee) are commercially significant [1]. These account for approximately 57% and 43% of the global coffee production, respectively [2]. Coffee is a leading commodity worldwide, involving around 100 million workers throughout its value chain [3]. However, the sustainability of the coffee sector is increasingly at risk due to climate change impacts, notably global warming and drought [4, 5]. To counteract this impact, the implementation of climate-smart strategies, such as agroforestry, is undeniably the most straight forward approach [5–7].

Theoretically, effective shaded coffee agroforestry systems (AFS) mitigate high temperatures and minimize evapotranspiration [7–9]. However, the success depends on the effective embedding of crop management practices (including soil, water, as well as compatible shade trees and coffee cultivars) within specific agro-ecological contexts [10, 11]. For example, the incidence of natural enemies of coffee biotic stressors may be either boosted or reduced depending on the vegetation structure and composition [12–16]. Another relevant aspect is the impact of shade which may have a negative [17], positive [18], or neutral [6] impact, depending on the interaction between genotype and environment, as well as the intensity of shade or irradiation [10, 11]. Altitude is also a crucial factor in coffee cultivation, driving the physical and chemical characteristics of coffee beans [6, 19–21]. Finally, the coffee microbiome emerges as a core component of the system [22–26], due to its preponderant role in plant evolution, health and productivity [27, 28].

Soils are among the most important reservoirs of biodiversity, hosting ca. 1/4 of living organisms in terrestrial ecosystems [29, 30]. These include bacteria, archaea, fungi, protists, and many other eukaryotes (e.g. nematodes, mites, ants, beetles, earthworms), which provide a set of supporting services (soil formation, and nutrient cycling), provision services (food, freshwater, fuel, fiber, biochemicals, genetic resources), and regulating services (climate regulation, pest and disease regulation, water regulation, remediation, and pollination) [31, 32]. Therefore, research on soil biodiversity is giving a step forward, envisioning the maximization of ecosystem goods and services, as well as the elucidation of biological, ecological and evolutionary processes [30]. In coffee, Caldwell et al. [22] reported high microbial diversity in soils from intensive, organic, and transition farms in Brazil, highlighting the potential of plant growth-promoting bacteria to improve coffee production and counteract environmental constraints. Tran [33] published a data set of the rhizosphere microbiome of *C. canephora* in the Central Highlands region of Vietnam, the second largest coffee producer in the world after Brazil. The reported taxonomical diversity was also considerably high, and reflected in the associated functions, particularly regarding biosynthetic processes. More recently [24], analyzed the effect of altitude on the diversity of microbial communities in the rhizosphere of *C. arabica* in Yunnan, the most expressive center of coffee production and trading in China. In line with the previous studies, the authors observed that microbial diversity and richness was high, and essentially driven by soil pH and altitude.

In this study, we conducted a comprehensive analysis of the soil physicochemical properties and microbial communities of *C. arabica* cultivated under AFS in the evergreen rainforest of Gorongosa Mountain, part of the Gorongosa National Park (GNP), Mozambique. GNP is one of the most interesting and valuable case studies for the development of climate mitigation and/or adaptation strategies [34, 35], owing to its exclusive biodiversity and anthropo-climate vulnerability [36, 37]. Specifically, we aimed to unveil the influence of elevation and canopy shade in bacteria, archaea, and fungi communities.

Materials and methods

Experimental design

The study was conducted in the Gorongosa Mountain, belonging to the Gorongosa National Park, Sofala province, Mozambique (Lat. 18° 24' 14"S, Long. 34° 06' 31.5"E). *Coffea arabica* plants were implanted 1.5 m apart within a row and 3 m between rows, at a density of ca. 2222 plants ha^{-1}. The split-plot design of [6] was used to assess the impact of altitude and/or light conditions on the coffee soil properties. This included three different altitudes (main plots): ca. 600 m (18° 30' 53" S, 34° 03' 05" E), ca. 800 m (18' 30' 04" S, 34° 02' 58" E), and ca. 900 m (18° 28' 54" S, 34° 02' 43" E) above sea level (a.s.l.); and three levels of light (sub-plots) per altitude: deep shade (DS, average diurnal PPFD of 127 ± 28 µmol m^{-2} s^{-1}), moderate shade (MS, 725 ± 48 µmol m^{-2} s^{-1}), and full Sun (FS, 1268 ± 52 µmol m^{-2} s^{-1}). The main canopy forest trees were *Khaya anthotheca* (Welw.) C. DC., *Erythrina lysistemon* Hutch., and *Albizia adianthifolia* (Schumach.) W.F.Wight.

Soil sampling

At each altitude and shade level, three coffee plants were randomly selected and soil samples were collected with an auger at a depth of 0–10 cm and 10–20 cm. One subset was air-dried, ground, sieved (2 mm particle size) and used for physicochemical analysis. The other part was used to prepare composite samples from the two depths, stored on ice during the field collection and once in the lab kept at −80 °C until DNA extraction.

Chemical and enzymatic analyses

Soil samples were characterized in terms of Olsen extractable phosphorus (P-Olsen), pH (in a suspension 1:2.5 with H_2O), soil organic carbon (SOC), and microbial biomass carbon and nitrogen (MBC and MBN, respectively), electric conductivity, extractable potassium (Egner Rhiem K), mineral nitrogen (NO_3^- and NH_4^+) and moisture using standard protocols [38–40]. MBC and MBN were determined with the fumigation-extraction method using calibration values of $K_{EC} = 0.45$ for C and $K_{EN} = 0.54$ for N [41, 42]. The quantification of C and N based on K_2SO_4 extraction was conducted using a near-infrared detector for carbon, and chemiluminescence for nitrogen. In both cases, samples underwent combustion at 950 °C in a Formac analyzer (Skalar, Breda, Netherlands).

The activities of β-glucosidase, acid phosphatase and alkaline phosphatase (phosphomonoesterases) were determined according to the protocol of [43], using p-nitrophenyl-β-D-glucopyranoside (*p*NG) as substrate for β-glucosidase, and p-nitrophenyl phosphate (*p*NP) as substrate for both phosphomonoesterases. Urease activity was determined as described by [44] without buffering. For all enzymatic activities, the absorbance values of the extracts were determined in a segmented flow analyzer system with a preliminary dialysis step to remove color and microparticle interferences. β-glucosidase, acid- and alkaline phosphatase activities were expressed in μg p-nitrophenol $h^{-1} g^{-1}$ dry soil, while urease activity was expressed in mg $N-NH_4^+ 2 h^{-1} kg^{-1}$ dry soil.

Descriptive statistics and statistical analyses for soil properties were performed using RStudio version 4.1.1 [45]. The heterogeneity of the variance was first tested, and the original data were normalized by log-transformation when necessary. A GLM analysis was used to analyze the effects of altitude, shade, and their interactions on the soil properties.

DNA extraction and amplicon sequencing

Microbial DNA was extracted from soil samples using the DNeasy PowerSoil Pro Soil DNA Isolation Kit (Qiagen, Germany City, MD, USA) following the manufacturer instructions. DNA integrity and concentration were determined by 1% agarose gel electrophoresis and fluorometric quantification using a fluorometer (Qubit 2.0, Invitrogen, CA, USA), respectively. Amplicon libraries targeting the V4 hypervariable region of the 16 S rRNA for bacteria (Bakt_341F: CCTACGGGNGGCWGCAG and Bakt_805R: GACTACHVGGGTATCTAATCC) and archaea (Bakt_341F: CCTACGGGNGGCWGCAG and Bakt_805R: GACTACHVGGGTATCTAATCC) and the ITS2 region for fungi (3 F: GCATCGATGAAGAACG CAGC and 4R: TCCTCCGCTTATTGATATGC) were used following the Amplicon Metagenomic Sequencing

Library Preparation guide (http://emea.support.illumina. com) [46]. Sequencing libraries were generated using the TruSEq DNA PCR-free sample preparation kit (Illumina, San Diego, CA, USA) following the manufacturer's instructions. The final libraries were sequenced using the Illumina Miseq300 PE to generate 300 bp paired-end reads through Macrogen sequencing services (Macrogen, Seul, Korea).

