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Characterization of the *Coffea arabica* microbiome: a comparative study of soil and fruit microbial communities under different managements

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Magister Scientiae

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THAYNARA LORENZONI ENTRINGER

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Dissertation submitted to the Agricultural Microbiology Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Magister Scientiae*.

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“Science and everyday life cannot and should not be separated.”
Marie Curie

ABSTRACT

ENTRINGER, Thaynara Lorenzoni, M.Sc., Universidade Federal de Viçosa, March, 2025. **Characterization of the *Coffea arabica* microbiome: a comparative study of soil and fruit microbial communities under different managements**. Adviser: Marliane de Cassia Soares da Silva. Co-advisers: Denise Mara Soares Bazzolli and Lucas Louzada Pereira.

Brazil, the world's largest coffee exporter, has had coffee cultivation as a key socio-economic driver since the 18th century. However, climate change and intensive agricultural practices have posed significant challenges, including reduced productivity and negative environmental impacts. In this context, sustainable alternatives, such as organic fertilization, have shown great promise in reconciling productivity with sustainability. In the second chapter, the microbiota of coffee fruits subjected to two fertilization treatments was evaluated: chemical fertilization (conventional) and organic fertilization. The results demonstrated that organic fertilization reduces reliance on chemical inputs, fosters the development of beneficial microbial communities, and enhances plant health. Furthermore, it contributes to quality consistency and sensory attributes. This more sustainable approach might also improve the resilience of coffee plants in the face of environmental challenges. In the third chapter, a metagenomic analysis of the soil microbiome revealed the influence of fertilization on microbial communities. Soils treated with organic fertilizers exhibited greater functional diversity of microorganisms, which play essential roles in nutrient cycling, pathogen suppression, and mitigating the effects of climate change due to their improved capacity to sequester carbon and reduce greenhouse emissions. This study is a pioneering effort to investigate the functional dynamics of the microbiome in Coffea arabica plantations in situ, examining the impacts of different fertilization regimes. The dissertation underscores that more sustainable practices, such as the use of organic fertilizers, combined with a deeper understanding of the coffee microbiome, are pivotal in addressing environmental and economic challenges, ensuring productivity, quality, and sustainability in coffee cultivation.

Keywords: Coffee; Microbiome ; Metagenomics

RESUMO

ENTRINGER, Thaynara Lorenzoni, M.Sc., Universidade Federal de Viçosa, março de 2025. **Caracterização do microbioma de *Coffea arabica*: um estudo comparativo das comunidades microbianas do solo e dos frutos sob diferentes manejos**. Orientadora: Marliane de Cassia Soares da Silva. Coorientadores: Denise Mara Soares Bazzolli e Lucas Louzada Pereira.

O Brasil, maior exportador mundial de café, tem na cafeicultura um motor socioeconômico essencial desde o século XVIII. No entanto, mudanças climáticas e práticas agrícolas intensivas vêm impondo desafios significativos, como a redução da produtividade e impactos ambientais negativos. Nesse contexto, alternativas sustentáveis, como a fertilização orgânica, têm se mostrado promissoras para conciliar produtividade e sustentabilidade. No segundo capítulo, avaliou-se a microbiota dos frutos do cafeeiro submetidos a dois tratamentos: fertilização química (convencional) e fertilização orgânica. Os resultados demonstraram que a fertilização orgânica reduz a dependência de insumos químicos, promove o desenvolvimento de comunidades microbianas benéficas e favorece a saúde das plantas, além de contribuir para a consistência da qualidade sensorial da bebida. Essa abordagem sustentável também pode aumentar a resiliência do cafeeiro frente a desafios ambientais. No terceiro capítulo, a análise metagenômica do solo mostrou como o tipo de fertilização afeta as comunidades microbianas presentes. O cultivo com fertilização orgânica apresentou maior diversidade funcional de microrganismos, fundamentais para o ciclo de nutrientes, a supressão de patógenos e a mitigação dos efeitos das mudanças climáticas. Este estudo é pioneiro ao investigar as dinâmicas funcionais dos microrganismos em plantações de Coffea arabica in situ, explorando os efeitos de diferentes regimes de fertilização. A presente dissertação evidencia que práticas sustentáveis, como a utilização de fertilização orgânica, aliadas ao aprofundamento do conhecimento sobre o microbioma do cafeeiro, são cruciais para enfrentar desafios ambientais e econômicos, assegurando produtividade, qualidade e sustentabilidade na cafeicultura.

Palavras-chave: Café; Microbioma; Metagenômica

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INTRODUCTION

The coffee industry is among the most significant global supply chains, with around two billion cups consumed daily, establishing coffee as one of the most widely consumed beverages worldwide. Coffee cultivation is concentrated in tropical regions, with Brazil alone responsible for nearly half of global production (CORREIA et al., 2024). It is estimated that the 2024 harvest will reach 58.81 million bags, of which 74.5% will be *Coffea arabica* and 25.5% *C. canephora* (CONAB, 2024). The state of Minas Gerais leads national production, with an estimated 30.18 million bags for the same year, followed by Espírito Santo, São Paulo, Bahia and Rondônia, respectively (CONAB, 2024).

Coffee is a product of terroir, that is, it is directly influenced by environmental aspects (e.g., soil and climate conditions and the microbiome of the rhizosphere) and human activities (e.g., harvesting, post-harvest processes). In addition to these factors, different agricultural procedures, together with different harvesting and post-harvesting methodologies and genetic characteristics, give the drink a unique identity, resulting in singular harvests in terms of both quality and quantity (WILLIAMS et al., 2022; JOHNSTON-MONJE et al., 2023; AGNOLETTI et al., 2024).

The growing global emphasis on sustainable agricultural production has stimulated a movement towards reducing reliance on external inputs and considering organic amendments as alternatives to synthetic agricultural fertilizers (PITTELKOW et al., 2015; GARBOWSKI et al., 2023; MARTINS, 2024). Agricultural management practices, such as fertilization choices, have a significant impact on the composition and functionality of the soil microbial community, directly affecting ecosystem's health, plant nutrition, and the sustainability of agricultural systems (AGUILAR-PAREDES et al., 2023). Recent studies highlight that organic fertilization, compared to conventional fertilization, can enhance microbial biodiversity by promoting greater abundance and diversity of beneficial bacteria and fungi, essential for nutrient cycling and disease suppression (GOUDA et al., 2018; PRATES JÚNIOR et al., 2019). Therefore, the adoption of sustainable management practices is crucial for maintaining soil health, with direct implications for agricultural productivity and environmental resilience (AGUILAR-PAREDES et al., 2023).

Studies comparing the effect of adding organic compounds to coffee-growing soil on the microbiota and its impact on coffee beverage quality are still incipient (PARTELLI et al. 2016; REGANOLD & WACHTER, 2015; SANTOS et al. 2018; JIANG et al. 2023).

Based on the hypothesis that organic fertilization can enhance both coffee plant health and beverage quality compared to conventional fertilization, this study aims to assess the effects of organic compost application on the microbial community associated with *Coffea arabica*. The present research compares conventional and organic fertilization practices, evaluating how these approaches influence the microbial community and, consequently, the sensory profile of the resulting coffee.

Studies on the use of metagenomic approaches to evaluate the coffee-associated microbiome are scarce. Rodrigues et al. (2020) compared the microbiomes of coffee plantations under organic and conventional production systems in tropical agroecosystems, but their study was limited to taxonomic identification based solely on amplicon sequencing. Thus, the present study pioneers the application of metagenomics to assess the coffee microbiome under different fertilization regimes, providing valuable insights into the functional dynamics of microorganisms. This research emphasizes the importance of soils managed with more sustainable and stable agricultural practices, better prepared to face the challenges posed by climate change.

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CHAPTER 1 - LITERATURE REVIEW

1.1 Coffee scenario

Brazil is the world's largest coffee exporter, followed by Vietnam, Colombia, Indonesia, and Ethiopia (VOLSI et al., 2019). Coffee was introduced to Brazil in 1721, and its development became closely tied to the nation’s economic trajectory due to the crop’s high commercial value and intensive exploitation (COSTA, 2020). Over time, coffee cultivation has evolved alongside Brazil’s historical and economical changes, with significant shifts in production locations (SILVA et al., 2021).

Brazil is the world's largest coffee exporter, followed by Vietnam, Colombia, Indonesia, and Ethiopia (VOLSI et al., 2019). Coffee was introduced to Brazil in 1721, and its development became closely tied to the nation’s economic trajectory due to the crop’s high commercial value and intensive exploitation (COSTA, 2020). Over time, coffee cultivation has evolved alongside Brazil’s historical and economic changes, with significant shifts in production locations (SILVA et al., 2021).

Initially, coffee was cultivated in northern Brazil - specifically in Pará - during the 18th century. It later spread to Rio de Janeiro and São Paulo (VOLSI et al., 2019; Costa, 2020). By 1850, coffee farming advanced toward Serra da Mantiqueira and Santos, and by the 20th century, it expanded further into São Paulo, southern Minas Gerais, Espírito Santo, Paraná, and Rondônia (VOLSI et al., 2019). Today, coffee remains a critical driver of socio-economic development, generating income and employment in rural areas (COSTA, 2020).

Following the end of large-scale government intervention in the 1990s, the Brazilian coffee sector underwent a transformation, modernizing its production processes and focusing on quality improvements (REICHMAN, 2018; DE RESENDE et al., 2021). Key strategies have included shifting production to regions with more favorable climates and entering the specialty coffee market through quality certifications, geographical indications, and organic production (VOLSI et al., 2019; COSTA, 2020; RESENDE et al., 2021).

However, climate change poses a significant challenge to coffee cultivation in Brazil. Studies predict a substantial reduction in suitable growing areas (KOH et al., 2020). For instance, in São Paulo, areas with low climate risk for coffee production could shrink by 20% with a 1 °C temperature increase, and by as much as 75% with a 5.8 °C rise (COLTRI et al., 2012). Additionally, most of the country’s 308,000 coffee farms are family-operated, leaving them particularly vulnerable to shifts in temperature and rainfall patterns (IBGE, 2017).

The Brazilian production of coffee in 2024 presents a scenario of an increase in the cultivated area and positive natural bienniality of the coffee tree; however, regarding productivity challenges influenced by climatic adversities, production did not increase as predicted. The total coffee area (including arabica and conilon) grew 0.8% compared to 2023, comprising 2.25 million hectares. However, the national average productivity estimated for 2024, at 28.8 bags per hectare, shows a drop of 1.9% compared to 2023 (CONAB, 2024).

For arabica coffee, the main export product of Brazilian coffee producers accounting for 56.7% of global coffee production, estimates indicate a world production of 99.9 million 60-kg bags (CONAB, 2024). However, the Brazilian coffee sector continues to face challenges due to climate change and consequent market fluctuations. This context emphasizes the need for management and resilience strategies to ensure the sustainability of the production chain, which is essential for the competitiveness of Brazilian coffee on the international market. Thus, the current scenario points to the importance of measures that protect production from climatic adversities, improving the health of the soil and the coffee tree.

1.2 The coffee tree

Coffee belongs to the genus *Coffea*, within the Rubiaceae family, and is primarily cultivated in tropical and subtropical regions worldwide (NAIK et al., 2021). There are approximately 100 species, but only *C. arabica*, *C. canephora*, and *C. liberica* are grown for beverage production. *C. arabica* and *C. canephora* account for about 70 and 30% of global production, respectively (DUONG et al., 2020; NAIK et al., 2021).

