Microbial community induced by host genotype in Coffea canephora: from field to fermentation

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Research Article

Keywords: Host genotype effects, Coffee Fermentation, Post-harvest processing, Beta diversity

Posted Date: March 12th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9021084/v1

Additional Declarations: No competing interests reported.

Abstract

The microbial community during coffee processing results from the interaction among host genetics, chemical composition, and post-harvest environmental selective pressures; however, the relative contribution of each factor remains poorly understood. In this study, we investigated how two genetically distinct *Coffea canephora* genotypes modulate bacterial and fungal communities from freshly harvested fruits to spontaneous and *Saccharomyces cerevisiae*-induced fermentations. Using high-throughput sequencing, beta diversity metrics, and variance decomposition by ASCA, we quantified the effects of genotype, processing, and their interaction. Processing acted as the main driver of microbial restructuring, clearly separating natural fruits from fermented samples, while genotype maintained a consistent influence on microbial community composition. ASCA models confirmed that processing explained the largest proportion of variance, followed by genotype and the genotype × process interaction. Taxonomic analyses revealed bacterial convergence toward profiles dominated by the genus *Weissella*, whereas fungal communities remained more genotype-dependent, including differences in the establishment capacity of *S. cerevisiae*. These patterns indicate that environmental factors and host-mediated selection jointly shape microbial profiles in *C. canephora*. Moreover, the genetic diversity of *C. canephora* supports distinct ecological responses during fermentation, providing a foundation for targeted fermentation strategies integrating plant genetics and microbial ecology.

INTRODUÇÃO

Microbial diversity associated with agricultural crops plays a central role in maintaining plant health, ecological balance, and the biochemical processes occurring throughout the production chain [1]. Within the coffee agroecosystem, fungi and bacteria colonize different plant compartments, including roots [2], leaves [3], and fruits [4], directly influencing plant physiology, protection against pathogens, and beverage quality. The endogenous microbiota of *Coffea* sp. fruits constitutes the foundation for the microbiological events that take place during fermentation [5].

Coffee fruit fermentation is a traditional practice historically used to facilitate mucilage removal and drying [6]. However, recent advances in microbial ecology and biotechnological applications have transformed this process into a strategic tool for modulating coffee sensory quality. The activity of native microbial communities during fermentation leads to the formation of metabolites that directly influence the sensory profile of the beverage [7]. Lactic acid bacteria and yeasts are key agents in this process, being responsible for chemical modifications that affect sensory attributes such as acidity, sweetness, body, and aromatic complexity [8, 9].

Thus, fermentation has become an important trend in modern coffee production. This approach is particularly promising for *Coffea canephora*, including the Robusta and Conilon varieties, which have historically occupied lower-value sensory markets but have recently gained recognition for their quality potential when subjected to appropriate post-harvest processing techniques, such as fermentation [10]. Nevertheless, the growing demand for fermented Robusta and Conilon coffees with distinct sensory

identity highlights the need to better understand the factors determining the dynamics and impacts of the fermentative process on the chemical and sensory quality of the beverage.

An often overlooked aspect in this context is the impact of plant genotype on the structuring of fruit-associated microbial communities. The allogamous reproductive system and self-incompatibility mechanism promote extensive genetic diversity in *C. canephora*, requiring the cultivation of distinct genotypes to ensure cross-pollination and fruit set [11–13].

This genetic variability may directly affect plant–microorganism interactions, influencing the diversity and dynamics of fruit-associated microbial communities from the field to post-harvest processing, including fruit fermentation. Therefore, different *C. canephora* genotypes may recruit distinct microbiota with equally distinct metabolic and fermentative capacities, potentially affecting final product quality and the reproducibility of post-harvest processing.

The interaction between plant genotype and microbial community has been widely investigated in olive [14], tomato [15], and wheat [16], revealing significant effects on microbiome structure as well as on agronomic and technological traits. However, a considerable knowledge gap remains regarding *C. canephora*, whose genetic diversity is still underexplored in microbiological studies.

Understanding how different genotypes modulate fruit-associated microbiota and how this modulation influences fermentation represents a critical step toward the development of optimization strategies aimed at improving *C. canephora* beverage quality. Moreover, microbiological variability driven by plant genotype imposes additional challenges to the standardization of the fermentative process, as distinct microorganisms exhibit specific metabolic rates, enzymatic profiles, and fermentative pathways [17].

Thus, genotypes exhibiting differentiated microbial behavior may require specific adjustments in post-harvest management, fermentation time, and environmental conditions, further increasing the complexity of implementing reproducible and scalable protocols. This genotype-associated microbial diversity may explain why coffee fruit fermentations conducted under identical physicochemical conditions can result in distinct chemical profiles and sensory attributes.

In this context, the objective of this study was to characterize the microbial community profile (bacteria and fungi) associated with different *C. canephora* genotypes, from fruits in the field through the fermentation stage, in order to understand how plant genetic background influences microbial diversity and the dynamics of different fermentation processes.