Taxonomic diversity

Paired-end sequence reads were demultiplexed using the MiSeq reporter software (Illumina Inc., CA, USA) and checked for quality using FastQC v.0.11.9 (Babraham Institute, Cambridge, UK). Paired-reads were trimmed at both 5′ and 3' ends eliminating poor quality nucleotides, denoised, merged, and chimeric sequences using the DADA2 denoiser [47] and then incorporated into QIIME 2 [48]. The resulting Amplicon Sequence Variant (ASV) count table was depleted of singletons, and representative sequences taxonomically classified using a trained classifier of the SILVA reference (Release 132) [49]. Alpha diversity analysis was conducted based on observed ASVs, Shannon entropy and Pielou's evenness indices, while community dissimilarity was assessed using Bray-Curtis distance, which was visualized through non-metric multidimensional scaling (NMDS) ordination. Permutational multivariate analysis of variance (PER-MANAOVA) was used to test for the significance of the microbial community dissimilarity across the different gradients of altitude and shades investigated. Canonical correspondence analysis (CCA) was also performed to determine the relationship of the microbial communities to soil physicochemical parameters along the shade and altitudinal gradients. Prior to computation of microbial diversity, the counts were normalized to 11,786 (bacteria), 2,414 (archaea) and 67,302 (fungi) ASV. The detection of biomarkers across gradients of altitude and shade was performed using Linear discriminant analysis Effect Size (LEfSe) [50]. Phylotypes with an LDA score ≥ 3.0 and a False Discovery Rate (FDR)-adjusted *P*-value ≤ 1.0 were considered to be differentially abundant. Except stated otherwise, data analysis and visualization were performed using R software v.4.1.1 [45].

Functional prediction

Prediction of bacterial community functions was done using Phylogenetic Investigation of Communities by Reconstruction of Unobserved States - PICRUSt2 [51]. The functional prediction was done by aligning the 16 S rRNA marker gene representative sequences to a reference multiple-sequence alignment and reference phylogeny utilizing HMMER [52], EPA-NG [53] and GAPPA [54]. Subsequently, gene families were predicted using a hidden state prediction tool – Castor [55], after

a normalization of the 16 S rRNA gene copies. The predicted gene families were thereafter collapsed into KEGG pathways using MinPath [56]. Differentially abundant pathways across the different altitudes were afterward determined using LefSe (LDA score ≥ 2.0; *P*-value ≤ 0.05).

Results

Chemical characteristics and enzymatic activities of soils

The level of shade only affected available phosphorus (P-Olsen; *P* = 0.014), MBN (*P* = 0.014) and the level of moisture (*P* = 0.006) (Table S1). In contrast, altitude exhibited a more pronounced effect on soil characteristics, influencing nearly all parameters except for pH and urease activity, i.e. P-Olsen (*P* = 0.008), SOC (*P* = 0.009), MBC (*P* = 0.0001), MBN (*P* = 0.0001), electrical conductivity (*P* = 0.002), Egner Rhiem K (*P* = 0.025), NO_3^- (*P* = 0.0001), NH_4^+ (*P* = 0.0001), moisture (*P* = 0.0001), β-glucosidase (*P* = 0.008), acid phosphatase (*P* = 0.0001), and alkaline phosphatase (*P* = 0.0001) (Table S1). Overall, at 800 m there were significantly higher levels of P-Olsen, SOC, and nitrate (NO_3^-), when compared to the 600 m and 900 m. MBC and MBN, as well as moisture content, were found to be lower at 800 m, highlighting a distinct environmental profile at this altitude (Table 1). Electrical conductivity and NH_4^+ increased with altitude, while the opposite was found for Egner Rhiem K (Table 1). Apart from urease, soil enzymatic activities also exhibited sensitivity to altitude changes (Table S1), with significantly higher activities of β-glucosidase and phosphatases (acid and alkaline) at 800 m (Table 1).

Coffea alpha diversity between different altitudes and shade trees

A total of 89,590 high quality reads were obtained for bacteria, 91,977 for archaea and 93,507 for fungi with high Q values and adequate GC contents (Table S2). For bacteria, the observed number of Operational Taxonomic Units (OTUs), Shannon entropy and Pielou's evenness revealed high species richness in all locations, although no significant differences were detected between the different levels of canopy shade (Fig. 1; Table S3). However, altitude had a strong influence on the bacterial richness with significantly lower values observed at the intermediate elevation of 800 m (Fig. 1; Table S4), while archaea richness increased with altitude (Fig. 1; Table S5; Table S6). In contrast, neither shade (Table S7) nor altitude (Table S8) had an impact on fungi richness, whether considering the observed number of OTUs, Shannon entropy, or Pielou's evenness (Fig. 1).

Coffea beta diversity between different altitudes and shade levels

Nonmetric multidimensional scaling analysis revealed a strong bacteria differentiation between altitude fields (Fig. 2A). These differences in the multivariate space were significant when considering the effect of altitude (PERMANOVA $R^2 = 0.493$, *P* = 0.001), but not when considering the effect of shade (PERMANOVA $R^2 = 0.052$, *P* = 0.797). The same was observed in the multivariate space of archaea (Fig. 2B) where altitude had a strong effect on the community structure (PERMANOVA $R^2 = 0.373$, *P* = 0.001), whereas no significant effect was detected when considering the level of shade

Table 1 Summary of the chemical properties and enzymatic activities of *Coffea arabica* soils. Mean values ± SD (*n* = 9) are indicated. Different superscripts indicate significant differences between altitudes, for each variable (ANOVA followed by Tukey-HSD, both for a 95% of confidence)

	600 m	800 m	900 m
Soil variables			
P-Olsen (mg kg^{-1})	5.40 ± 2.02 [a]	8.34 ± 2.37 [b]	5.94 ± 5.43 [a]
pH (H$_2$O)	5.41 ± 0.33 [a]	5.17 ± 0.33 [a]	5.67 ± 0.25 [a]
SOC (g kg^{-1})	64.60 ± 9.47 [a]	74.50 ± 12.56 [b]	66.37 ± 14.54 [a]
MBC (mg C kg^{-1})	146.32 ± 37.07 [b]	85.15 ± 21.53 [a]	138.28 ± 33.94 [b]
MBN (mg N kg^{-1})	18.65 ± 6.16 [b]	12.65 ± 4.21 [a]	21.64 ± 5.21 [b]
Electrical conductivity (µS cm^{-1})	84.92 ± 25.36 [a]	113.40 ± 61.08 [b]	149.17 ± 62.43 [c]
Egner Rhiem K (mg kg^{-1})	158.91 ± 20.78 [c]	140.46 ± 50.89 [b]	119.54 ± 47.91 [a]
N-NO$_3^-$ (mg kg-1)	9.35 ± 3.54 [a]	15.50 ± 7.04 [a]	8.21 ± 3.59 [a]
N-NH$_4^+$ (mg kg^{-1})	19.42 ± 2.68 [a]	27.22 ± 8.56 [b]	30.14 ± 8.63 [c]
Moisture (g kg^{-1})	68.31 ± 4.30 [b]	57.20 ± 4.55 [a]	66.68 ± 6.36 [b]
Enzymatic activities			
Urease (µg N-NH$_4^+$ g^{-1} h^{-1})	39.25 ± 10.67 [a]	32.54 ± 9.31 [a]	35.53 ± 7.53 [a]
β-glucosidase (µg p-nitrophenol g^{-1} h^{-1})	58.20 ± 12.81 [a]	65.73 ± 10.87 [b]	54.03 ± 12.85 [a]
Acid phosphatase (µg p-nitrophenol g^{-1} h^{-1})	310.98 ± 64.91 [a]	686.61 ± 149.97 [c]	530.51 ± 123.39 [b]
Alkaline phosphatase (µg p-nitrophenol g^{-1} h^{-1})	122.43 ± 42.42 [a]	189.96 ± 42.47 [b]	139.04 ± 28.15 [a]