Coffee originated on the Abyssinian plateau (Ethiopia) and was introduced to Arabia in the 11th century (NAIK et al., 2021). Currently, seven countries cultivate coffee on large scales: Brazil, Vietnam, Colombia, Indonesia, Ethiopia, India, and Honduras (ADHIKARI et al., 2020; NAIK et al., 2021). Additionally, around 25 million small-scale farmers, mainly in developing countries, depend on the coffee sector for their livelihoods (ICO, 2018; DUONG et al., 2020).

The seed takes between one to two months to germinate, and the plant requires three to four years to reach maturity and bloom. The lifespan of a coffee tree is approximately 80 years, with an average height of 3.3 meters. The flowers are white and aromatic. Arabica coffee undergoes self-pollination, while conilon coffee typically relies on cross-pollination (ADHIKARI et al., 2020). Within two weeks, the flowers develop into coffee cherries, and harvesting takes place when the fruit turns either dark red or yellow, depending on the variety (NAIK et al., 2021). After harvesting, the cherries go through various processes to remove the outer layers of the fruit and reduce moisture content to levels suitable for storage. Three different processing methods - dry, semi-washed, and washed - are employed based on the species, geographic location, and size of the farm (SCHWAN et al., 2012). The quality of the coffee beans influences market prices, and any defects in the beans reduce the overall quality of the coffee (PATERSON et al., 2014).

1.3 Fertilization and coffee quality

With the objective of increasing productivity, the modernization of coffee production involved the introduction of new varieties, increased planting density, exclusive use of chemical fertilizers, and intensive application of agrochemicals (DUONG et al., 2020). However, intensive systems result in increased environmental contamination, soil degradation, and biodiversity loss (GUILLEMOT et al., 2018).

The need to develop sustainable approaches to coffee cultivation has become increasingly important in the context of climate change (DUONG et al., 2020). Hence, research focusing on the coffee-associated microbiome is essential, particularly for understanding its role in promoting healthier, more balanced, and resilient soils that are less vulnerable to pests and diseases (COMPANT et al., 2019; AFRIDI et al., 2022).

The past century has witnessed significant climate change, with global temperatures rising by an average of 1 °C since the onset of the industrial revolution, already affecting coffee cultivation (ADHIKARI et al., 2020; KOH et al., 2020). Beyond temperature increases, shifts in precipitation patterns are expected, leading to an intensified water cycle and altered rainfall distribution (ADHIKARI et al., 2020; KOH et al., 2020). These climatic changes will have far-reaching consequences for agriculture, affecting plant growth conditions, reducing the suitability of cultivated areas, and potentially expanding the range and impact of pests and diseases (BUNN et al., 2015; OVALLE-RIVERA et al., 2015; GROENEN, 2018).

A critical challenge for modern agriculture is its dependence on fertilizers, which are deeply tied to global geopolitical dynamics. The production of nitrogen fertilizers, for instance, relies heavily on natural gas, making it vulnerable to energy market fluctuations and international conflicts. Crises such as trade sanctions, wars, and export restrictions disrupt the global supply chain, driving up fertilizer prices and placing economic pressure on farmers worldwide (HARVEY et al., 2021; TEIXEIRA et al., 2021).

In conventional agricultural management, heavy use of agrochemicals and fertilizers is common to ensure crop productivity and pest control (DE RESENDE et al., 2021). However, this dependency on imported inputs exposes the sector to global economic fluctuations, reinforcing the need for sustainable and cost-effective alternatives. Adopting management strategies based on organic fertilization can mitigate these risks, enhancing the resilience of agricultural systems amidst uncertainties in energy markets and geopolitical landscapes (DE RESENDE et al., 2021; HARVEY et al., 2021).

Composting with sanitized and well-matured organic waste offers a sustainable solution for soil recovery. In addition to being economically viable, organic fertilization provides several advantages over synthetic fertilizers, including improved soil health and enhanced microbial biodiversity, which promote water retention and balanced nutrient cycles (REGANOLD; WACHTER, 2016). These benefits not only reduce soil erosion but also increase resilience to climate change and intensive management, supporting sustainable agricultural production in the long term (HUSSAIN et al., 2021).

1.4 Microorganisms associated with the coffee tree

Soil management in coffee cultivation significantly influences the associated microbiota. In addition to the bacteria, fungi, and yeasts found in the environment, the endophytic microbiota of coffee plants has gained increasing attention for its diversity and potential benefits to coffee quality (GOMES et al., 2024). These endophytic microorganisms are valuable genetic resources for biotechnology and are a focus of bioprospecting due to their potential for biological control and plant growth promotion (SILVA et al., 2022).

The soil microbiome, including beneficial bacteria and fungi, plays a crucial role in plant nutrition and protection, which ultimately affects coffee quality (AVELINO et al., 2005; HAILE; KANG, 2019). Effective soil management practices, along with a thorough understanding of the interactions among microorganisms, plants, and the environment, are essential for producing high-quality coffee (PRATES JÚNIOR et al., 2021). Soil microorganisms can promote plant growth, control pathogens, fix nitrogen, and solubilize phosphate, while also enhancing tolerance to abiotic stress and acting as antagonists to phytopathogens affecting coffee plants (MULETA et al., 2013; HADDAD et al., 2014; BARRA-BUCAREI et al., 2019).

Additionally, the microbial community is influenced by post-harvest processing techniques, which are critical to produce specialty coffees (MARTINEZ et al., 2019; ZHANG et al., 2019). The sensory quality of coffee can be affected by microbial activity during fermentation, which alters the chemical composition of the beans. The microorganisms involved in this process can either be endophytic or originate from the fermentation environment (including soil, air, and equipment). They consume available substrates and produce metabolites and enzymes that influence the flavor of the final beverage (BRAGA et al., 2023).

1.5 Amplicon and metagenomic sequencing in diversity analysis of environmental samples

Assessing the effects of management practices on the microbiota associated with coffee plants is essential from a sustainable agriculture perspective. Studying the structure and function of microbial communities using molecular biology tools can support more sustainable decision-making in coffee management (GIROUX et al., 2022). Advances in high-throughput sequencing have transformed microbiome research, producing large volumes of data and offering new perspectives on microbial diversity across various environments. The microbiome is a complex system comprising microorganisms, their genomes, and the environment they inhabit, playing key roles in biological processes and nutrient cycles (LIU et al., 2021).

Amplicon sequencing is frequently used due to its low cost and straightforward application. It is suitable for basic taxonomic studies but is typically limited to genus-level resolution. This method involves amplifying specific DNA regions using targeted primers and is recommended for samples with low biomass or contamination from host DNA (LIU et al, 2021). Common tools for processing these data include QIIME 2, DADA2, and UPARSE. The recent transition from operational taxonomic units to amplicon sequence variants has improved accuracy, with DADA2 being particularly noted for its effectiveness in reducing noise in sequencing data (LIU et al, 2021; CALLAHAN et al., 2016).

Metagenomic sequencing offers a comprehensive approach to studying microbial communities without the need for cultivation, providing detailed taxonomic and functional insights (LIU et al., 2021). This technique supports the creation of metagenome-assembled genomes (MAGs), which are formed by grouping sequences with similar genomic characteristics and represent potential new microbial genomes. It allows for the discovery of new species and clarifies the functional roles of microbial communities within dynamic ecosystems. The assembly and annotation of these genomes involve multiple computational tools for classification, gene prediction, and functional annotation. Metagenome-assembled genomes enhance the ability to map sequencing data, advancing the understanding of microbial functional diversity and its connection to the environment. The development of new algorithms and more integrated workflows has significantly improved the accuracy and efficiency of analyses. As sequencing technologies and bioinformatics continue to evolve, future studies are expected to provide deeper insights into microbial diversity and function, supporting efforts to understand and conserve ecosystems over time (YANG et al., 2021).

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CHAPTER 2 - AMPLICON ANALYSIS OF COFFEE FRUIT UNDER DIFFERENT FERTILIZATION REGIMES

2.1. Introduction

Brazil is the largest coffee producer and exporter in the world, accounting for 56.7% of global coffee production. In 2024, coffee production in Brazil expanded due to an increase in the cultivated area and benefited from the positive biennial cycle, which alternates years of high production. However, climatic adversities have limited productivity growth. In Minas Gerais, the leading coffee-producing state, droughts and high temperatures, particularly in 2021 and early 2024, have intensified challenges for producers (CONAB, 2024).

The quality of coffee cherries is influenced by various factors. Some of them are intrinsic to the growing region, such as altitude, climate, and the soil’s geological composition, while others are shaped by management systems and post-harvest practices (SANCHEZ, 2013). Practices that support the soil’s microbial community are essential for coffee tree health, as they help create a more resilient ecosystem against climate change, promote healthier plant growth, and reduce the incidence of pests and diseases (PRATES JÚNIOR et al., 2021; LIU et al., 2021).

Conventional production systems that rely exclusively on chemical fertilizers face substantial economic and environmental challenges. Economically, they depend on production chains vulnerable to geopolitical instability (ABEBE, 2022). Environmentally, the production of nitrogen fertilizers via the Haber-Bosch process emits greenhouse gases such as CO_2 and N_2O, contributing to global warming (SMITH et al., 2020). Additionally, extracting minerals like phosphate rock and potash causes environmental degradation and contaminates nearby areas. In the soil, improper use of these fertilizers results in water pollution, eutrophication, and soil degradation due to reduced organic matter content and increased acidity (PAHALVI et al., 2021).

Meanwhile, organic fertilization has emerged as a cost-effective and sustainable alternative. Organic waste often enriches the soil with organic carbon, stimulating microbial activity and increasing biomass (HAN et al., 2021; RUI et al., 2009). Composted waste tends to have more enduring effects because its carbon compounds are more stable, supporting long-term soil health and quality improvement (Bastida et al., 2008). In other words, the humified carbon compounds in composted waste protect soil organic matter from degradation (SPACCINI; PICCOLO, 2020). In the long term, combining composted waste with mineral fertilizers can promote soil carbon sequestration, enhance nitrogen retention (CHEN et al., 2024), and improve soil aggregation (NATH et al., 2023). This combination increases soil multifunctionality and health compared to applying only conventional fertilization (WALDER et al., 2023).

Therefore, this study aims to evaluate the microbial community in two cultivation systems: one utilizing conventional (chemical) fertilization and the other using organic fertilization, based on a compound from chicken litter. The hypothesis is that reduction of the chemical fertilization with the organic compound can meet the plant's mineral needs and promote the development of a microbial community beneficial to the coffee tree. It is anticipated that this community will support plant growth and pathogen control, positively impacting coffee tree health and, in the medium and long term, enhancing the coffee drink quality consistency.

2.2 Materials and Methods

2.2.1 Sampling

Samples of *Coffea arabica*, variety Catuaí Vermelho, from the municipality of Araponga, Minas Gerais, Brazil, at an altitude of approximately 900 meters (20° 45' 48.1“ S, 42° 33' 59.5” W), were evaluated. Two areas were analyzed, each measuring approximately 4,000 m², located at the same altitude and slopes facing west. The soil in the first area received conventional fertilization (i.e., fertilization with industrially produced micro and macronutrients). The soil in the second area was fertilized over four years with organic compost from a controlled process of composting chicken litter, supplying all the crop's needs except nitrogen. The conventional crop is 15 years old and is pruned every four years. During the last harvest, 1200 kg of NPK fertilizer was applied in the 25-5-20 ratio, divided into two equal doses of 600 kg each. The crop with organic fertilization, on the other hand, is 22 years old and is pruned every two years. In the last harvest, each hectare of soil received 400 kg of urea and 6,000 kg of compost. Phosphate and potassium fertilization, as well as liming, were not carried out due to the good availability of these nutrients and the suitable pH conditions, as found in the chemical analysis of the soil. Cherry fruits were sampled during the harvest period in May 2023. In each treatment area, 10 sampling points were selected. From each point, samples were taken from 5 plants, and, for each plant, 5 samples of ripe fruit were collected.