Materials and methods

Raw material collection

The experiment was conducted on a coffee farm located in the municipality of Jaguaré, northeastern Espírito Santo State, Brazil (18°55'42.98" S; 40°10'41.10" W; altitude 93 m). According to the Köppen

climate classification, the region is classified as Aw, characterized as a tropical humid climate with a dry winter, an average annual temperature of 23.5°C, and a mean annual precipitation of 1,291 mm [18].

Two *C. canephora* var. Conilon genotypes (LBI and Verdim TA) were selected for this study. These genotypes are cultivated on the same farm, within the same field plot, and under identical agronomic management conditions (Fig. 1). In addition, the agronomic potential and performance of these genotypes have been previously evaluated [19–21].

Figura 1 Croqui da área de coleta com a localização dos genótipos na lavoura e genótipos utilizados no estudo. LBI (Partelli et al., [22]) e Verdim TA (Partelli et al., [23]).

The experimental design consisted of a sampling arrangement of two genotypes × five distinct points within the field plot × five plants per point, totaling 25 plants per genotype.

Fruit sampling followed the protocol described by Gomes et al. (2024). For the analysis of initial microbial diversity, five ripe fruits with peduncle were collected per plant. Fruits from each plant were pooled to form a composite sample, and each plant was considered an experimental unit. Thus, a total of 25 fruits per genotype were analyzed.

The composite samples were placed in sterile plastic bags and stored at − 20°C until microbiological analyses were performed.

Post-harvest processing

In addition to the fruits collected for characterization of the microbiota present on the plant, fruits were also harvested for the implementation of post-harvest processes and subsequent analysis of microbial dynamics throughout fermentation.

For this stage, 60 kg of fruits per genotype were collected and subjected to two different post-harvest processes (Table 1): spontaneous fermentation and Saccharomyces cerevisiae-induced fermentation. The objective was to monitor the evolution of the microbial community from freshly harvested fruits to distinct fermentation regimes, allowing the assessment of the effect of anthropogenic intervention on microbiome structuring.

Fruits were manually harvested, avoiding any contact with the soil. After harvesting, green and floating fruits were discarded. The selected material exhibited approximately 90% cherry-stage ripeness. Subsequently, the fruits were washed and sorted using a stainless steel washer and a manually operated tilting separator.

After standardization, the fruits were stored in hermetic GrainPro© bags until the onset of fermentation processes. The lot was then divided and subjected to the two post-harvest processing methods described in Table 1.

Table 1
Post-harvest processing methods applied to *Coffea canephora* var. Conilon fruits from the LBI and Verdim genotypes.

Process	Description
Fermentation induced by Saccharomyces cerevisiae	4 L of peeled Cherry Coffee, without mucilage removal, followed by inoculation of 1 g/dL of *S. cerevisiae*, plus 2 L of water. Adapted from Pereira et al. [24]
Natural process or Spontaneous fermentation	4 L of natural coffee put directly to rest in a suspended yard for the drying process.

Fermentation was conducted for 36 h under anaerobic conditions, with or without the addition of *S. cerevisiae* (commercial strain Fleischmann, Heilsbronn, Germany). Each fermenter (polyethylene container) received four liters of ripe coffee fruits and two liters of water. The container was hermetically sealed at time zero and remained closed throughout the entire fermentation period. The initial concentration of S. cerevisiae was 10^7 CFU/mL.

In the Natural treatment, coffee fruits were directly subjected to drying on a covered suspended patio (African bed system) under indirect sunlight exposure. Drying was carried out for 15 to 18 days using an elevated structure covered with transparent plastic sheeting. During this period, ambient temperature was continuously monitored, with an average of 19.54°C, daytime maxima reaching 45.72°C, and nighttime minima of 9.07°C. Thermal monitoring was performed using an Arduino Uno R3 system coupled to a DHT22 AM2302 sensor, with data transmission via an HC-06 RS232 Bluetooth module and storage on an SD card.

After post-harvest processing, five coffee fruits from each treatment (Table 1) were collected for microbial diversity analysis. These samples were placed in plastic bags and stored at − 20°C until microbiological analyses were performed.

DNA Extraction, PCR, and Sequencing

DNA Extraction, PCR, and Sequencing
A total of 250 mg of each sample (freshly harvested fruits from the field and post-harvest processed samples) was subjected to DNA extraction using the Precellys® 24 homogenizer (Bertin Instruments) at 4,000 rpm for 50 s and the NucleoSpin Soil kit (Macherey-Nagel, Germany). The integrity and quantity of the extracted DNA were assessed by electrophoresis on 0.8% agarose gel stained with ethidium bromide and by UV–visible spectrophotometry at 260 and 280 nm.

For microbial community assessment, the 16S rRNA gene (V3/V4 region) and ITS1 region were amplified by polymerase chain reaction (PCR) using the primer pairs 341F (5'-CCTAYGGGRBGCASCAG-3') and 806R (5'-GGACTACNNGGGTATCTAAT-3'), and ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS2 (5'-GCTGCGTTCTTCATCGATGC-3'), respectively. PCR products were evaluated by electrophoresis on 2% agarose gel using SYBR Green staining. Samples showing bands between 400 and 450 base pairs (bp)

were used for library construction with the NEBNext® Ultra™ DNA Library Prep Kit for the Illumina platform.