P-Olsen: available phosphorous; SOC: soil organic carbon; MBC: microbial biomass carbon; MBN: microbial biomass nitrogen; Egner Rhiem K: extractable potassium; NO$_3^-$: nitrate; NH$_4^+$: ammonium
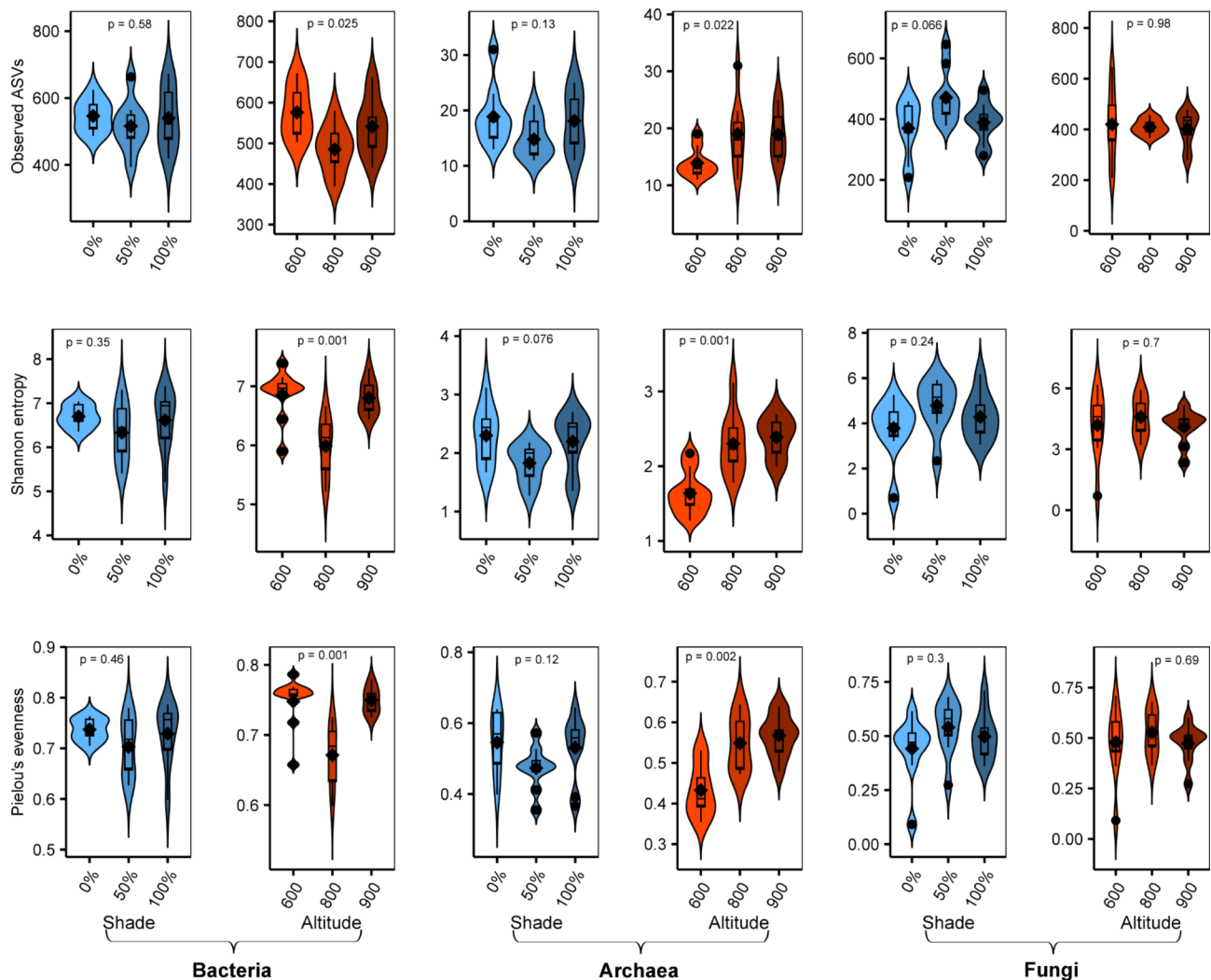
Fig. 1 Observed ASVs, Shannon–Wiener index of diversity and Pielou's evenness in soil samples of *C. arabica* grown under different altitudes (600 m, 800 m, and 900 m) and under different levels of canopy shading (0%, 50% and 100%) considering Bacteria, Archaea, and Fungi. Black-filled dots on the boxplots depict mean values. Comparisons between altitude and shade levels are based on Kruskal-Wallis (Bonferroni adjusted $P < 0.05$)

(PERMANOVA $R^2 = 0.047$, $P = 0.745$). Altitude also influenced fungi beta diversity (Fig. 2C), as significant differences were found between altitude levels (PERMANOVA $R^2 = 0.177$, $P = 0.001$), but not when considering the effect of shade (PERMANOVA $R^2 = 0.110$, $P = 0.068$).

Altitude alone accounted for 46% of the bacterial community variation ($R^2 = 0.02$; $P = 0.001$). However, the effect of shade on the bacterial community was not significant ($R^2 = 0.01$; $P = 0.113$). In the case of the archaeal community, altitude played an even more prominent role, explaining 61% of its variation ($R^2 = 0.01$; $P = 0.001$), while the impact of shade remained insignificant ($R^2 = 0.01$; $P = 0.277$). Regarding the fungal community, altitudinal changes significantly affected its composition ($R^2 = 0.158$; $P = 0.001$). Additionally, canopy cover had some influence on the fungal community, though to a lesser extent ($R^2 = 0.038$; $P = 0.016$). Notably, there was still 81%

of the variation in the fungal community that remained unexplained by either altitude or canopy cover.

To further determine the microbial community – soil physicochemical relationship, we performed a canonical correspondence analysis (CCA). The results indicated that the model for bacteria explained 33% of the community variation (adjusted $R^2 = 0.33$; $P = 0.001$). Both altitude ($f = 6.87$; $P = 0.001$) and shade ($f = 1.53$; $P = 0.05$) significantly influenced the community assembly. Also, several soil physiochemical parameters, including NH_4^+, pH, EC, MBN, MBC and moisture significantly influenced the dissimilarities of the bacterial community along the altitudinal and shade gradients. Accordingly, there was high relative abundance of the bacterial families *Flavobacteriaceae* and *Ligionellaceae* at 900 m altitude, while *Streptomycetaceae* and *Shingobacteriaceae* were dominant at 600 m altitude (Fig. 3B).
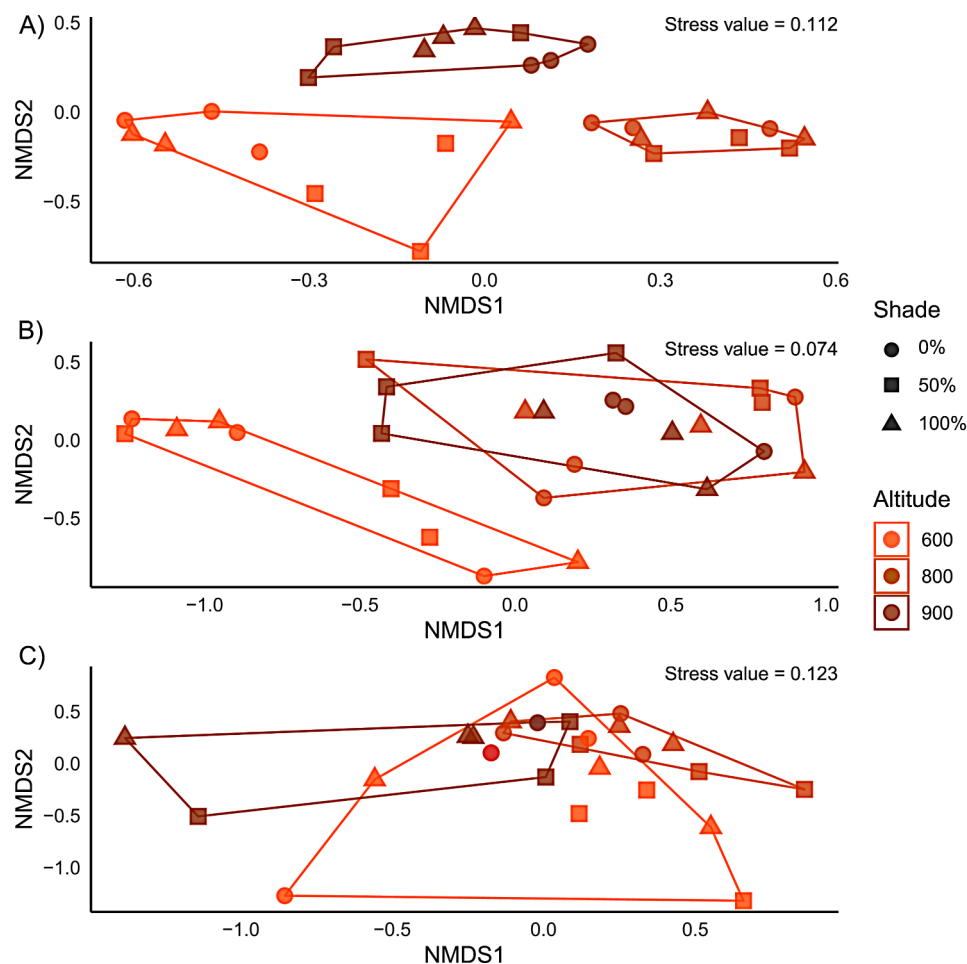
Fig. 2 Nonmetric multidimensional scaling (NMDS) analysis of Bacteria (**A**), Archaea (**B**) and Fungi (**C**) community differences in soil samples of *C. arabica* grown under different altitudes (600 m, 800 m, and 900 m)