2.2.2 DNA extraction, PCR amplification and sequencing

A total of 250 mg of fruit (sourced from 5 units) was subjected to DNA extraction using the Bioer – Biospin Omni Genomic DNA Extraction Kit (Bioflux, China). Prior to extraction, the fruits were ground in liquid nitrogen. During the initial phase of extraction, the material underwent cell lysis with the Precellys® 24 homogenizer (Bertin Instruments) at 4,000 rpm for 50 seconds. Subsequent steps were carried out according to the manufacturer's instructions. At the end of the process, the integrity and quantity of the extracted DNA were assessed through agarose gel electrophoresis (0.8%) stained with ethidium bromide.

To evaluate the microbial community, the DNA from the 16S (V3/V4) and ITS1 regions was amplified via polymerase chain reaction (PCR) using the primer pairs 341F (5’CCTAYGGGRBGCASCAG-3’)/806R (5’-GGACTACNNGGGTATCTAAT-3’) and ITS1F (5’-CTTGGTCATTTAGAGGAAGTAA-3’)/ITS2 (5’-GCTGCGTTCTTCATCGATGC-3’), respectively. The PCR products were examined using agarose gel electrophoresis (2% w/v) stained with ethidium bromide. Samples with bands corresponding to sizes between 400 and 450 base pairs (bp) were selected for library preparation using the NEBNext® Ultra™ DNA Library Prep Kit for the Illumina platform, following the manufacturer’s recommendations. The samples were barcoded, and the quality of the libraries was assessed using the Qubit 2.0 fluorometer (Thermo Scientific) and the Agilent Bioanalyzer 2100 system. Finally, the libraries were sequenced using the Illumina NovaSeq 6000 platform by Novogene (Hong Kong, China) with 250 base pairs for each read.

2.2.3 Amplicon data analysis

The reads had their adapters and barcodes removed, and the remaining fragments were subjected to quality control by removing low-quality sequences, chimeras, and singletons (sequences present only once in the sample). High-quality remaining sequences were evaluated using the Divisive Amplicon Denoising Algorithm

(DADA2; CALLAHAN et al., 2016) to determine the Amplicon Sequence Variants (ASVs), which were annotated using bacterial (SILVA 138; QUAST et al., 2013) and fungal (UNITE v. 2024-04-04; KÕLJALG et al., 2020; ABARENKOV et al., 2023) taxonomy databases. After this stage, contaminant sequences originating from mitochondria, chloroplasts, or other non-fungal eukaryotic taxa were removed. All subsequent analyses were conducted using R software (R Core Team, 2020).

2.2.4 Ecological metrics

To assess the fungal and bacterial community composition (β-diversity) in the fruits samples, a principal coordinate analysis (PCoA) was constructed using the Bray–Curtis dissimilarity between samples. The α-diversity metrics (Chao1 index, Pielou evenness, and Shannon diversity) were calculated using Vegan (LAHTI; SHETTY 2017).

2.2.5 Post-harvest and sensory analysis

The post-harvest process was carried out at the Coffee Analysis and Research Laboratory (LAPC) located at the Instituto Federal do Espírito Santo - Venda Nova do Imigrante. The harvested ripe coffee cherries were taken immediately to the greenhouse and distributed evenly on a drying rack for 1 to 2 weeks until the moisture content dropped to 10-12%.

Samples were processed following the recommendations of the Specialty Coffee Association. Eight to 24 h after the roosting process, the coffee was ground in a disc mill (Bunn Coffee Mill, model G3A HD), with granulometry between 70 and 75% of the particles passing through a sieve of 20 mesh (US Standards). The sensory analysis panel comprised six Q-graders (PEREIRA et al., 2018). Each coffee sample was evaluated using five cups, with the ideal concentration determined as 8.25 grams of ground coffee per 150 mL of water. The infusion was performed after the water reached 92–95 °C. The Q-Graders began the evaluations when the temperature of the cups reached 55 °C and the time of tasting was 4 min after the infusion. Fragrance/Aroma, Flavor, Aftertaste, Acidity, Body, Balance, Sweetness, Clean Cup, Uniformity, Overall, and Final Score are the variables used in the descriptive sensory analysis following the SCAA protocol. Two statistical tests were used to assess the consistency and quality of sensory attributes. Bartlett's test was applied to check the variance of the data, which indicates the stability of the sensory attributes evaluated in each treatment. The F-test, an analysis of variance (ANOVA), was used to compare the mean sensory scores between the two types of handling, to identify possible significant differences in the parameters analyzed. To investigate how variations in the microbial community might influence the sensory characteristics of coffee produced, a Pearson correlation analysis was conducted in R software (R Core Team, 2020) between microbial community traits (i.e. richness, evenness, and diversity) and the sensory attributes of coffee described above.

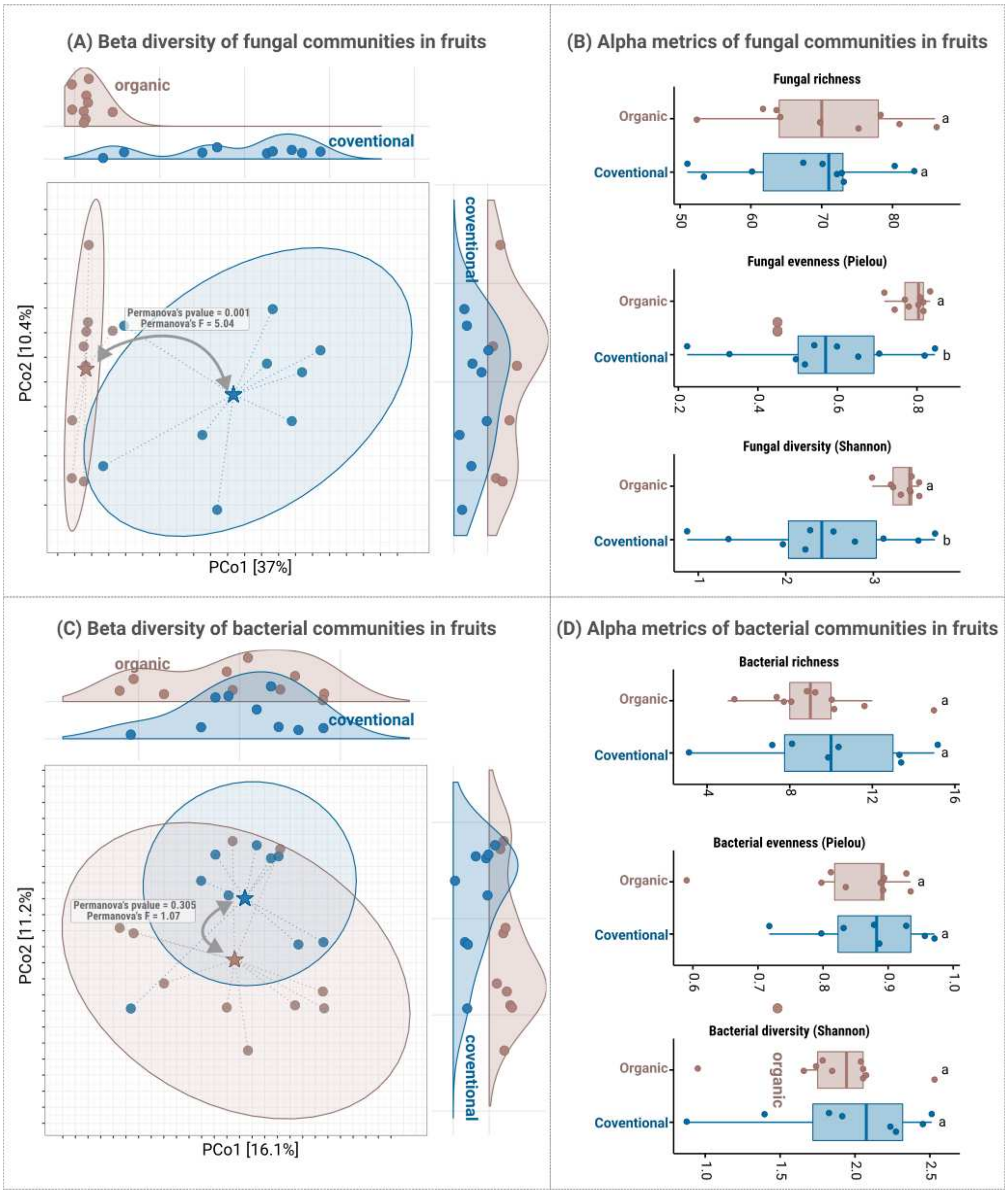
2.3 Results

2.3.1 Alpha and beta metrics

According to the beta diversity analysis of the fungal community (Figure 1A), samples from the area with organic fertilization exhibited greater homogeneity compared to those from the area with conventional fertilization. Additionally, the fungal communities in the two areas showed no overlap, suggesting that they are ecologically distinct. In contrast, for the bacterial community (Figure 1C), the beta diversity analysis revealed an overlap between samples from the two areas, indicating shared species, with both exhibiting low homogeneity.

In the alpha diversity analysis for fungi (Figure 1B), statistically significant differences were observed: the Shannon index was higher in the area with organic fertilization, indicating greater diversity; the equitability (Pielou) was also higher in the organic system, reflecting a more uniform distribution of species. For bacteria (Figure 1C), however, the indices of richness (number of species), equitability (Pielou), and diversity (Shannon index) showed no statistically significant differences between the two systems.

Figure 1 - Beta and alpha diversity of coffee fruit's fungal and bacterial communities of *Coffea arabica* under two fertilization regimes (conventional and organic). (A,C) Principal Coordinate Analysis (PCoA) of beta diversity based on the Bray–Curtis distance matrices comparing the community distribution between the two groups. The blue points represent the conventional treatment, while the brown points represent the organic treatment. Stars represent the centroids for each treatment. Permutational analysis of variance (PERMANOVA) was conducted to determine the magnitude of the differences between the treatments. (B,D) Alpha diversity of the fungal and bacterial communities: Richness, Evenness (Pielou), and Diversity (Shannon). Each point represents a composite sample; the organic treatment is indicated in brown and the conventional treatment in blue. The letters (a, b) represent significant differences ($p < 0.05$) according to the F test.