Each amplified sample was indexed with barcodes, and library quality was assessed using a Qubit 2.0 fluorometer (Thermo Scientific) and an Agilent Bioanalyzer 2100 system. Libraries were sequenced on the Illumina NovaSeq 6000 platform using paired-end 250 bp reads.

Bioinformatics Analyses

Adapters and barcodes were removed from the sequencing reads. The remaining fragments were subjected to quality control by removing low-quality sequences, chimeras, and singletons (sequences present only once in a given sample). High-quality sequences were then processed using the Divisive Amplicon Denoising Algorithm (DADA2) [25] to determine Amplicon Sequence Variants (ASVs). Taxonomic assignment of ASVs was performed using bacterial [26] and fungal [27] reference databases. Subsequently, contaminant sequences of mitochondrial, plastidial, or non-fungal origin were removed. All previous steps were carried out using the QIIME2 platform [28].

Microbial diversity indices, relative abundance of microorganisms, and principal component analysis (PCA) were performed using the R software environment [29].

Results

Principal component analysis (PCA) revealed a structured pattern in the beta diversity of bacterial and fungal communities associated with the two evaluated genotypes (LB1 and Verdim) across the three processing conditions (natural, spontaneous fermentation, and induced fermentation) (Fig. 2).

For bacterial community beta diversity (Fig. 2A), the first two principal components jointly explained 62.13% of the observed variability, indicating strong discriminative power of the distance matrix.

Figura 2 Principal Coordinates Analysis (PCoA) of the beta diversity structure of bacterial (A) and fungal (B) communities associated with the LB1 and Verdim genotypes under the natural (N), spontaneous fermentation (SF), and Saccharomyces cerevisiae-induced fermentation (SC) treatments.

Along PC1 (52.6%), a clear separation was observed between bacterial communities associated with natural fruits and those subjected to fermentative processes, regardless of genotype (Fig. 2A). Natural samples consistently clustered on the negative side of this axis, whereas samples from spontaneous or induced Fermentation were positioned on the positive side, reflecting pronounced shifts in community structure following the transition from the fruit environment to the fermentative environment.

The ordination of fungal communities (Fig. 2B) revealed structured patterns of dissimilarity between genotypes and processing conditions, with 59.65% of the total variability explained by the first two principal components.

ASCA analysis applied to the microbial communities revealed consistent patterns in the contribution of genotype, processing, and their interaction to the multivariate structure of both bacterial and fungal communities (Fig. 3).

For the bacterial community (Fig. 3A), the genotype factor explained 12.93% of the total variance, resulting in a clear separation between LB1 and Verdim, with the two genetic materials occupying distinct regions of the ordination space even before accounting for processing effects.

Processing showed the largest contribution, explaining 25.62% of the variability. The three treatments—natural, spontaneous fermentation, and induced fermentation—formed well-defined and minimally overlapping clusters, reflecting substantial reorganization of bacterial structure as fruits transitioned from the natural state to fermentative environments. The genotype × processing interaction accounted for an additional 19.19% of the variance, distributing the six factorial combinations into distinct regions of the multivariate space and demonstrating that bacterial responses to different processing types are modulated by plant genetic background, with no relevant overlap among groups.

Figura 3 ASCA models representing the effects of genotype, processing type, and the genotype × process interaction on the structure of bacterial (A) and fungal (B) communities.

For the fungal community (Fig. 3B), although genotype also influenced composition, its contribution was lower, explaining 6.89% of the total variance. Nevertheless, LB1 and Verdim exhibited consistent distinctions along PC1, occupying separate regions of the ordination space.

The evaluation of taxonomic composition of bacterial and fungal communities along the intervention gradient revealed markedly distinct patterns between genotypes and across processing stages (Figs. 4A and 4B).

In the case of bacteria, both genotypes exhibited similar trajectories as they progressed from natural fruits to fermentative stages, converging toward a profile dominated by the genus *Weissella*, whose relative abundance increased markedly during spontaneous and induced fermentations. Despite this overall convergence, intergenotypic differences were maintained.

During induced fermentation with *S. cerevisiae*, although the genus *Weissella* remained dominant in both genetic materials, the Verdim genotype displayed a more balanced distribution, with approximately 30% of the relative abundance distributed among other bacterial taxa, whereas LB1 exhibited lower relative diversity, with values below 15% for the remaining taxa. In natural coffee samples, both genotypes were characterized by the predominance of the genus *Methylobacterium*, although LB1 also showed substantial abundance of the genus *Sphingomonas*, suggesting that the natural fruit stage supports greater intragenotypic heterogeneity, while fermentation tends to reduce these differences and promote a more homogeneous profile between genetic materials.

Figura 4 Relative abundance profiles of the 15 most abundant microbial genera in two Coffea canephora genotypes (LB1 and Verdim) across the natural, spontaneous fermentation, and yeast-induced

fermentation treatments.

The taxonomic composition of the fungal community exhibited a more heterogeneous pattern between genotypes along the same intervention gradient. In natural fruits, LB1 showed predominance of the genus *Candida*, whereas Verdim displayed a more evenly distributed community among different genera, with a slight predominance of *Trichoderma* sp.