The CCA model for the archaeal community explained 34% (adjusted $R^2=0.34$; $P=0.001$) of the community dissimilarities along the shade and altitudinal gradients, though only altitude (shade: $f=1.55$, $P=0.09$; altitude: $f=6.83$; $P=0.001$) had a significant influence on the archaeal community. The dominance of *Methanobacteriaceae* at 800 m correlated with the concentration of NO_3^-. Other parameters that influenced the archaeal community were similar to those influencing the bacterial community. The CCA analysis further revealed that a significantly smaller variation of the fungal community dissimilarities was explained by the constrained variables compared to bacteria and archaea (adjusted $R^2=0.16$; $P=0.001$). However, contrary to the archaeal community, both altitude ($f=3.04$; $P=0.001$) and shade ($f=1.54$; $P=0.001$) significantly influenced the fungal community assembly, while the influence of $N-NH_4^+$, pH, EC, MBN, MBC and moisture were consistent, regardless of microbial domain.

Coffea dominant and differentially abundant phylotypes
The composition of the microbial communities was dominated by several distinct phyla (Fig. 4). For bacterial communities, four predominant phyla were identified: *Proteobacteria* (24%), *Verrucomicrobia* (23%), *Actinobacteria* (19%), and *Acidobacteria* (15%). In contrast, the archaeal community was overwhelmingly dominated by *Thaumarchaeota*, which constituted 98% of its population. Among the fungi, *Mucoromycota* was the most prevalent (54%), followed closely by Ascomycota (40%) (Fig. 4). At this taxonomic rank, there were no differentially abundant archaea or fungi across the different altitudes and shades, but for bacteria, some phyla had distinct patterns across altitudes. For instance, higher altitudes increased the relative abundance of *Acidobacteria* (LDA=5.57; adjusted $P=0.005$), *Proteobacteria* (LDA=5.46; adjusted $P=0.006$) and *Planctomycetes* (LDA=4.97; adjusted $P=0.001$). At the lowest altitude of 600 m, the abundance of *Bacteroidetes*, *Chloroflexi*, *Nitrospirae*, and *Synegistetes* were significantly higher,
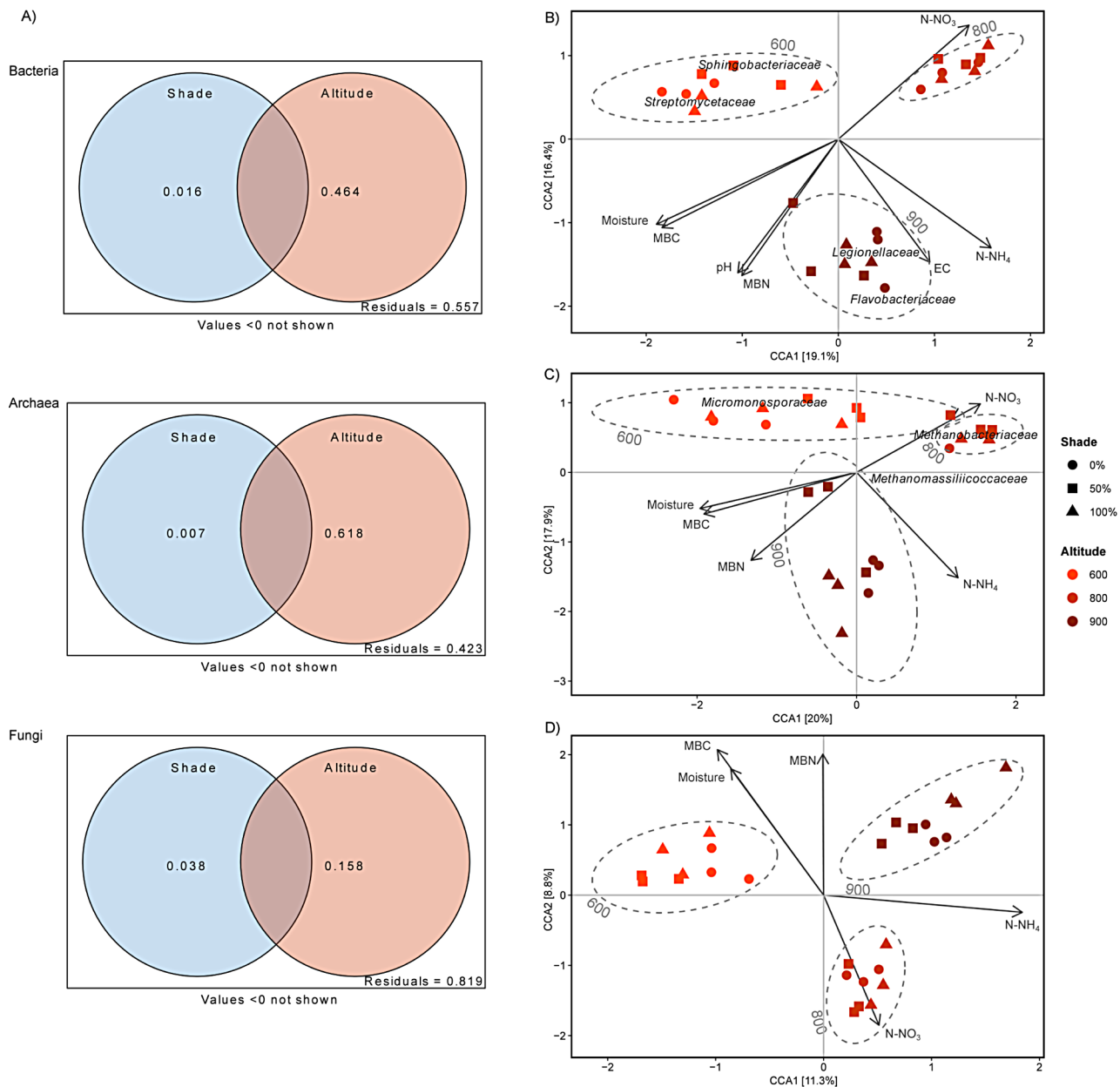
Fig. 3 Bacterial, archaea and fungal community differentiation explained by shade and altitude based on variation partitioning analysis (**A**). Canonical correspondence analysis (CCA) explaining bacterial (**B**), archaeal (**C**), and fungal (**D**) communities relationship to soil physicochemical parameters along a shade and altitudinal gradient. The ellipses represent a 95% confidence interval in multivariate space according to each group's centroid

while the biomarkers of the mid-level altitude (800 m) included *Verrucomicrobia* and *Actinobacteria* (Fig. 4).