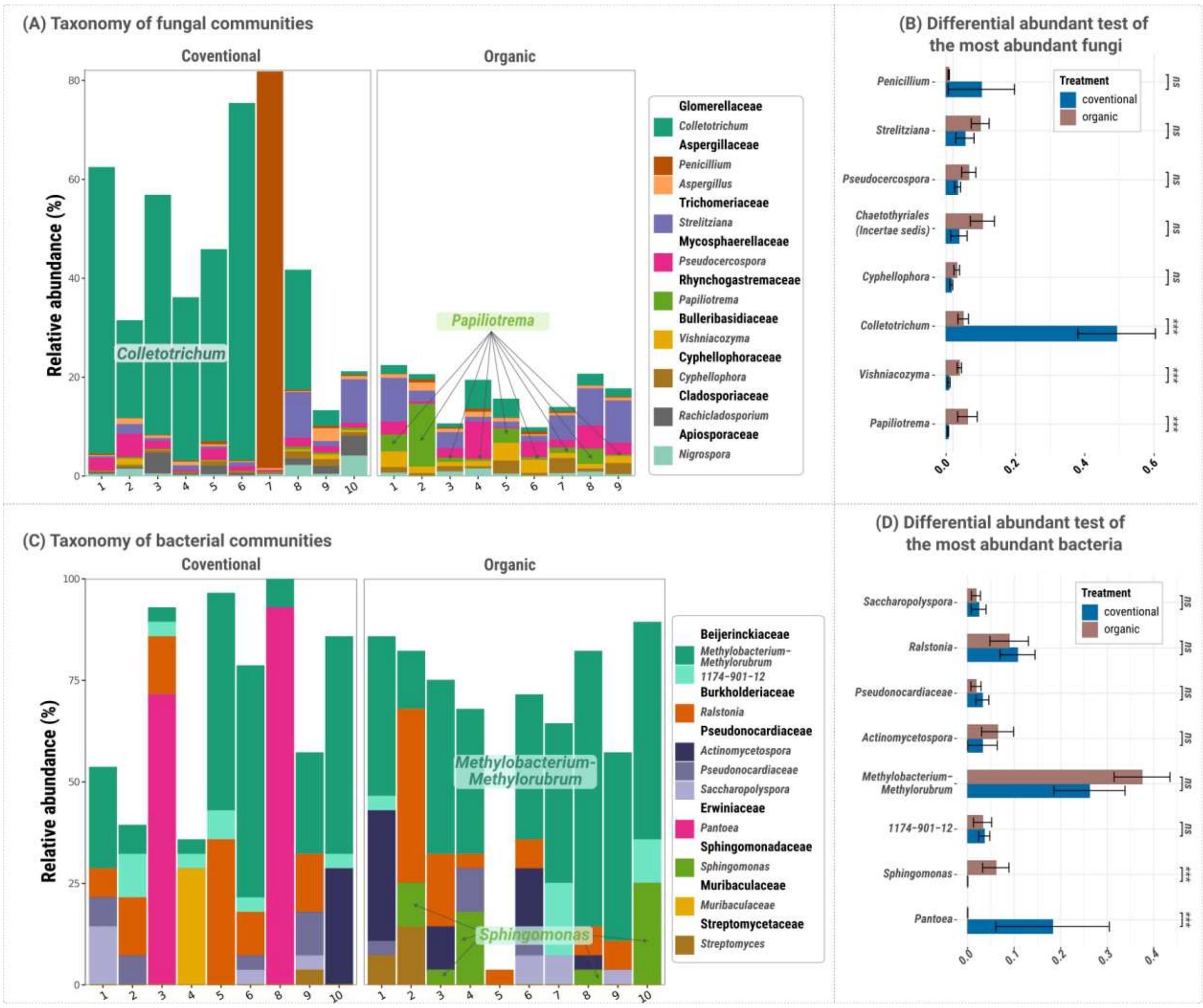


2.3.2 Taxonomic composition

Regarding the relative abundance of fungi, there was a significantly greater presence of the genus *Colletotrichum*, which includes several pathogenic species, in the fruit from conventional cultivation compared to organic cultivation. *Colletotrichum* was also predominant in most conventional samples (Figures 2A and B). In contrast, the genera *Vinishiacozyma* and *Papiliotrema* were more abundant in the organic regime, while the genus *Colletotrichum* presented a significant reduction in this treatment (Figures 2A and B). Other fungal genera, such as *Penicillium*, *Strelitziana*, *Pseudocercospora*, *Chaetothyriales*, and *Cyphellophora* were also among the most abundant groups, but did not differ between fertilization regimes (Figures 2A and B).

Moreover, considering the relative abundance of bacterial communities (Figures 2C and D), the organic fertilization regime showed a greater abundance of the genus *Sphingomonas*. In contrast, the genus *Pantoea* had a significantly greater presence in the conventional fertilization regime. The other predominant bacterial groups, i.e., *Saccharopolyspora*, *Ralstonia*, *Pseudonocardiaceae*, *Actinomycetospora*, and *Methylobacterium/Methylorubrum*, did not differ between treatments.

Figure 2 - Relative abundance of fungal and bacterial communities of *Coffea arabica* under two fertilization regimes (conventional and organic). (A) Top 10 genus and (B) differential abundance test of the most abundant fungi. (C) Top 10 genus and (D) differential abundance test of the most abundant bacteria. Conventional fertilization is represented in blue and organic fertilization in brown ($p < 0.05$).



Due to the contrast in the fungal abundance between organic and conventional fertilization regimens, especially for yeasts related to biocontrol, we improved the annotation of ASVs at the genus and species level. We identified over 2000 fungal ASVs across samples from both systems. Upon filtering, 41 yeast genera were identified after manual curation. Among these, six were classified as Ascomycota and 35 as Basidiomycota. Notably, three yeast genera (*Apiotrichum*, *Vinishiacozyma*, and *Papiliotrema*) were significantly more abundant in the organic system ($p < 0.05$; Wilcox's test). Furthermore, the predominant fungi associated with the coffee fruits were basidiomycetes (83%) (Figure 3). At the species level, 59 species were identified after manual curation and improvement of ASV annotation. *Papiliotrema flavescens* and *P. tapputti* significantly differed between the two fertilization regimens, consistent with the results at the genus level (Figures 3 and 4).

Figure 3 - Heatmap of yeast genera found in fruits of *Coffea arabica* under two fertilization regimes (conventional and organic). Red and gray stars denote, respectively, significant ($p < 0.05$) and nonsignificant ($p \geq 0.05$) differences between the two treatments according to Wilcox's test.

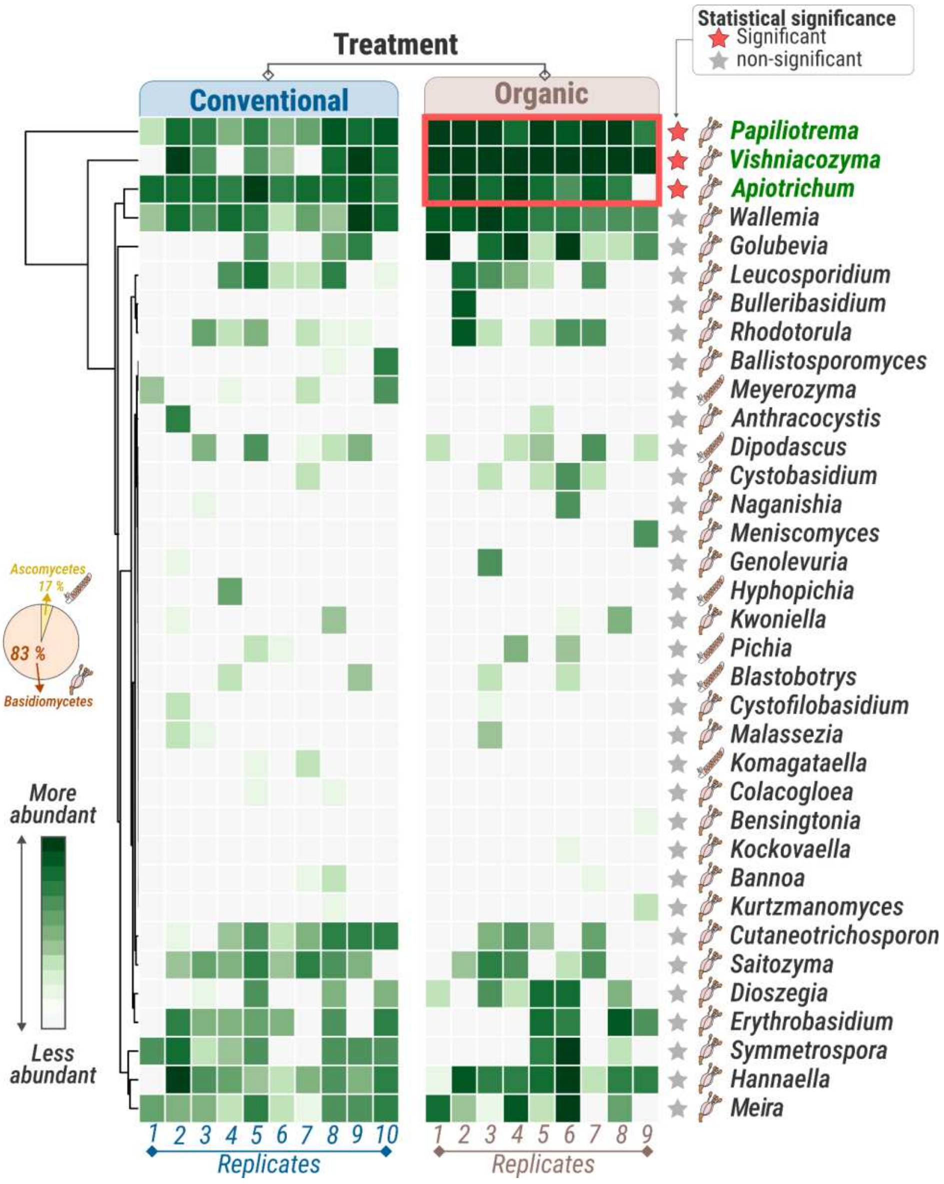
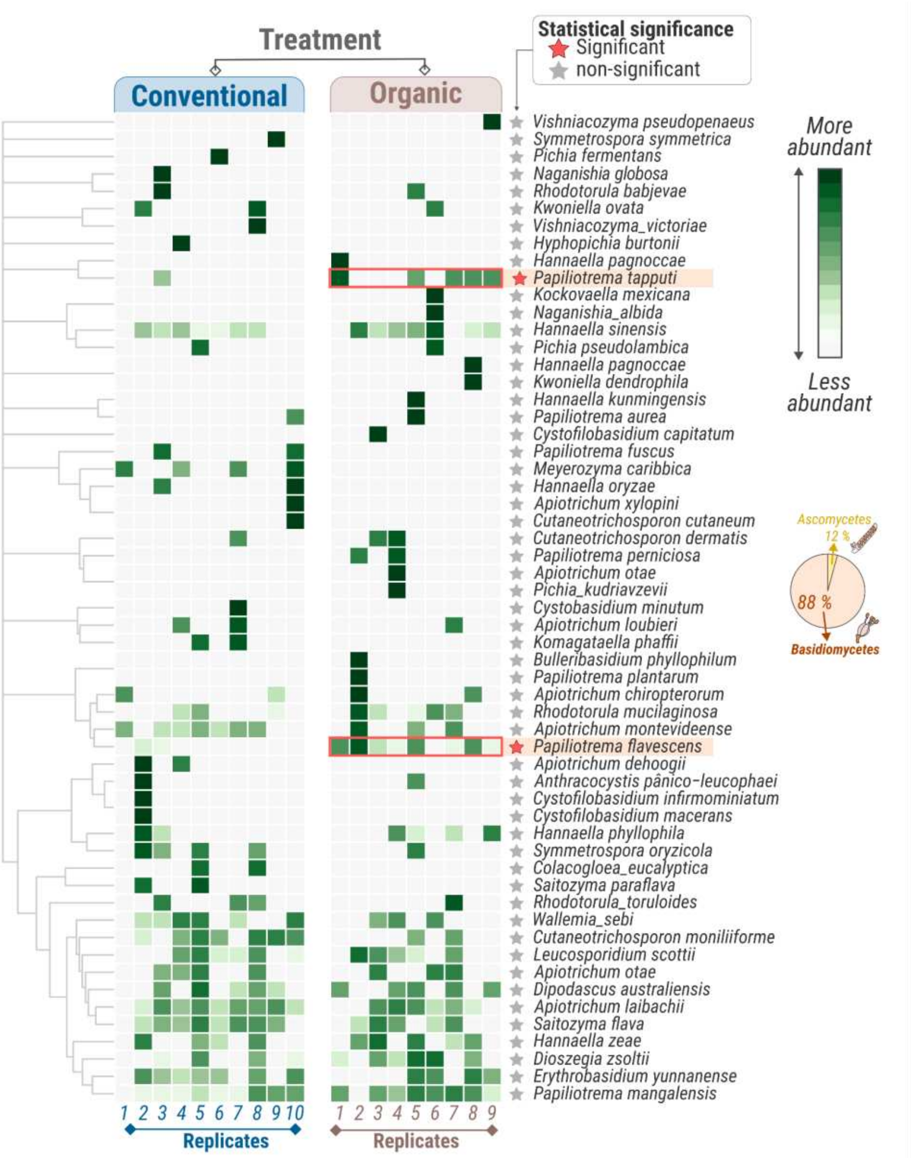