At the spontaneous fermentation stage, LB1 exhibited an overall reduction in relative abundances, particularly for *Komagataella* and *Trichoderma*, whereas Verdim showed a more diverse profile, with notable increases in the same genera, in addition to further enrichment of *Hannaella* and *Colletotrichum*.

Finally, during induced fermentation with *S. cerevisiae*, a marked difference between genotypes was observed: Verdim responded to the process with an almost complete predominance of *Saccharomyces*, indicating strong establishment of this group, whereas LB1 maintained residual or undetectable levels of this genus, suggesting limited fungal incorporation during the induced intervention. These patterns indicate that although processing exerts a strong influence on both microbial communities, the fungal response appears to be more dependent on plant genetic background than the bacterial response.

Discussion

he structuring of microbial communities associated with *Coffea canephora* fruits along the post-harvest intervention gradient reveals a system governed by the interaction between environmental selective pressures and constraints imposed by host genotype [30].

Beta diversity analysis demonstrated that post-harvest processing acts as the primary structuring force, promoting an abrupt reorganization of microbial profiles and reducing heterogeneity between genotypes (Fig. 2). In addition to this marked separation between post-harvest conditions, PC2 (9.53%) highlighted further differences attributable to host genotype (Fig. 2A). Even within the same processing category, LB1 and Verdim samples formed distinct bacterial clusters along this axis, indicating genotype-specific compositional signatures. Notably, bacterial communities from natural fruits of both genotypes exhibited clear separation along PC2, while fermentation-derived communities maintained this distinction, albeit with greater multivariate proximity.

Collectively, the observed pattern demonstrates that (i) processing type represents the main axis of variation in bacterial community structure and (ii) within each processing state, genotype continues to shape microbial composition, resulting in genetically defined clustering along both principal components. Thus, the fermentation-induced convergence observed in *C. canephora* genotypes (Figs. 2, 3, and 4) aligns with findings from other plant systems subjected to strong disturbances, in which environmental conditions may partially override host genetic influence during microbial community assembly [31, 32].

However, even under conditions of elevated environmental pressure (as in fermentation), the two evaluated genotypes maintained distinct profiles along the main ordination axes, indicating that intrinsic host characteristics continue to exert a modulatory role, as reported in studies demonstrating consistent genotypic effects in shaping the microbiome of perennial and fruit-bearing plants [33, 34].

Similar to the pattern observed for bacteria (Fig. 2A), PC1 captured a consistent separation of the fungal community between natural fruits and samples subjected to fermentative processes (Fig. 2B). Unprocessed fruits clustered in the negative region of the axis, whereas fermented samples were predominantly concentrated in the positive region.

Beyond this primary division between processing states, the multivariate arrangement highlighted an additional genotype-associated pattern along PC2. Notably, Verdim samples subjected to induced fermentation formed a highly discrete cluster, clearly separated from the other conditions, indicating a singular compositional configuration of the fungal community in this context. This isolation was not observed for the same treatment applied to the LB1 genotype, whose induced fermentation samples remained more closely associated with communities derived from spontaneous fermentation.

Furthermore, variance decomposition using the ASCA model deepens this interpretation by quantifying the relative effects of genotype, processing, and their interaction on microbial community assembly. For bacteria, the genotypic effect accounted for a relevant proportion of the variance, supporting the concept that plants differ in their capacity to recruit and sustain specific microbiomes, whether due to metabolic, physiological, or structural differences [35, 36]. Studies in grapevine, olive, and other woody crops have demonstrated that mucilage composition, secondary metabolite profiles, and epidermal chemistry directly influence microbial colonization before and after harvest, resulting in communities that are not only taxonomically distinct but also functionally differentiated [34, 37].

The significant contribution of processing confirms that fermentative environments can reduce diversity while favoring groups with physiological plasticity capable of tolerating osmotic stress, oxygen fluctuations, and the accumulation of fermentative metabolites [38]. However, the significant genotype × processing interaction indicates that these selective pressures do not act uniformly across all genetic materials, suggesting that intrinsic genotypic characteristics modulate the intensity of processing effects, thereby generating microbial profiles specific to each host–environment combination [39, 40].

In the case of fungal communities, the genotypic effect was smaller but still detectable, which is consistent with literature suggesting that fungi are generally more sensitive to extreme environmental conditions than to metabolic differences among genotypes within the same plant species [41, 42]. The high dispersion observed in fungal interactions in this study indicates that, for this group, the response to processing is more stochastic and potentially dependent on early colonization events, a phenomenon widely discussed in microbial ecology as the “founder effect” [43].

Nevertheless, the contrasting response to *S. cerevisiae*-induced fermentation between LB1 and Verdim suggests that, for certain taxa, host genotype may decisively influence colonization success or failure,

possibly through differences in mucilage chemical composition affecting adhesion, nutrient diffusion, or metabolic compatibility between the host and introduced microorganisms [44].