At the genus level, bacterial communities showed a predominance of *Chthoniobacter* (22%), followed by *Rhodoplanes* (8%), *Acidobacterium* (6%) and *Arthrobacter* (5%) (Fig. 5). *Nitrososphaera* was the dominant archaea (85%), especially at an altitude of 600 m while the abundance of *Nitrosopumilus* (13%) increased at higher altitudes (Fig. 5). In the case of fungi, *Linnemannia* (41%) was predominant in all altitudes, followed by *Mortierella* (10%), *Fusarium* (7%) and *Penicillium* (5%) (Fig. 5).

At the genus taxonomic rank, 103 bacterial phylotypes were differentially abundant across altitudinal levels (Fig. 6). In the case of bacteria, *Gaiella* (LDA 5.45; FDR-adjusted $P < 0.01$) and *Natranaerobaculum Natranaerobaculum* (LDA 5.06; FDR-adjusted $P = 0.03$) were among the most predominant biomarker phylotypes at the lowest altitude of 600 m, *Chthoniobacter* (LDA 5.80; FDR-adjusted $P < 0.01$) and *Stella* (LDA 4.89; FDR-adjusted $P < 0.01$) were predominant biomarkers at 800 m, while *Occallatibacter* (LDA 5.30; FDR-adjusted $P = 0.01$) and *Paludibaculum* (LDA 4.89; FDR-adjusted $P < 0.02$) were
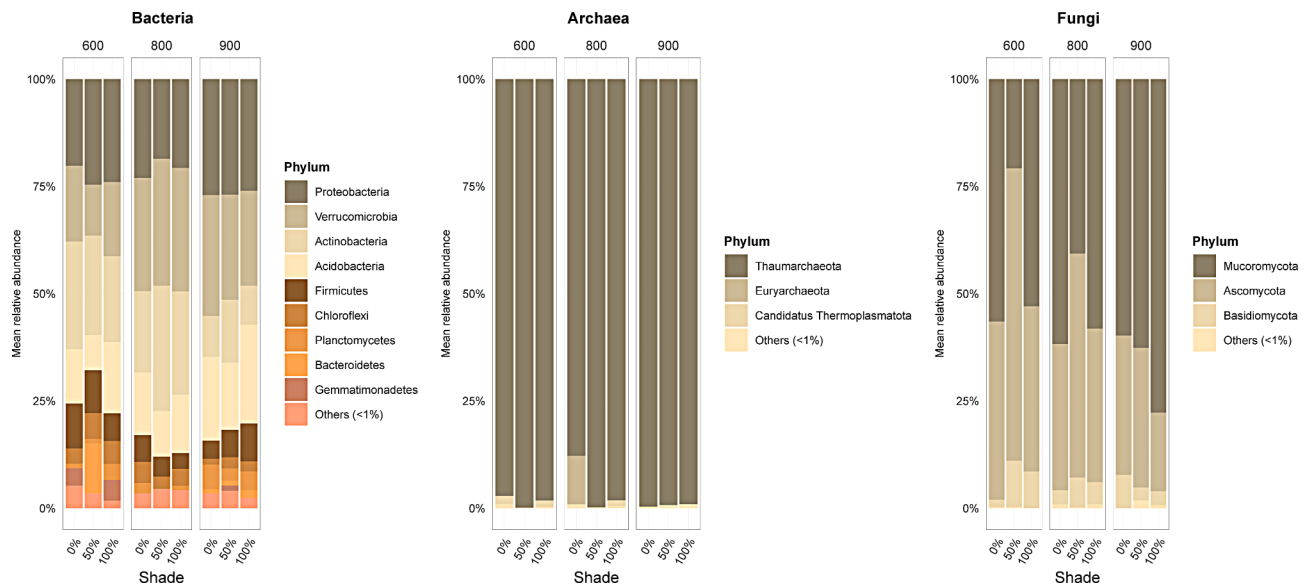
Fig. 4 Phylum rank taxonomic profile of the microbial communities of *C. arabica* grown under different altitudes (600 m, 800 m, and 900 m) and under different levels of shade from native trees (0%, 50% and 100%)
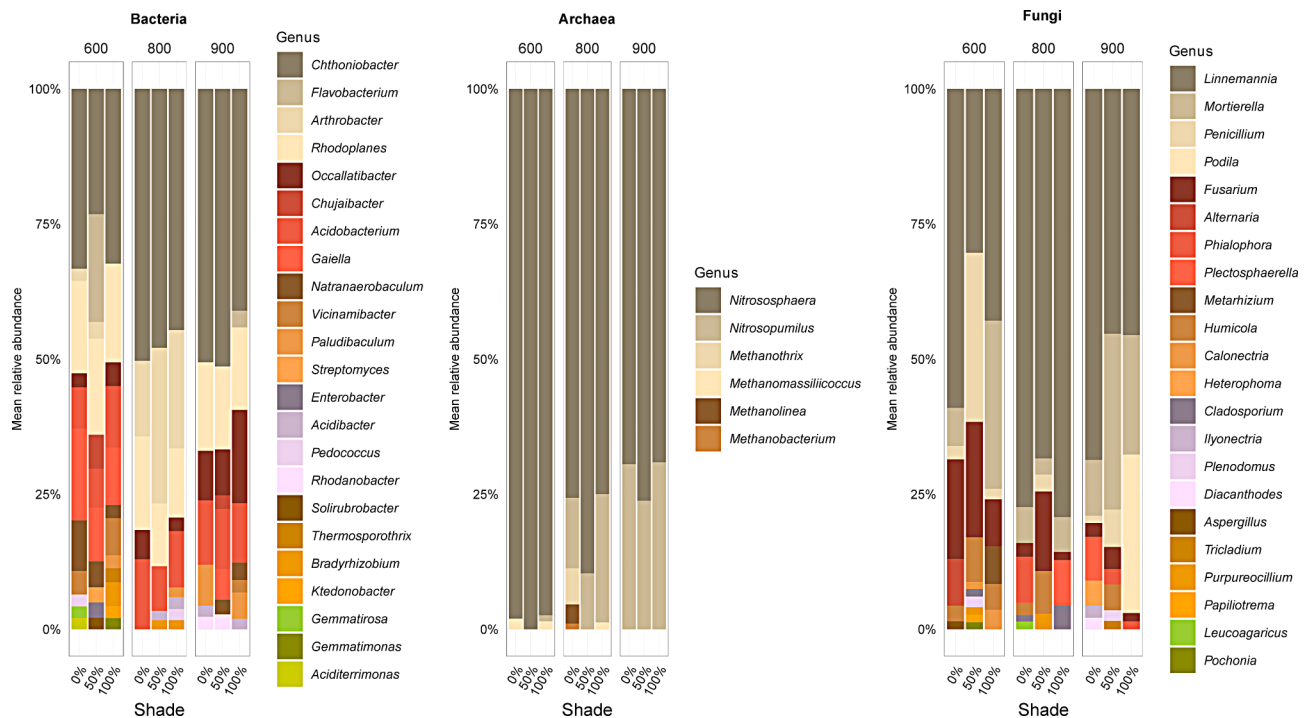

Fig. 5 Genus level taxonomic profile of the microbial communities of *C. arabica* grown under different altitudes (600 m, 800 m, and 900 m) and different levels of shade from native trees (0%, 50% and 100%). Only phylotypes with a relative abundance of at least 1% in any of the samples are presented

predominant biomarkers at 900 m. Only four archaea phylotypes were differentially abundant at the different altitudinal levels: *Nitrososphaera* (LDA 6.11; FDR-adjusted $P<0.01$) and *Methanomassiliicoccus* (LDA 5.12; FDR-adjusted $P=0.60$) at 600 m and *Nitrosopumilus* (LDA 6.15; FDR-adjusted $P<0.01$) and *Methanocaldococcus* (LDA 3.60; FDR-adjusted $P=0.20$) at 900 m (Fig. 6).