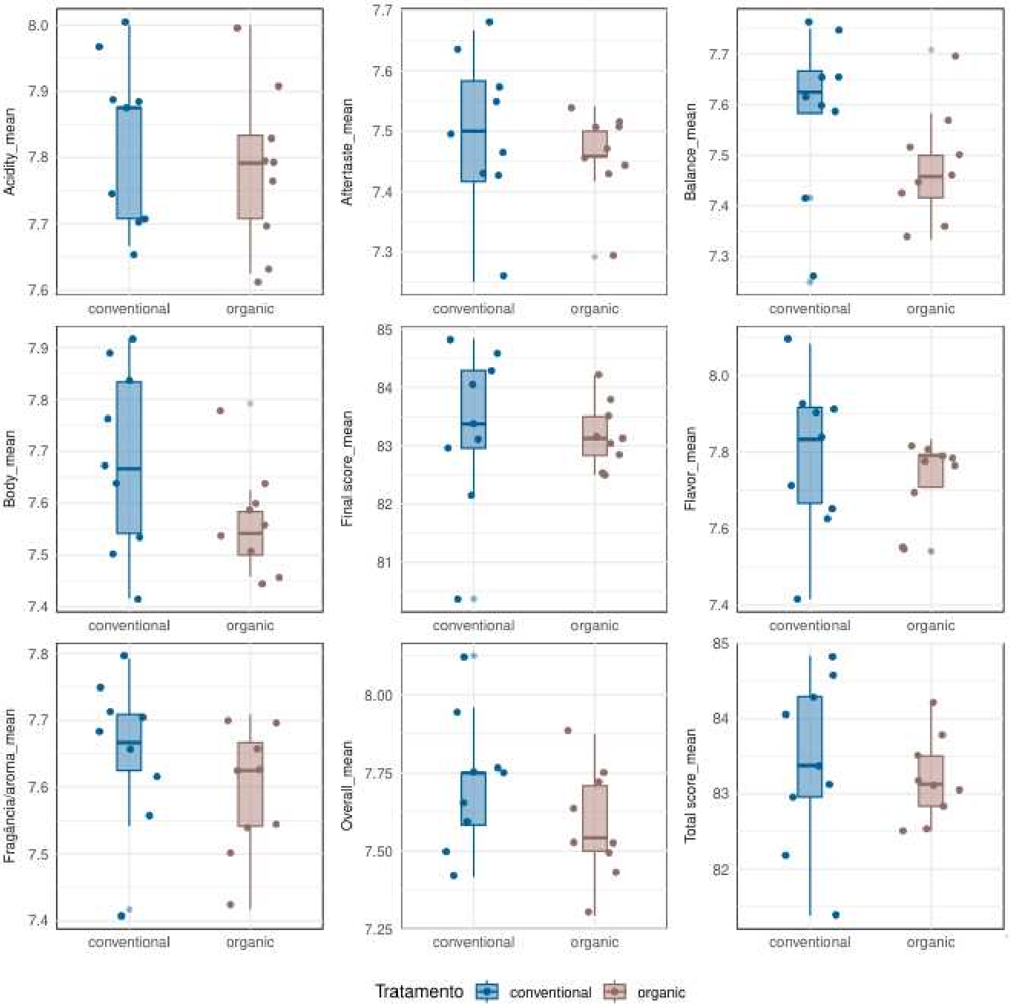
Figure 4 - Heatmap of yeast species found in fruits of *Coffea arabica* under two fertilization regimes (conventional and organic). Red and gray stars denote, respectively, significant ($p < 0.05$) and nonsignificant ($p \geq 0.05$) differences between the two treatments according to Wilcox's test.



2.3.3 Sensory analysis

Furthermore, the coffee fruit collected were used for post-processing and sensory quality assessment (described in section 2.2.4). The results show that, although there were no significant differences between the means of the sensory attributes of conventionally and organically grown coffees, the organic system stood out for showing less variation in the scores (Figure 5).

Figure 5 - Sensory attributes of coffee drinks from conventional (blue) and organic (brown) of *Coffea arabica* under two fertilization regimes (conventional and organic). The analysis of the sensory data included Bartlett's test and the F-test (ANOVA), both considering $p < 0.1$.



In the organic fertilization system, a positive correlation was observed between the fungal richness and diversity with the body of coffee beverage (Figure 6). Additionally, there was a negative correlation between fungal evenness and acidity. On the other hand, no significant correlations were detected between bacterial alpha metrics and these sensory attributes (Figure 7).

Figure 6 - Correlation between sensorial attributes and alpha metrics of fungal communities of *Coffea arabica* under two fertilization regimes (conventional and organic). Cells with one (*) or (**) asterisks indicate statistical significance at the levels of 0.05 and 0.01, respectively. Only significant correlation pairs are highlighted.

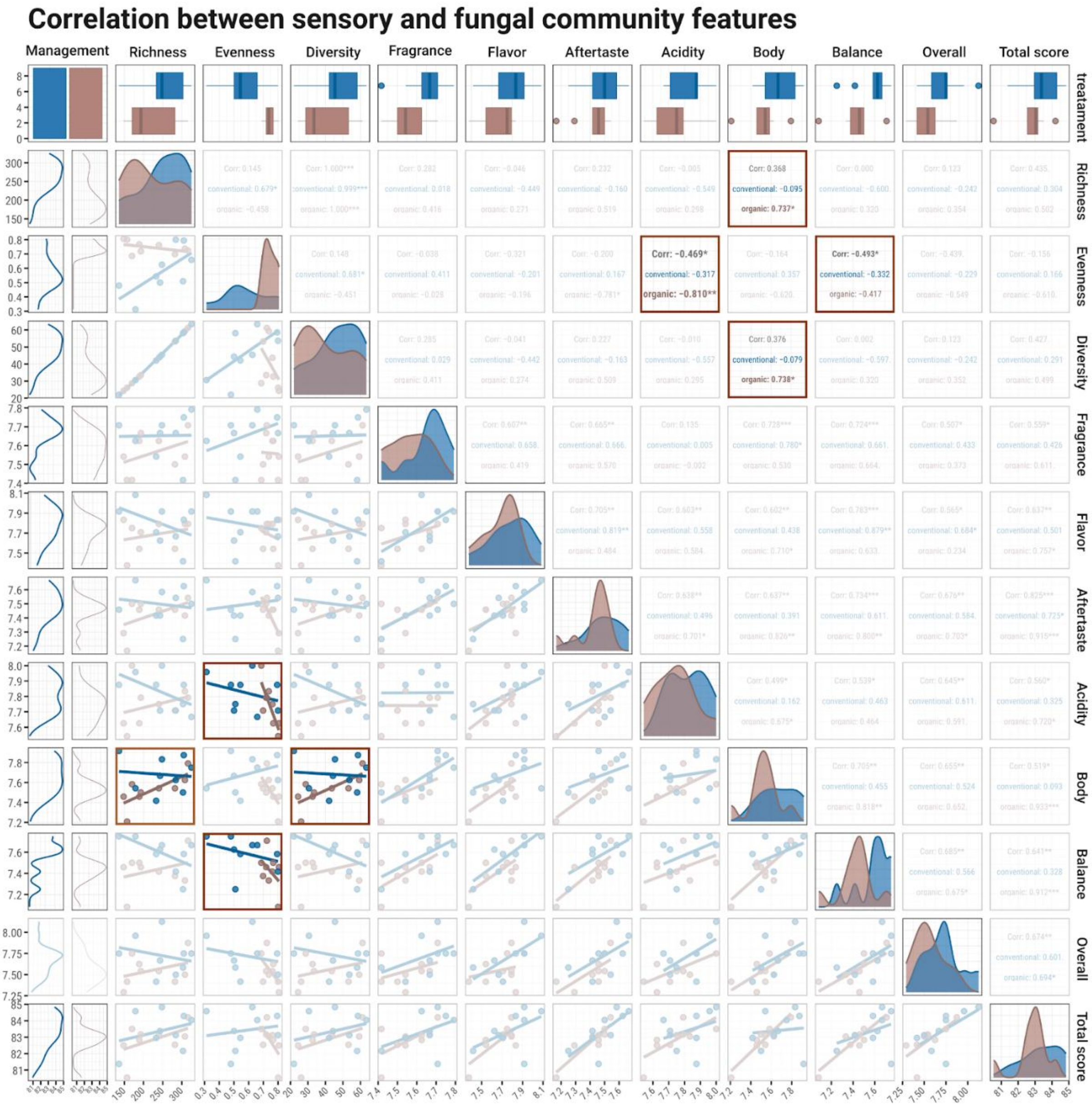
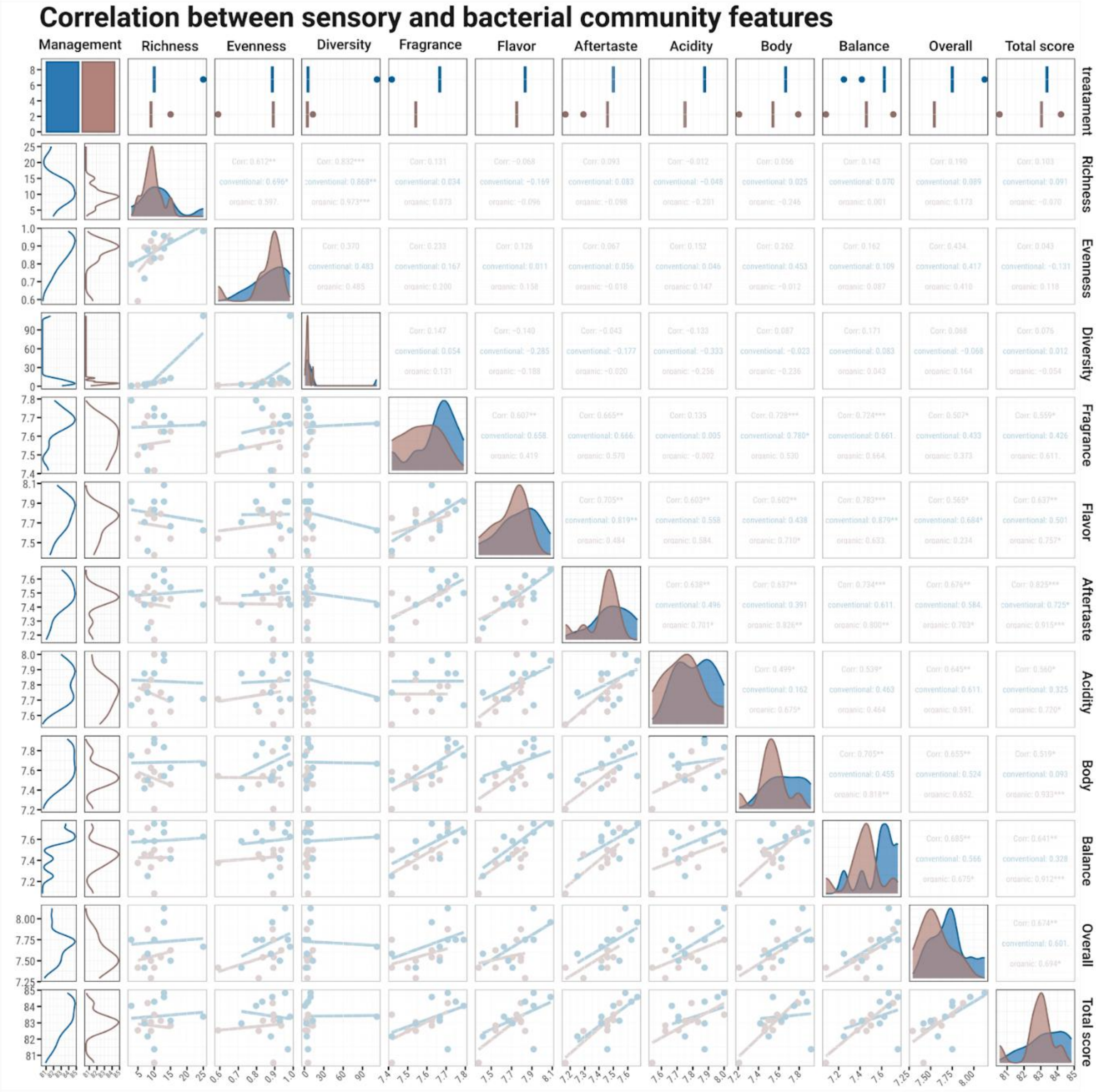