Taxonomic analysis further completes this framework by demonstrating that bacterial convergence toward *Weissella*-dominated profiles during fermentation does not entirely eliminate genotypic effects, particularly in induced fermentations, in which Verdim maintained greater residual diversity compared to LB1. This pattern suggests that, even under intense selective pressures, certain genotypes retain characteristics that enable the coexistence of microbial subcommunities, potentially resulting in greater functional stability or enhanced metabolite diversity [45,46].

For fungi, the initial prevalence of differentiated communities—with the genus *Candida* dominating in genotype LB1 and *Trichoderma* or other groups occupying larger proportions in genotype Verdim—demonstrates that natural microbial recruitment in fruits is strongly influenced by host genetics, a phenomenon previously documented in plant species with complex genetic structures [44,47]. The greater fungal diversity observed in Verdim during spontaneous fermentation and the strong establishment of *S. cerevisiae* during induced fermentation point to a system in which host genotype regulates not only initial community composition but also the capacity of microorganisms to establish during later stages of microbial succession.

Taken together, these results outline a scenario in which the coffee-associated microbiome is not merely a component passively reorganized by the post-harvest environment, but rather a system simultaneously shaped by host genotype and ecological pressures imposed by fermentation. This complex interaction has direct implications for the development of novel biotechnological processes in the coffee sector. The extensive genetic diversity of *C. canephora*, often greater than that observed in *C. arabica*, provides not only variation in agronomic traits but also a reservoir of potential microbial strategies. The manner in which each genotype responds to fermentation suggests that microbial interventions, such as targeted inoculations or optimization of fermentation stages, may be more effective when tailored to specific genetic materials.

Thus, this study demonstrates that an integrated understanding of coffee-associated microbial ecology and host genetic background is essential for advancing more efficient, reproducible, and genotype-adapted post-harvest strategies in *C. canephora*. The interaction between genetics and microbiome not only explains part of the natural variability observed in fermentative processes but also offers opportunities for innovation in microbial management in coffee, ranging from the refinement of artisanal fermentations to the development of more predictable and robust industrial protocols.

Conclusions

Post-harvest processing is the primary driver of microbial structuring, consistently reorganizing beta diversity across treatments.

C. canephora genotype maintains a detectable influence, contributing significantly to the explained variance and preserving distinct microbial profiles.

Bacterial communities converge toward *Weissella*-dominated profiles during fermentation, regardless of genotype.

Fungal communities exhibit genotype-dependent responses, including contrasts in the adhesion and establishment of *S. cerevisiae*.

Genotype and processing jointly define specific microbial profiles, providing a foundation for future targeted fermentation strategies in *C. canephora*.

Declarations

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding

This research was funded by Sul Serrana of Espírito Santo Free Admission Credit Cooperative—SICOOB, the Cooperativa Agrária dos Cafeicultura de São Gabriel - COOABRIEL, the Coordination for the Improvement of Higher Education Personnel—CAPES, the Research and Innovation Support Foundation of Espírito Santo—FAPES, the National Council of Scientific and Technological Development—CNPq, the Federal Institute of Espírito Santo for supporting the research through the PRPPG no. 12/2021—Research Productivity Program—PPP.

Author Contribution

W.S.G. conceived the study, designed the research, and wrote the main manuscript text. T.G.R.V. and M.C.S.S. performed the bioinformatic and statistical data analyses. J.M.R.L. contributed to manuscript revision and critical corrections. F.L.P., A.P.M., and L.L.P. supervised the project, contributed to project administration, and reviewed the manuscript. All authors read and approved the final manuscript.

Acknowledgement

The authors would like to thank the Federal University of Espirito Santo — UFES, the Sul Serrana of Espirito Santo Free Admission Credit Cooperative — Sicoob (23186000886201801), the Coordination for the Improvement of Higher Education Personnel — CAPES, the Research and Innovation Support

Foundation of Espírito Santo — FAPES (Edital: Nº 019/2022), the National Council of Scientific and Technological Development — CNPq, the Federal Institute of Espirito Santo for supporting the research through the PRPPG no. 10/2019 — Research Productivity Program — PPP, the Capixaba Institute for Research, Technical Assistance and Rural Extension — INCAPER. As well as the coffee grower João Marré.

Data Availability

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request and with appropriate justification.