Additionally, among the classified fungal phylotypes, 30 genera were differentially abundant across altitudinal levels. Of these, *Exophiala* (LDA 4.27; FDR-adjusted $P=0.05$) and *Cladophialophora* (LDA 4.21; FDR-adjusted $P=0.04$) were among the predominant biomarker phylotypes at 600 m, *Gliocladiopsis* (LDA 4.44; FDR-adjusted $P=0.05$) and *Cystofilobasidium* (LDA 4.12; FDR-adjusted
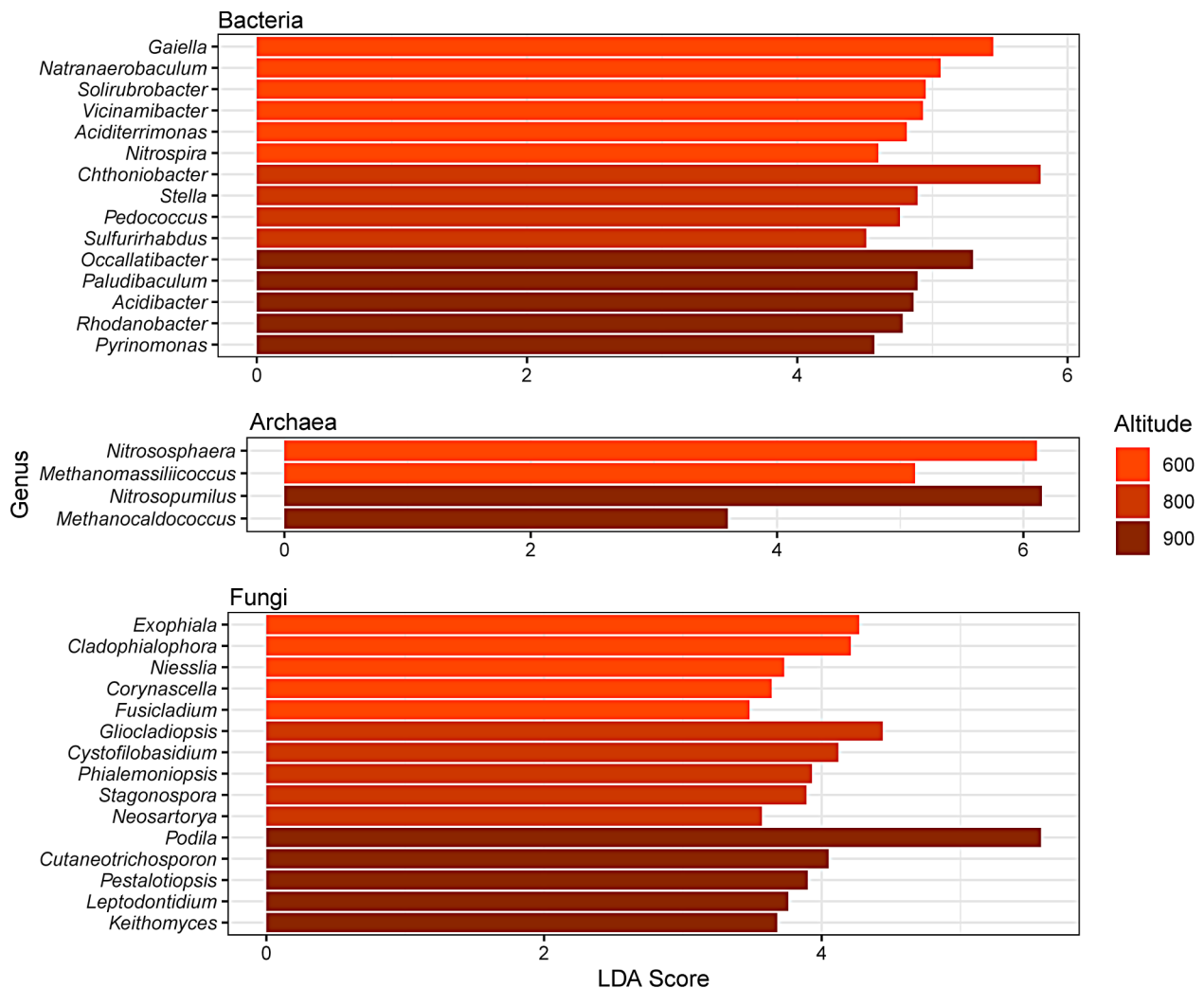
Fig. 6 Differentially abundant Bacteria, Archaea and Fungi at the genus rank, across altitudes (600 m, 800 m, and 900 m). The differentially abundant data for bacteria and fungi (LDA ≥ 2.0; FDR-adjusted P ≤ 1.0) are a subset that was sorted according to adjusted p-values, LDA scores and altitudinal groupings. The complete list of differentially abundant genera is presented in Tables S9 and SS10

P<0.01) at 800 m and *Podila* (LDA 5.58; FDR-adjusted P=0.01) and *Cutaneotrichosporon* (LDA 4.05; FDR-adjusted P<0.01) at 900 m (Fig. 6).

The prediction of bacterial community functions revealed significant variations across different altitudes, highlighting distinct metabolic adaptations that potentially support plant growth and productivity. As detailed in Fig. 7, several ecologically relevant KEGG pathways were identified as differentially abundant at 900 m, 800 m, and 600 m, each corresponding to specific environmental stimuli and microbial interactions, organic matter decomposition and fermentation, and the breakdown of plant-derived compounds, respectively. The LDA scores and FDR-adjusted P-values for these pathways are presented in Table S10. At the highest elevation of 900 m, bacterial communities showed a predominance of pathways associated with the response

to environmental stimuli and microbial interactions. Key pathways included those related to oxidative stress response, bacterial chemotaxis, and biofilm formation.

At the mid-level elevation of 800 m, the bacterial community was enriched in pathways involved in the decomposition of organic matter, fermentation processes, and bioremediation. Notable pathways included those related to amino acid and carbohydrate metabolism, butanoate and propanoate metabolism, and the degradation of aromatic compounds and chlorocyclohexane.

At the lowest elevation of 600 m, the bacterial community function was characterized by pathways related to the breakdown of complex plant-derived compounds. Pathways such as cellulose and lignin degradation, starch and sucrose metabolism, and phenylpropanoid biosynthesis were significantly represented.
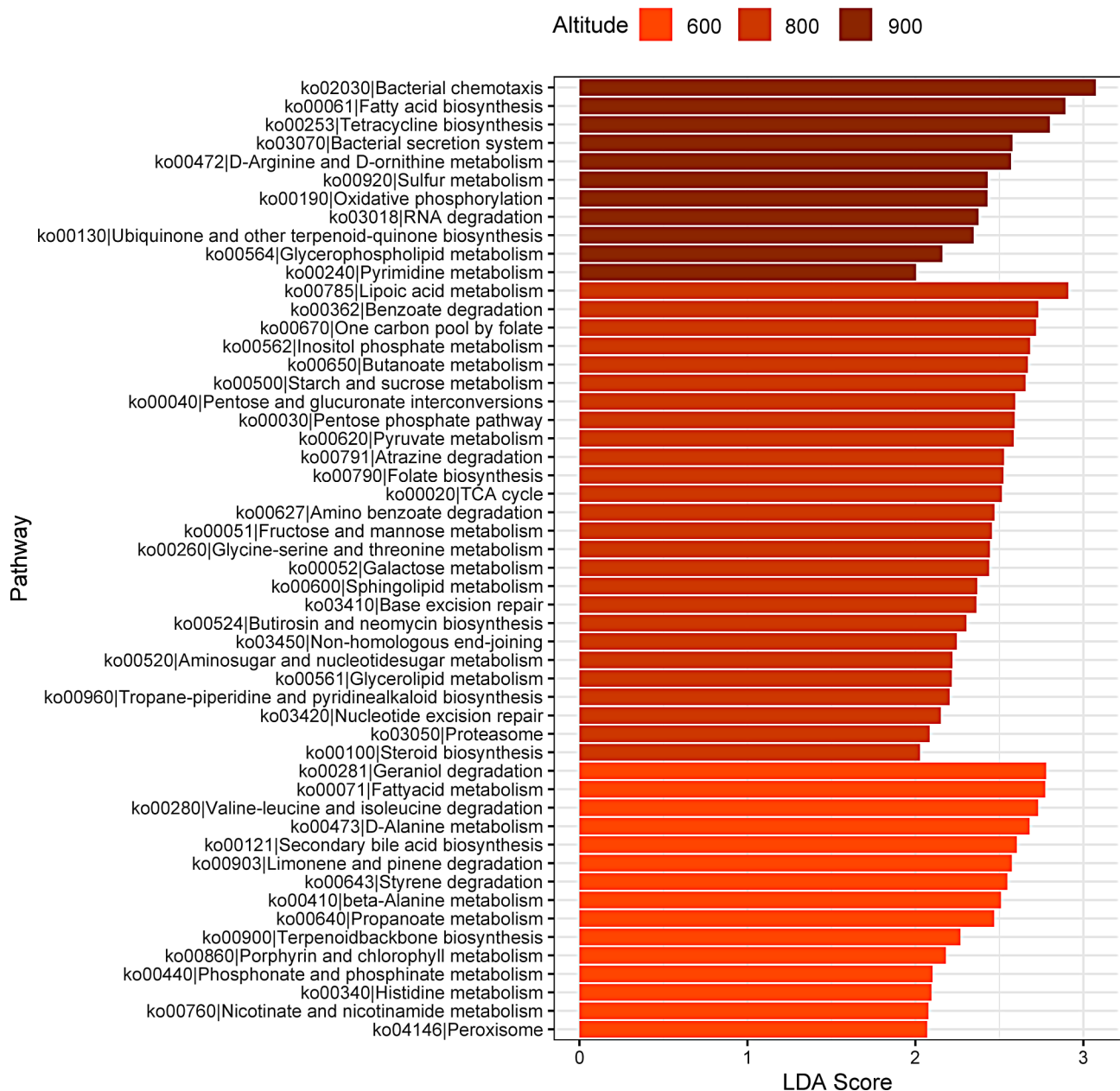
Fig. 7 PICRUSt2 predicted differentially abundant KEGG pathways along the altitudinal gradient ($P \leq 0.01$; LDA ≥ 2.0)