Figure 7 - Correlation between sensorial attributes and alpha metrics of bacterial communities of *Coffea arabica* under two fertilization regimes (conventional and organic). Cells with one (*) or (**) asterisks indicate statistical significance at the levels of 0.05 and 0.01, respectively.



2.4 Discussion

In the current study, we evaluated the microbial community associated with coffee fruits from trees under two fertilization regimens: conventional (chemical) and organic (compound from chicken litter). We hypothesized that the reduction of most of the conventional fertilization for the organic compound would favor the beneficial microbial community in the coffee fruits.

Analyses of beta and alpha diversity revealed greater homogeneity and fungal diversity in the area with organic fertilization, particularly reflected in the Shannon index and equitability (Pielou) (Fig. 1A and B). In contrast, bacterial communities exhibited shared species between the systems, with no statistically significant differences in richness, equitability, and diversity indices (Fig. 1C and D). Despite the similarity in species richness - ranging from 50 to 90 potential species in each area - the organic system demonstrated greater uniformity in species distribution. This finding suggests that, in the conventional system, certain species are more dominant, whereas this dominance is less pronounced in the organic system, resulting in a more balanced microbial community. Consequently, although the two systems have similar species richness, the fungal diversity, as indicated by the Shannon index, was higher in the organic system. In summary, the microbial community in the organic system was more stable and exhibited greater fungal diversity. Consistently, the meta-analysis conducted by Shu et al. (2022) showed that organic fertilization leads to higher fungal diversity and shapes the structure of the community compared to conventional fertilization, positively affecting microbial functionality and cultivation yields.

In the conventional system, the fungus *Colletotrichum* was predominant and significantly more abundant compared to the organic system (Fig. 2). *Colletotrichum* is responsible for different plant diseases and substantial productivity losses (MACHENAHALLI et al., 2021). The cultivation of *Coffea arabica* worldwide is affected by phytopathogenic fungi, including those of the genus *Colletotrichum*, the causative agent of anthracnose (SÁNCHEZ et al., 2020; ALHUDAIB et al., 2023). Affected plants may show leaf death and necrosis, while unripe and ripening fruit show dark brown lesions. *C. aeschynomenes, C. siamense, C. karstii, C. phyllanthi, C. saudianum, C. coffeae-arabicae*, *C. gloeosporioides,* and *C. siamense* are among the species that have been associated with coffee and have been implicated with diseased plants (ALHUDAIB et al., 2023; EL-KOMY et al., 2023); however, we could only identify ASVs for this fungus at the genus level.

In contrast, in the organic system, the yeast genera *Vishniacozyma, Papiliotrema*, and *Apiotrichum* showed greater abundance compared to the conventional regimen. Nevertheless, *Apiotrichum* was also abundant in the conventional system. *Papiliotrema flavescens* and *P. tapputti* were also significantly more abundant in the organic regimen at the species level (Figures 2, 3 and 4).

Papiliotrema has been studied for its ability to act as a biocontrol agent against various plant pathogens, including during post-harvest processing and storage, as well as for plant growth promotion. Its antifungal properties can help protect crops against diseases, reducing the need for chemical fungicides (LIU et al., 2024). For example, *P. flavescens* releases volatile organic compounds (VOCs) that promote plant growth by increasing lateral root formation, fresh weight and chlorophyll content (LIU et al., 2024). *Apiotrichum* and *Vishniacozyma* also have biocontrol potential but have been less studied compared to *Papiliotrema* (PRETSCHER et al. 2018). Besides, *Apiotrichum* can also degrade and detoxify mycotoxins (SUN et al., 2020). Overall, this result suggests that organic fertilization can contribute to the development of a healthier and more functional microbiota, while conventional fertilization can increase the incidence of pathogenic fungi. This predominance of yeast with biocontrol potential might have also contributed to the lower abundance of *Colletotrichum* in the organic treatment.

The importance of the fungal community was also demonstrated by its correlation with sensory attributes (Figure 6). Fungal alpha diversity and richness were positively correlated to the body of coffee beverage, while it was negatively related to acidity in the organic fertilization system. In line with these results, de Sousa et al. (2023) and Entringer et al. (2024) have shown that fungal diversity affected the sensory attributes of the coffee beverage and contribute to its quality.

Regarding the predominant bacteria described here, *Pantoea* stood out in fruits from conventional fertilization, while *Methylobacterium-Methyloborum* and *Sphingomonas* were highlighted in organic fertilization (Figure 2). Species of *Methylobacterium* can enhance nutrient availability, regulate phytohormone levels, and provide protection against plant pathogens, supporting plant growth and resilience (ZHANG et al, 2021). Some *Pantoea* species also have plant growth-promoting characteristics; however, others are associated with plant diseases, which can pose a risk to agricultural productivity under this management regimen (WALTERSON; STAVRINIDES, 2015). Meanwhile, *Sphingomonas* has been associated with the remediation of soil contamination and with the production of plant growth-associated hormones; this genus has been also reported to improve plant resilience under stress conditions (ASAF et al. 2020). Hence, the expressive presence of *Sphingomonas* in the fruits from the organic regimen exemplifies the potential of organic fertilization to promote more sustainable agricultural practices associated with beneficial outcomes for the plant.

Finally, coffee produced from fruit cultivated with organic fertilization exhibited less variability in sensory scores compared to conventional fertilization (Figure 5). Notably, in both areas studied, the scores classified the coffees as specialty grade, a distinction largely attributed to the region's terroir. Terroir plays a pivotal role in specialty coffee production, shaped by factors such as location, altitude, microclimate, temperature, photoperiod, and the mineralogical and physical properties of the soil (WILLIAMS et al., 2022). In the municipality of Araponga, Minas Gerais, the unique interplay of these elements endows the plantations with a naturally favorable terroir for producing high-quality coffees (SILVA, 2014).

The reduced variability in scores observed within organic samples aligns with the more stable microbial community identified in the fruit from the organic system (Figure 1). This stability may partially account for the diminished variation in sensory analyses. Besides, the correlation analysis indicated that the beneficial fungal community detected in the organic fertilization system might contribute to beverages with a full body and lower acidity (Figure 6). This can translate to increased density and complexity in the sensory experience, reflecting the interaction between microbial components and flavor profiles. As a result, farms utilizing organic fertilization can by less susceptible to annual fluctuations in coffee quality caused by edaphic and climatic disturbances, offering a more consistent and dependable approach compared to conventional methods. This makes it an attractive option for producers seeking more stable production over the years.

2.5 Conclusion

The organic fertilization regimen favors the development of microorganisms with beneficial potential for coffee plants, including the fungi *Papiliotrema, Vinishiacozyma,* and *Apiotrichum*, and the bacteria *Sphingomonas*, while the conventional fertilization is associated with a higher abundance of the fungus *Colletotrichum* and the bacteria *Pantoea*. Furthermore, fruits from the organic system exhibit a more stable microbial community and less variation in sensory attributes. Organic fertilization regimes, as well as other sustainable cultivation practices, are essential for developing strategies that consider not only productivity, but also long-term ecological health. In addition, the microorganisms identified in this study can be isolated from the evaluated environment for further research into their potential in bioformulations.

2.6 Data availability

All the sequencing data is deposited on the BioProject PRJNA1248875.

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CHAPTER 3 - METAGENOMIC ANALYSIS OF COFFEE SOIL UNDER DIFFERENT FERTILIZATION REGIMES

3.1. Introduction

Coffee production represents one of the most important production chains worldwide, making coffee one of the most consumed beverages in the world. Coffee cultivation is concentrated in tropical regions, with Brazil accounting for almost half of the world production (CORREIA, 2024). For the 2024 harvest, the production is estimated at 58.81 million bags, 74.5% of *Coffea arabica* and 25.5% of *C. canephora* (CONAB, 2024). The state of Minas Gerais leads Brazilian production, with a predicted production of 30.18 million bags, followed by Espírito Santo, São Paulo, Bahia and Rondônia (CONAB, 2024).

Soil microbial diversity, shaped throughout evolution, is essential for the sustainability of agricultural production (WALL et al., 2015; HARTMANN; SIX, 2023). This diversity, considered an effective metric for predicting soil health (WILHELM et al., 2022), has been threatened by the intensive use of agrochemicals, reducing the efficiency of cropping systems (JAT et al., 2021). The impact is aggravated by abiotic stresses, which are also intensified by climate change, compromising agricultural sustainability (DUONG et al., 2020). Changes in soil structure affect the survival and metabolism of microorganisms, influencing the organic matter content, nutrient cycling and plant-microorganism interactions (JACOBY et al., 2017; HARTMANN; SIX, 2023).

The microorganisms in the soil and associated with plants play an essential role in the defense against pathogens through competition, antimicrobial release, induction of plant systemic resistance and/or release of secondary metabolites (KEFI et al., 2015; SALEEM et al., 2017). However, the loss of ecosystems' functional components, including those that contribute to soil fertility and pest control, has led to the intensive use of fertilizers and pesticides to sustain high yields, especially in commercial agriculture (WHITE et al., 2019; JAT, 2021). In this context, the conservation and strengthening of biological systems is crucial for sustainable agriculture (JAT et al., 2021).

The growing global attention to sustainable agricultural production has driven the search for practices that reduce dependence on external inputs, promoting the use of organic alternatives to chemical fertilizers (PITTELKOW et al., 2015; GARBOWSKI et al., 2023; MARTINS, 2024). Recent studies highlight that organic fertilization, compared to chemical fertilization, can increase microbial biodiversity by promoting a greater abundance and diversity of beneficial bacteria and fungi, which are essential for nutrient cycling and disease suppression (GOUDA et al., 2018; PRATES JÚNIOR et al., 2019). Thus, adopting sustainable practices is fundamental for maintaining soil health, with direct implications for agricultural productivity and environmental resilience (SOUSA et al., 2022; AGUILAR-PAREDES et al., 2023).