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Figures

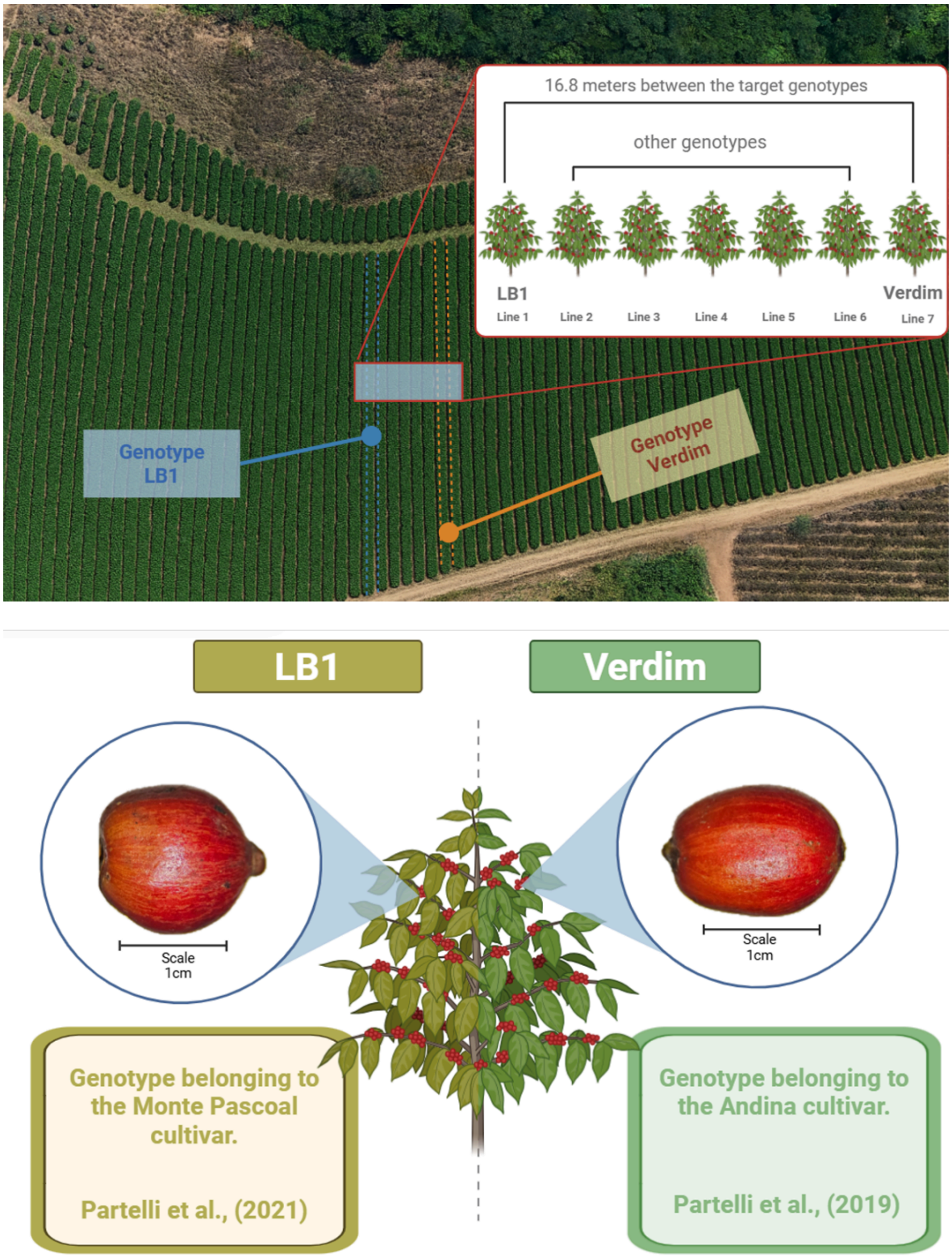

Figure 1

Croqui da área de coleta com a localização dos genótipos na lavoura e genótipos utilizados no estudo. LBI (Partelli et al., [22]) e Verdim TA (Partelli et al., [23]).