Discussion

The soil microbiome encompasses diverse microorganisms residing near plant roots, playing a pivotal role in ecosystem functioning. It not only shapes the structure and composition of biodiversity but also critically influences plant health and fitness. Its interactions with plants significantly contribute to various ecosystem processes, including nutrient cycling, soil fertility, and plant disease resistance, thereby underpinning the overall health and resilience of ecosystems. Recent research has devoted special attention to the nexus plants-microbes-environment, as an essential component of climate-smart,

resilient, and sustainable agriculture [57–59]. In this study, a comprehensive analysis of the soils under the canopy projection of *Coffea arabica* was conducted to evaluate the shifts in microbial composition and associated functions along a gradient of elevation (600 m, 800 m, and 900 m) and shade (0%, 50%, 100%). A set of soil variables (organic carbon, microbial biomass carbon and nitrogen, available phosphorus, extractable potassium, nitrate, ammonium, moisture, pH, electrical conductivity), and microbial enzyme activities (urease, β-glucosidase, acid- and alkaline phosphatases) were analysed. In addition, metabarcoding was performed

to study microbial communities (bacteria, archaea, and fungi), and their putative functions.

Most soil parameters were highly variable (high standard deviation) and only influenced by altitude, with a particularly differentiated pattern at 800 m, where available phosphorus (P-Olsen), soil organic carbon (SOC) and nitrate (NO_3^-) were significantly higher, and microbial biomass carbon and nitrogen (MBC and MBN, respectively) and moisture were significantly lower when compared to 600 m and 900 m (Table 1). Considering that soil elementary composition along the elevation and shade gradients was quite homogeneous (fine clay, acidic, non-saline, non-calcareous, 8–14% organic matter; Table S11), this pattern might be driven by the vegetation composition, i.e. *C. arabica*, shade tree species and other natural vegetation [60, 61], which in turn shapes the microbial communities [62]. Gota et al. [63] reported that the influence of agroforestry species on soil chemical properties is specific to each species and is not altered by altitude changes. Accordingly [60], reported that shade trees were the main drivers of soil composition in cocoa agroforestry systems (AFS), improving pH, NH_4^+, NO_3^-, total C and N, biomass and P-Olsen contents. However, soil function improvement varied across AFS, being low with fruit species (*Canarium* and *Dacryodes*), moderate with tree legumes (*Albizia*), and high with timber trees (*Milicia* and *Ceiba*). In the present study, the canopy shade trees in the experimental plots located at 800 m were mostly legumes (*Albizia adianthifolia* and *Erythrina lysistemon*), both included on the top list of N_2-fixing fertilizers [64], while at 600 m and 900 m, non-legume trees were also dominant. In addition, the differences observed between the three altitudes might also be associated with climate variations, particularly rainfall and temperature, which shape vegetation composition and structure, implying differences in the amount and composition of litter [65, 66]. This hypothesis is corroborated by the enzyme activity assays, particularly β-glucosidase, and phosphatases, which presented higher activities at 800 m, i.e. 65.73±10.87, 686.61±149.97, and 189.96±42.47 μg p-nitrophenol g^{-1} h^{-1}. Enzymatic activities in soils are important for the decomposition of organic matter and mineralization of nutrients, and are useful indicators of soil biological activity and deductively of soil health [67, 68].

Metabarcoding analysis revealed high species richness in bacterial communities, with the lowest indexes recorded at 800 m (Fig. 1; Table S4). This might explain the lowest MBC and MBN values obtained at this altitude. As for the soil parameters, altitude was the most important driver of bacterial communities, explaining 46% of the variation (R^2=0.493, *P*=0.001), while shade did not produce significant changes (R^2=0.01; *P*=0.113) (Figs. 2A and 3). The predominant phyla (*Proteobacteria*,

Verrucomicrobia, *Actinobacteria*, and *Acidobacteria*) and the average Shannon diversity indexes were similar to those reported previously for *Coffea* spp. soils, i.e. between 6 and 7 [22, 24–26]. Although the abundance of various phyla varied along the elevation gradient (Fig. 4), key plant growth-promoting (PGP) functions such as, nutrient cycling, organic matter decomposition, phosphate solubilization, soil aggregation, and biocontrol, were consistently present across all clusters, in line with the studies of [69, 70]. Accordingly, the predominant genera, i.e. *Chthoniobacter*, *Rhodoplanes*, *Acidobacterium*, and *Arthrobacter* (Fig. 5), incorporated these major PGP attributes [71–73]. While the presence of *Rhodoplanes* and *Arthrobacter* in *Coffea* soils has been previously reported, the presence of *Chthoniobacter* and *Acidobacterium* seems to be exclusive of our agro-ecological system [23, 26, 74, 75]. A set of 103 biomarker phylotypes were differentially abundant across the altitude gradient (Fig. 6), from which the most prominent were: *Gaiella* and *Natranaerobaculum* at 600 m; *Chthoniobacter* and *Stella* at 800 m; and *Occallatibacter* and *Paludibaculum* at 900 m. All of them are included in the group of PGP bacteria with vital functions in forest ecosystems. The genus *Gaiella* is bound to microbe-microbe interactions [76, 77], playing an important role in biocontrol, e.g. of *Fusarium oxysporum* in tomato [78] and strawberry [79]; some members also contribute to nitrogen cycling through the reduction of nitrate to nitrite [80]. *Natranaerobaculum* is typical of hypersaline soda environments [81], but its occurrence in forest soils seems to be related to health biomarkers [82]. *Chthoniobacter* is involved in nutrient cycling and produces secondary metabolites [83, 84], associated with the control of bacterial wilt [85] and growth of beneficial bacterial communities [84]. Although *Stella* and *Paludibaculum* bacteria are not common soil taxa, their ecological function might be related to organic matter decomposition [86]. Additionally, *Paludibaculum* may also enhance plant chlorophyll content [87]. *Occallatibacter* may play a relevant function in denitrification [88] as well as soil remediation [75]. Despite the fact that the basic PGP functions are likely maintained along the elevational gradient, taking into account that P-Olsen, SOC and NO_3^- were significantly higher at 800 m, we hypothesize that the microbial consortium at this altitude is more effective in nutrient cycling. Interestingly, despite the presence of legume trees and unlike what has been reported in similar studies in coffee [23, 25, 26, 89], the presence of diazotrophs (Nitrogen-fixing bacteria) was not prominent in this study. However, collectively, a considerable set of symbiotic (*Bradyrhizobium*, *Mezorhizobium*, and *Rhizobium*) and non-symbiotic (e.g. *Agrobacterium*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Clostridium*, *Microvirga*,

Nitrospirillum, Paraburkholderia) genera is represented in the bacterial pool.