Despite the economic and social importance of coffee, few studies have been carried out on the coffee-associated metagenome. Although metagenomic studies regarding the coffee-associated microbiome under different fertilization regimes are incipient, SOUSA et al. (2022) evaluated the rhizosphere microbiome of different coffee species and their role in improving plant health. RODRIGUEZ et al. (2020), on the other hand, compared the microbiomes of coffee plantations under organic and conventional systems, but used amplicon sequencing, which restricts analysis primarily to taxonomy, and functional potential remains largely unexplored. Thus, this study is the first metagenomic analysis of the soil of *C. arabica* plants *in situ,* to evaluate the coffee soil microbiome under different fertilization regimes. The results provide insights into the functional dynamics of microorganisms and highlight the relevance of soils managed with more sustainable and resilient agricultural practices in the face of climate change.

3.2. Materials and Methods

3.2.1 Sampling

Samples of *Coffea arabica*, variety Catuaí Vermelho, cultivation soil from the municipality of Araponga, Minas Gerais, Brazil, at an altitude of approximately 900 meters (20° 45' 48.1“ S, 42° 33' 59.5” W) were evaluated. Two areas were analyzed, each measuring approximately 4,000 m², located at the same altitude and with the same sun exposure. The soil in the first area received conventional fertilization (i.e., fertilization with industrially produced micro and macronutrients). The soil in the second area was fertilized over four years with organic compost from a controlled process of composting chicken litter, supplying all the crop's needs except nitrogen, which was supplied by chemical fertilizer. The conventional crop is 15 years old and is pruned every four years. During the last harvest, 1200 kg of NPK fertilizer was applied in the 25-5-20 ratio, divided into two equal doses of 600 kg each. The crop with organic fertilization, on the other hand, is 22 years old and is pruned every two years. In the last harvest, each hectare of soil received 400 kg of urea and 6,000 kg of compost. Phosphate and potassium fertilization, as well as liming, were not carried out due to the good availability of these nutrients and the suitable pH conditions, as found in the chemical analysis of the soil. The soil was sampled during the harvest period in May 2023. In each treatment area, 10 sampling points were selected. From each point, samples were taken from five plants and, for each plant, three samples of soil.

3.2.2 DNA extraction and sequencing

A total of 250 mg of soil was subjected to DNA extraction using the NucleoSpin Soil kit (Macherey-Nagel, Germany). Prior to extraction, we removed any roots to avoid DNA contamination by the host or any other surrounding plants. During the initial phase of extraction, the material underwent cell lysis with the Precellys® 24 homogenizer (Bertin Instruments) at 4000 rpm for 50 seconds. Subsequent steps were carried out according to the manufacturer's instructions. At the end of the process, the integrity and quantity of the extracted DNA were assessed using 0.8% (w/v) agarose gel electrophoresis stained with ethidium bromide and a Nanodrop spectrophotometer, respectively. To ensure the quality of the metagenomic sequencing, the DNA samples needed to exhibit proper integrity and a concentration suitable for the sequencer. Additionally, the A260/280 and A260/230 absorbance ratios were expected to be near their optimal values of 1.8 and 2.0, respectively. The sequencing libraries were prepared using NEBNext®Ultra™ DNA Library Prep Kit for Illumina according to the manufacturer's recommendations. The library quality was evaluated using a Qubit® 2.0 Fluorometer (Thermo Scientific) and an Agilent Bioanalyzer 2100 system. Finally, the Illumina NovaSeq 6000 platform was used to sequence the libraries to produce 250 bp paired-end reads by Novogene (Hong Kong, China).

3.2.3 Soil analysis

A total of 250 g of soil from each of the 20 sampling points (10 points from soil under organic fertilization and 10 points from soil under conventional fertilization) was sent for laboratory analysis.

The analyses included: pH (determined in water, KCl, and $CaCl_2$ at a ratio of 1:2.5, using a pH meter); phosphorus (P), potassium (K), and micronutrients, extracted in a bi-acid solution (hydrochloric acid 0.05 N + sulfuric acid 0.5 N). Phosphorus was quantified in the supernatant after adding ammonium molybdate and ascorbic acid, with the blue coloration measured in a spectrophotometer (CELM, Model E 225 D) at 725 nm; K was determined by flame emission spectrophotometry (Micronal, Model B 462); micronutrients (Fe, Zn, Mn, Cu) were analyzed in the supernatant by atomic absorption spectrophotometry (Varian, Model Spectra A 20). Calcium (Ca), magnesium (Mg), and aluminum (Al) were extracted with a normal potassium chloride solution (soil to solution ratio of 1:10). Ca and Mg were quantified using atomic absorption spectrophotometry with strontium chloride (0.5% w/v), while Al was determined via titration with 0.05 N sodium hydroxide in the presence of bromothymol blue as an indicator.

To measure potential acidity (H+Al), extraction was performed using 1 N calcium acetate at pH 7.0, followed by titration with 0.05 N sodium hydroxide in the presence of phenolphthalein as an indicator. Additionally, sulfur (S) was extracted with acetic acid and calcium phosphate, then determined by spectrophotometry (420 nm wavelength) using barium chloride. Finally, total organic carbon (C.Org) was measured through colorimetric oxidation: a 1.0 mL aliquot of TFSA solution was transferred to a white polyethylene cup, followed by the addition of 10 mL of a digesting solution consisting of $Na_2Cr_2O_7 \cdot 2H_2O$ 4N and H_2SO_4 10 N. The mixture was shaken on a horizontal shaker for 10 min, left to rest for 1 h, then 50 mL of distilled water was added, and decanted overnight. The reading was performed using a colorimeter (red filter) at 650 nm (transmittance). All procedures were conducted following standardized methods (EMBRAPA, 1997; DEFELIPO; RIBEIRO, 1981).

Statistical analysis was performed, beginning with principal component analysis (PCA) to identify the attributes with the greatest contributions to the components and to simplify the structure of the analyzed variables. To account for the non-normal distribution of the data (Kolmogorov-Smirnov test), a non-parametric Wilcoxon test was used to compare the differences between fertilization regimens.

3.2.4 Metagenomic analysis

3.2.4.1 Read processing

This stage focuses on the initial quality control of sequencing data to ensure the reliability of subsequent analyses. Data quality was evaluated using FastQC (ANDREWS, 2010), and the processing of reads was carried out with Trimmomatic

(BOLGER et al., 2014). First, reads with quality scores below the predefined threshold were filtered out to eliminate low-quality data. Next, artificial sequences, such as adapters and primers, were identified and removed. To prevent interference, contaminating reads - such as DNA from the host (*Coffea arabica*) - were also excluded.

3.2.4.2 Read taxonomic assignment

The taxonomic assignment stage aims to identify the taxonomic groups present in the sample based on the obtained sequences. For this, we used the Kraken2 tool (WOOD et al., 2019) and its extension Bracken (LU et al., 2017). The first performs taxonomic classification by comparing sequences to reference databases and the second estimates more accurately the abundance of each taxon. Herein, the PlusPFP database of Kraken2 was used with a confidence threshold of 0.4 and other parameters set as default. Kraken2's approach relies on directly matching sequences with known genomes, generating a taxonomic profile that lists the identified organisms and their relative abundances. Thus, the taxonomic distribution was visualized in a Pavian plot (FLORIAN et al., 2020) constructed from the median abundance of each organism detected in each sample derived from Bracken results.

3.2.4.3 Contig assembly and binning

Contigs were assembled to reconstruct longer DNA fragments from the sequenced reads, facilitating more detailed structural and functional analyses and enabling the investigation of genes and organisms present in the sample. This process was performed using the MetaSPAdes tool (NURK et al., 2017), a specialized version of SPAdes designed to handle metagenomic data. Before binning, the reads were aligned to the contigs of each sample using Strobealign (SAHLIN, 2022). For the binning stage, three complementary tools were employed: CONCOCT (ALNEBERG et al., 2013), MaxBin (WU et al., 2014), and MetaBAT2 (KANG et al., 2019), all configured to group contigs with a minimum length of 1,000 base pairs (bp).

3.2.4.4 Bin refinement, dereplication and quality analysis

To refine the bins, we used MetaWRAP (URITSKIY et al., 2018). This tool combines bins generated by different binning approaches (*i.e.* MaxBin2, MetaBAT2, and CONCOCT), selects the best candidates, and merges redundant bins to improve the final quality. The process aims to maximize completeness and minimize contamination, following the MIMAG (Minimum Information about a Metagenome-Assembled Genome) guidelines for genomes assembled from metagenomes (MAGs), which have ≥ 50% completeness and ≤ 10% contamination. Finally, bins were dereplicated using dRep (OLM et al., 2017). After each step, bin quality was assessed using CheckM (PARKS et al., 2015) and CheckM2 (CHKLOVSKI et al., 2023).

3.2.4.5 Bin taxonomic assignment, annotation and abundance mapping

After refining the bins, taxonomic assignment was performed using GTDB-Tk (Genome Taxonomy Database Toolkit) (CHAUMEIL et al., 2020), which compares the bins against standardized taxonomic databases, enabling accurate classification into hierarchical taxonomic levels. Subsequently, we annotated genes in each bin using PROKKA (SEEMANN, 2014), a tool that identifies genes and predicts their biological functions, providing comprehensive insights into the genomic features of each bin. For a deeper understanding of the metabolic potential, functional profiling was further enriched using DRAM (Distilled and Refined Annotation of Metabolism) (SHAFFER et al., 2020), allowing detailed annotation of metabolic pathways and genes associated with biogeochemical cycles. To assess the potential related to plant growth promoting genes (PGPG), we recovered the HMM profile of nitrogen fixing genes (*nif*H, *nif*D, *nfl*H, *nfl*D, *nif*K, *vnf*K, *vnf*H, *vnf*D, *anf*D, *anf*H, *anf*K) from (BELLANGER, 2024), and build HMM profiles for genes related to exopolysaccharide (EPS) production (*eps*D, *eps*E, *eps*F, *eps*H, *eps*I, *eps*J, *eps*L, *eps*M, *eps*N, *eps*O), nodulation (*nod*A, *nod*B, *nod*C, *nod*E, *nod*F, *nod*J, *nod*N, *nod*N_like, *nod*O, *nod*P, *nod*S, *nod*T, *nod*U, *nod*V, *nod*W, *nod*X, *nod*Y, *nod*Z, *noe*A, *nol*M), reactive oxygen species (ROS) scavenging (*sod3*, *sod*C, *sod*N), iron acquisition (*lip*A, *lip*B, *lip*M, *lpl*A), salinity stress-potassium transport (*kdp*A, *kdp*B, *kdp*C, *kdp*D, *kdp*E), plant embryogenesis-spermidine (*pup*, *puu*A, *puu*B, *puu*C, *puu*D, *puu*E), IAA-related tryptophan metabolism (*trp*A, *trp*B, *trp*C, *trp*CF, *trp*D, *trp*DG, *trp*E, *trp*F, *trp*G, *trp*GE, *trp*R, *trp*S), and phosphorus solubilization (*pho*A, *pho*C, *pho*D, *pho*E, *pho*H, *pho*N, *pho*P, *pho*R, *pho*S, *pho*T, *pho*U, *pho*W, *phy*, *phy*A, *phy*C, *pst*A, *pst*B, *pst*C, *pts*S). In this case, we first retrieved sequences for each gene from Uniprot (UNIPROT CONSORTIUM, 2024), aligned them with ClustalW (THOMPSON et al., 1994), and built the HMM profile using HMMER 3 (POTTER et al., 2018). To search for the genes of interest on each bin annotation, we used the search function from HMMER 3.

Finally, abundance mapping was performed to estimate the relative abundance of each bin across the samples. This was achieved by aligning the original reads to MAGs using Strobealign (SAHLIN, 2022), ensuring a precise and efficient analysis of coverage and relative abundance for each sample.