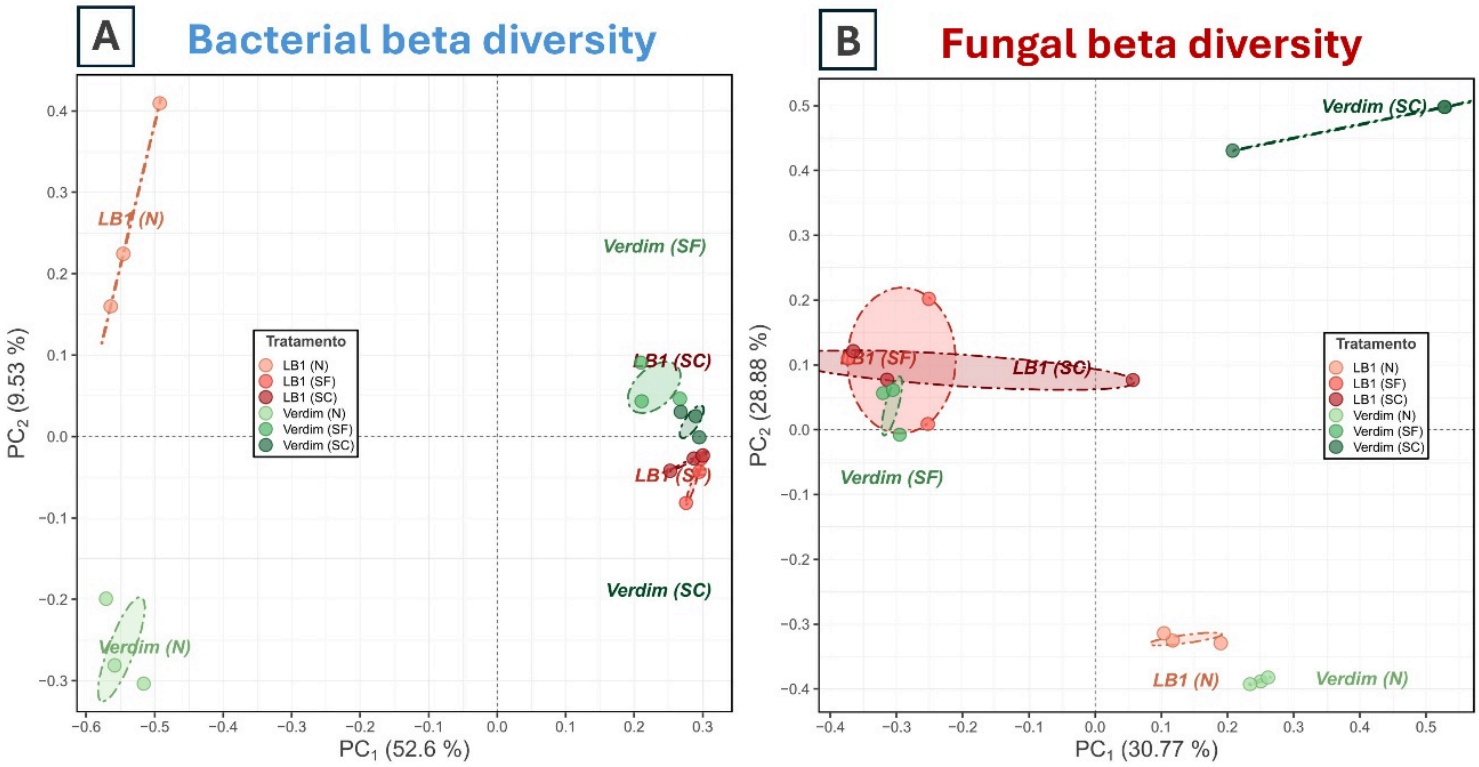

Figure 2

Principal Coordinates Analysis (PCoA) of the beta diversity structure of bacterial (A) and fungal (B) communities associated with the LB1 and Verdim genotypes under the natural (N), spontaneous fermentation (SF), and Saccharomyces cerevisiae-induced fermentation (SC) treatments.