The richness and diversity of archaea was considerably lower than that of bacteria, and fungi. Similarly to the soil parameters and bacterial communities, the patterns of richness and diversity also varied with altitude. However, in this case, the lowest values of richness and diversity were recorded at the lowest altitude of 600 m. *Thaumarchaeota*, typically including ammonia-oxidizing archaea (AOA), was the dominant phylum along the altitudinal gradient, similar to the observations of [90] for alpine forest soils [22], for the coffee rhizosphere, and [91] for the Himalayas. Additionally, this is the most prominent endophytic phylum in several crops, including coffee [92]. *Euryarchaeota*, which is associated with methanogenesis [90], was detected only at 800 m and under full sun (0% shade) and less represented (<1%) in all other comparison groups. The irregular and low abundance (<10%) of this phylum has also been reported in other soils, e.g. along an elevation gradient in alpine forest soils [90], and in the rhizosphere of full-sun coffee in consociation with other food crops [22]. Both phyla are associated with a wide range of PGP functions, e.g. improvement of plant growth, tolerance to biotic and abiotic stress, and nutrient solubilization and assimilation [93]. In line with the findings of [90] and [22], in this study, *Nitrososphaera* (phylum *Thaumarchaeota*) was the most abundant genus (70–100%) in all comparison groups. *Nitrosopumilus*, another AOA belonging to *Thaumarchaeota*, was the second most abundant genus (<30%), particularly at 800 m and 900 m. These two genera play a crucial role in the nitrogen cycle (converting NH_3^+ into NO_2^-), i.e. bioavailability, soil fertility, and environmental regulation of nitrogen.

Fungi richness and diversity was considerably high (410.26±91.32 observed ASVs and 4.29±1.19 Shannon indexes, on average) (Fig. 1) and within the range of the values reported for the soil microbiome of *C. arabica* (e.g [24, 94]. Alpha-diversity of fungi was neither affected by shade (Table S7), nor altitude (Table S8), while beta-diversity (Fig. 2C) was driven by altitude (R^2=0.177, *P*=0.001), but unaffected by shade (R^2=0.110, *P*=0.068). *Mucoromycota* and *Ascomycota* were the dominant phyla (>90% in total) (Fig. 5). As for the other microbial domains, both phyla include soil mycorrhizal fungi, saprotrophic decomposers and endophytes, with key functions in e.g. nutrient cycling and assimilation, or plant protection [95, 96]. *Linnemannia* (*Mucoromycota*) was the predominant genus in all comparison groups. Interestingly, this genus has never been reported previously in *Coffea* [22–25, 73, 97–99]. *Linnemannia* and *Mortierella* (the second most abundant genus in our study) have been reported as strong elicitors of plant growth in maize [99], wheat [100], and Arabidopsis [101, 102], likely driven by

phytohormones [102]. In the *Ascomycota* phylum, the most prevalent genera were *Fusarium* and *Penicillium*. These genera are linked to a broad spectrum of ecological functions, including decomposition, nutrient cycling, biocontrol, and bioremediation [103, 104]. Such functions are visible among the 30 biomarkers differentially abundant across the three altitudes, from which *Exophiala* and *Cladophialophora* (600 m), *Gliocladiopsis* and *Cystofilobasidium* (800 m), and *Podila* and *Cutaneotrichosporon* (900 m) were the predominant genera (Fig. 6). For instance, *Exophiala* promotes plant stress tolerance and growth [105]; *Cladophialophora* is associated with plant protection and productivity [106]; *Gliocladiopsis* enhances tolerance against biotic ad abiotic factors [107, 108]; and *Cysttofilobasidium* is also a potential biocontrol agent [109]. While the ecological roles of *Podila* and *Cutaneotrichosporon* remain less defined, the presence of *Podila* in alpine forest soils has been documented [110], and *Cutaneotrichosporon* shows promise in biotechnology for the food and cosmetic industries [111], as well as in biofuel and bioplastic production [112].

The KEGG pathway data across different altitudes revealed distinct metabolic adaptations of microbial communities to their respective environments (Fig. 7). Firstly, at 800 m the metabolic pathways to lipoic acid and benzoate degradation indicated a rich organic matter environment and a microbial ability to utilize diverse organic compounds [113]. This altitude also shows active fermentation processes, as evidenced by butanoate metabolism and pyruvate metabolism pathways, hinting at the decomposition of organic materials. Notably, the atrazine degradation pathway suggests a capability to bioremediate certain pollutants, reflecting potential exposure to agricultural chemicals in this mid-altitude environment [114]. This observation suggests a robust microbial activity adapted towards organic matter turnover and pollutant degradation, which aligns with the higher levels of soil organic carbon and nitrate observed at this altitude, corroborating the hypothesis formulated above regarding the higher effectiveness of the microbial consortium at this altitude.

At 900 m, the high abundance of pathways such as bacterial chemotaxis and secretion systems (Fig. 7) suggests a heightened sensitivity to environmental stimuli and an advanced capacity for microbial interactions, possibly aiding adaptation to cooler, more variable high-altitude conditions. The biosynthesis of tetracycline and ubiquinone is usually related to microbial defense and stress response, vital for survival in a potentially challenging high-altitude environment [115]. Key pathways such as oxidative phosphorylation and sulfur metabolism highlight the efficiency in energy production and nutrient cycling, crucial in nutrient-limited high-altitude conditions.

At the lowest altitude of 600 m, the dominance of pathways related to the breakdown of plant-derived compounds, such as geraniol, limonene, and pinene degradation [116] suggests a closer interaction between plants and microbes, likely due to the more direct influence of vegetation at this altitude. Basic cellular processes are represented by pathways like D-alanine metabolism and steroid biosynthesis, suggesting a diverse microbial community engaged in various ecological functions. Moreover, phosphonate and phosphinate metabolism pathways at this altitude underline roles in phosphorus cycling, essential for both plant growth and microbial activity within soil ecosystems [117]. Altogether, the microbial community at this altitude seems highly adapted to decomposing plant materials, thereby facilitating nutrient release and availability for plant uptake.

Overall, the functional prediction analysis underscores the adaptive metabolic strategies employed by bacterial communities across different altitudes. These strategies are integral to supporting the growth and productivity of *C. arabica* by enhancing nutrient cycling, organic matter decomposition, and resilience to environmental stressors. The differential abundance of these KEGG pathways not only reflects the unique environmental conditions at each altitude but also highlights the potential of utilizing these microbial functions for sustainable coffee cultivation and ecosystem management.

Conclusion
In summary, this study highlights the significant impact of elevation on the microbiome of *Coffea arabica* soils within agroforestry systems. At different elevations, particularly at 800 m with predominantly legume trees, distinct microbial communities and soil chemistry profiles emerged, suggesting elevation-specific microbial functions likely related to nutrient cycling and plant growth. Despite the variations in microbial biomass and diversity, plant growth-promoting functions remained consistent across the altitudinal gradient. The study offers valuable insights into sustainable coffee cultivation, emphasizing the role of the soil microbiome in ecosystem health. Understanding these complex microbial interactions paves the way for developing climate-smart agricultural practices that leverage natural processes for improved crop resilience and productivity, aligning with the goals of sustainable agroecosystem management.

Supplementary Information
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Supplementary Material 1

Data availability
The datasets generated and analysed during the current study are available in the Sequence Read Archives (SRA) of the National Centre for Biotechnological Information (NCBI) under the accession number PRJNA924052 [https://www.ncbi.nlm.nih.gov/sra/?term=PRJNA924052; https://www.ncbi.nlm.nih.gov/bioproject/924052]. The remaining data and materials are included in this article (Figures, Tables, and Supplementary information).

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
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Competing interests
The authors declare no competing interests.

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