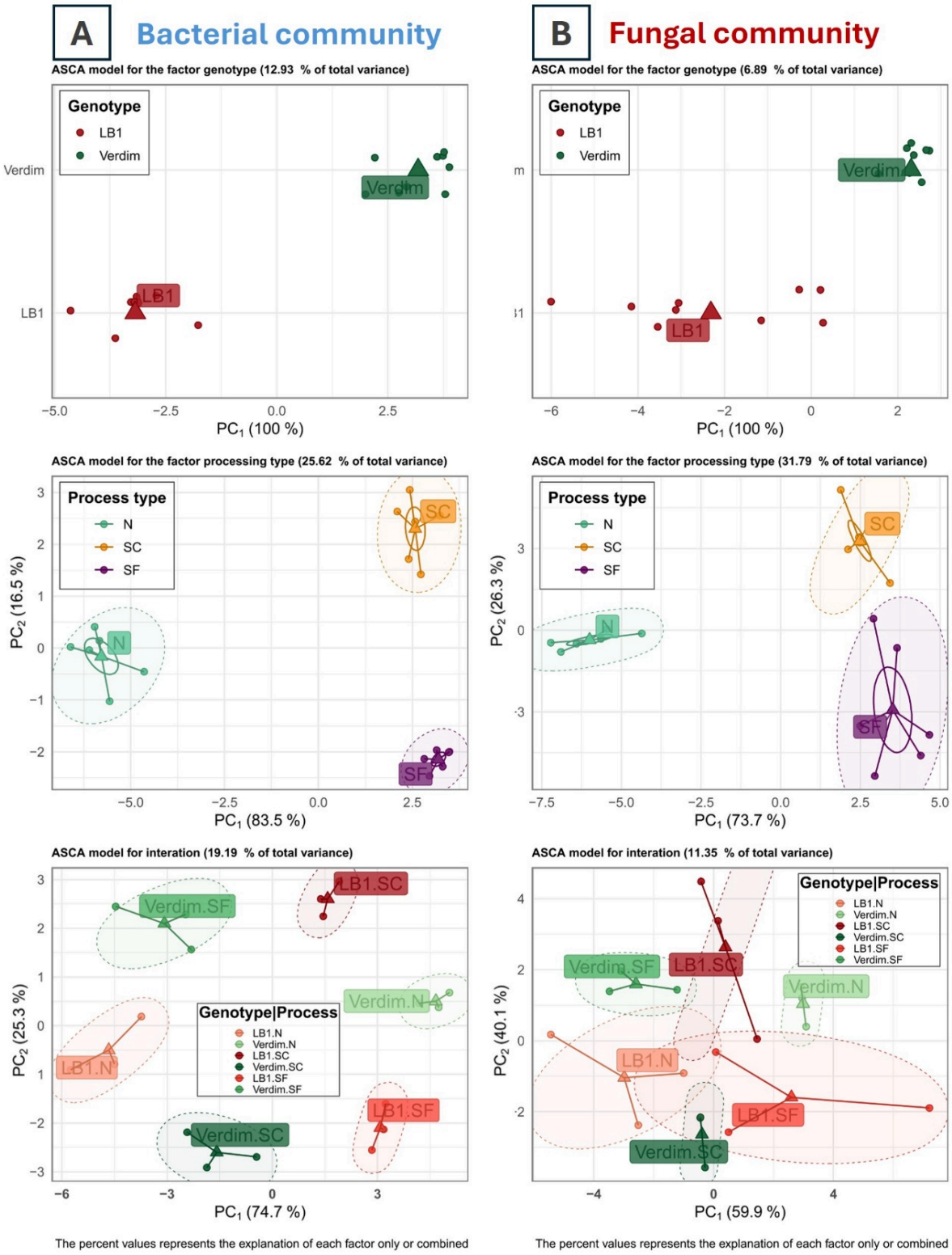

Figure 3

ASCA models representing the effects of genotype, processing type, and the genotype × process interaction on the structure of bacterial (A) and fungal (B) communities.

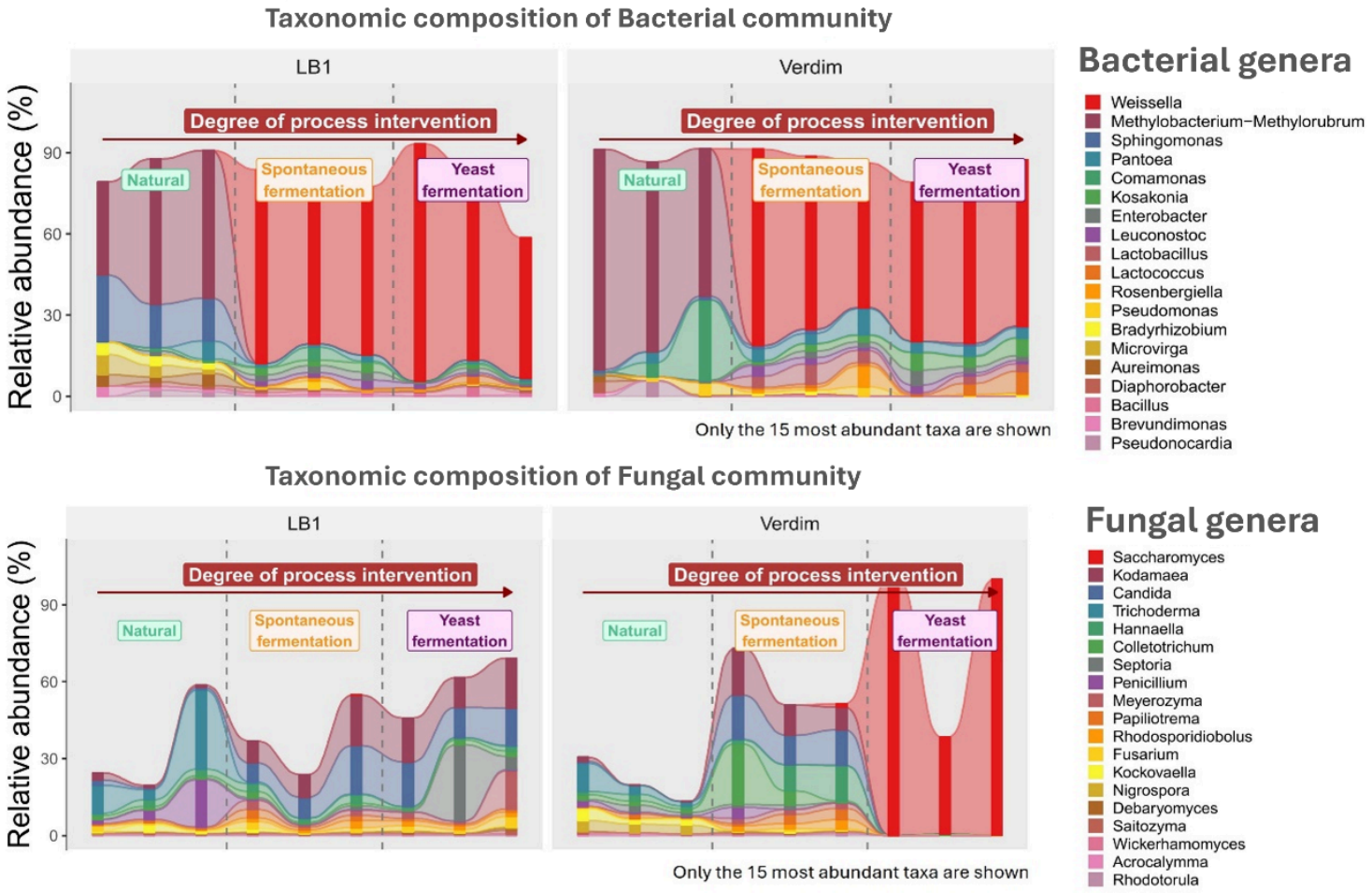

Figure 4

Relative abundance profiles of the 15 most abundant microbial genera in two Coffea canephora genotypes (LB1 and Verdim) across the natural, spontaneous fermentation, and yeast-induced fermentation treatments.