3.2.5 Ecological metrics

To assess the community structure (Fungi, Archaea, and Bacteria) from the read analysis (β-diversity) in soil samples, a principal coordinate analysis (PCoA) was constructed using the Bray–Curtis' dissimilarity between samples. Multidimensional analysis was applied to correlate community patterns with soil parameters. The α-diversity metrics for both read and bin data (Chao1 index, Pielou evenness, and Shannon diversity) were calculated using Vegan (LAHTI; SHETTY, 2017).

3.3 Results

3.3.1 Read analysis

The soil under organic fertilization presented higher effective and cationic exchange capacities, boron, calcium, magnesium, manganese, organic matter, phosphorus, sulfur, and zinc content and lower iron and remaining content compared to the conventional fertilization regimen (Figure 1). The archaeal community of the organic fertilized soil was correlated to sulfur, phosphorus, calcium, organic matter, magnesium, manganese, zinc, and boron contents, the sum of exchangeable bases, and the cation exchange capacity; meanwhile, in the conventional fertilized soil, the community was related to the remaining content, pH, iron content and base saturation (Figure 2A). A similar trend was observed for the bacterial and fungal communities (Figures 2B and C), with an additional correlation of the conventionally fertilized soil community to copper and potassium and the correlation of the organic system community to pH and base saturation.

Figure 1 - Edaphic attributes of soil of *Coffea arabica* under two fertilization regimes (conventional and organic). The data presented a non-normal distribution (Kolmogorov-Smirnov test), and differences between fertilization regimens (conventional – blue; organic – brown) were determined by the Wilcoxon test. A $p < 0.05$ was considered statistically significant. One (*), two (**) and three asterisks (***) indicate statistical significance at the level of 0.05, 0.01 and 0.001, respectively.

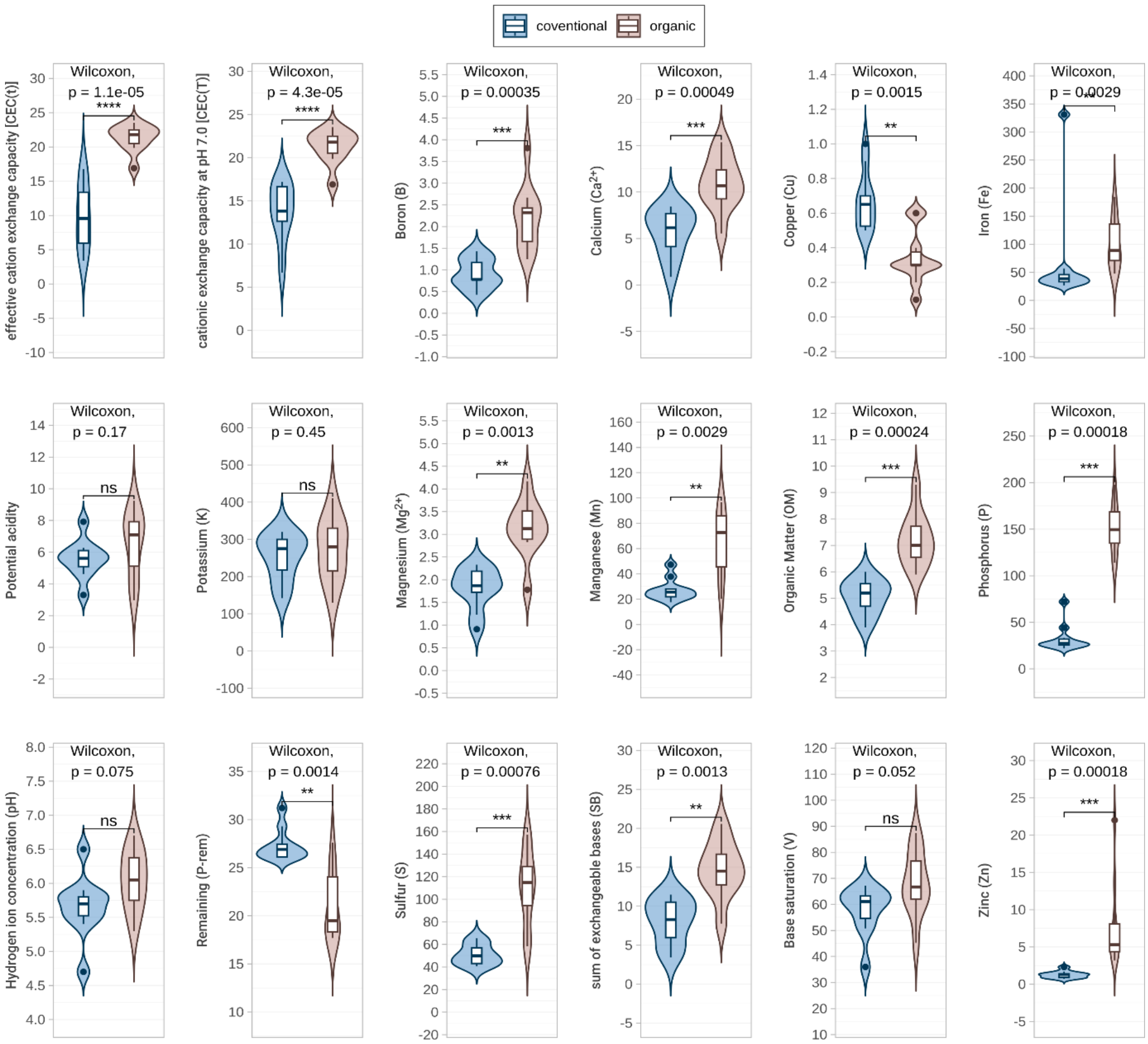
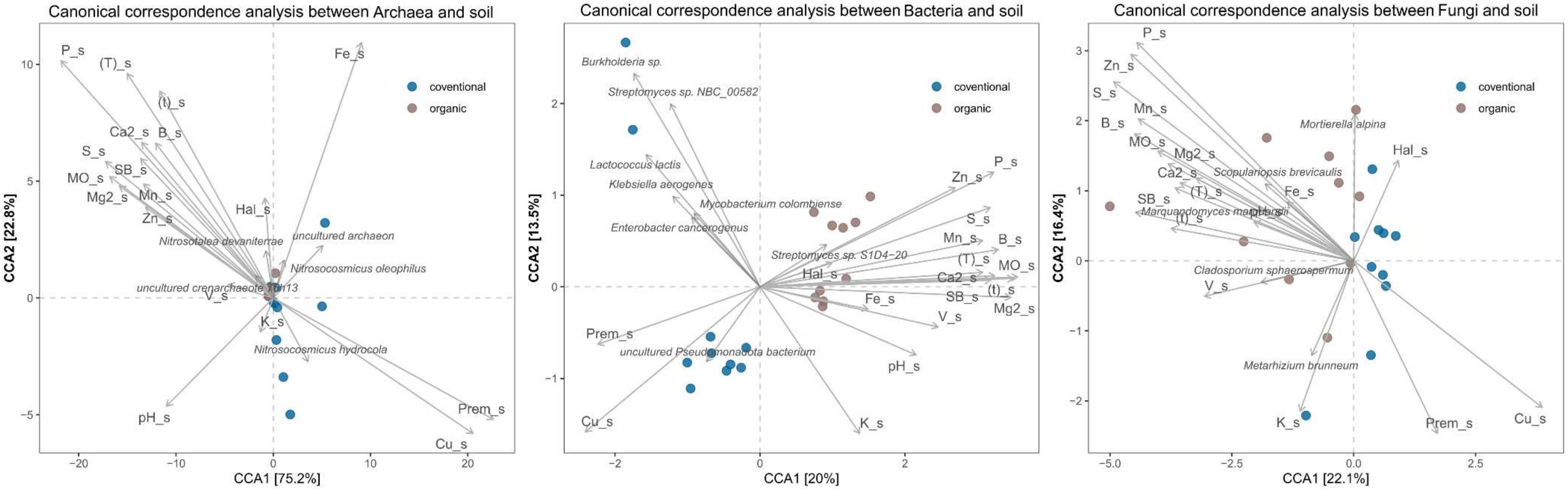


Figure 2 - Canonical correspondence analysis between soil attributes and archaea, bacterial and fungal communities of *Coffea arabica* under two fertilization regimes (conventional and organic). Principal Coordinates Analysis (PCoA) of beta diversity based on Bray–Curtis distance matrices based on the abundance of reads across samples, illustrating community distribution across the two groups. Blue points indicate conventional fertilization, and brown points indicate organic treatment. Multidimensional analysis was applied to correlate community patterns with evaluated soil parameters. (t) = "effective cation exchange capacity [CEC(t)], (T) = cationic exchange capacity at pH 7.0 [CEC(T)], Hal = Potential acidity, SB = sum of exchangeable bases (SB), Ca2 = Calcium, Mg2 = Magnesium, Cu = Copper, Fe = Iron, K = Potassium, B = Boron, Mn = Manganese, Zn = Zinc, V = Base saturation, MO = Organic Matter, P = Phosphorus, Prem = Remaining, S = Sulfur (S).



Canonical correspondence analysis between Archaea and soil

Canonical correspondence analysis between Bacteria and soil

Canonical correspondence analysis between Fungi and soil

The alpha metrics based solely on the reads indicated a greater richness for the archaeal community in the organic-fertilized soil and a higher evenness and Shannon diversity for conventional fertilization. The bacterial community presented a higher evenness and Shannon diversity for the conventional system, but did not differ in richness. In contrast, the fungal community presented greater richness for the conventional system, while the organic system presented higher evenness (Figure 3). However, since only 0.9-1.3% of reads could be identified in the Kraken2 analysis, these results should be considered carefully. As demonstrated below (Bin analysis), most reads were mapped to bins representing organisms that were not, or poorly, identified using Kraken2.

In both treatments, several microbial families, genera, and species were consistently identified, highlighting a core set of microorganisms shared between the communities (Figures 4 and 5). Among the most common families, *Nitrobacteriaceae*, *Rhizobiaceae*, and *Lysobacteraceae* were particularly prominent. The genera detected in both areas included *Streptomyces*, *Mycobacterium*, *Amycolatopsis*, *Burkholderia*, *Paraburkholderia*, *Variovorax*, and *Pseudomonas*. Additionally, the species *Amycolatopsis rhabdoformis*, *Paraburkholderia sabiae*, and *Variovorax sp.* were observed in both treatments. The overall community structure was similar across the treatments, likely due to the homogeneous environmental conditions, such as comparable geology, climate, and altitude. Furthermore, both fertilization regimes showed a high abundance of non-cultivable microorganisms, suggesting a rich microbial diversity that remains largely unexplored.

In the area with conventional fertilization, notable species included *Streptomyces griseorubiginosus*, *Mycobacterium intracellulare*, *Amycolatopsis sp.*, *Fusarium oxysporum*, and *Purpureocillium lilacinum*. The *Rhizobiaceae* and Enterobacteriaceae families, along with the genera *Fusarium*, *Sphingobium*, and *Burkholderia*, were also abundant in this treatment. In contrast, in the area under organic fertilization, the predominant species were *Streptomyces caniferus* and *Mycolicibacterium gilvum*. The Sphingomonadaceae family and the genera *Nitrospira* and *Lysobacter sp.* were also notably present in this treatment.

Figure 3 - Ecological metrics of coffee soil's archaeal, bacterial and fungal communities of *Coffea arabica* under two fertilization regimes (conventional and organic): Richness, Evenness (Pielou) and Diversity (Shannon). Each point represents a composite sample; the organic fertilization is presented in brown and the conventional fertilization in blue. The numbers represent significant differences ($p < 0.05$) according to the F test.

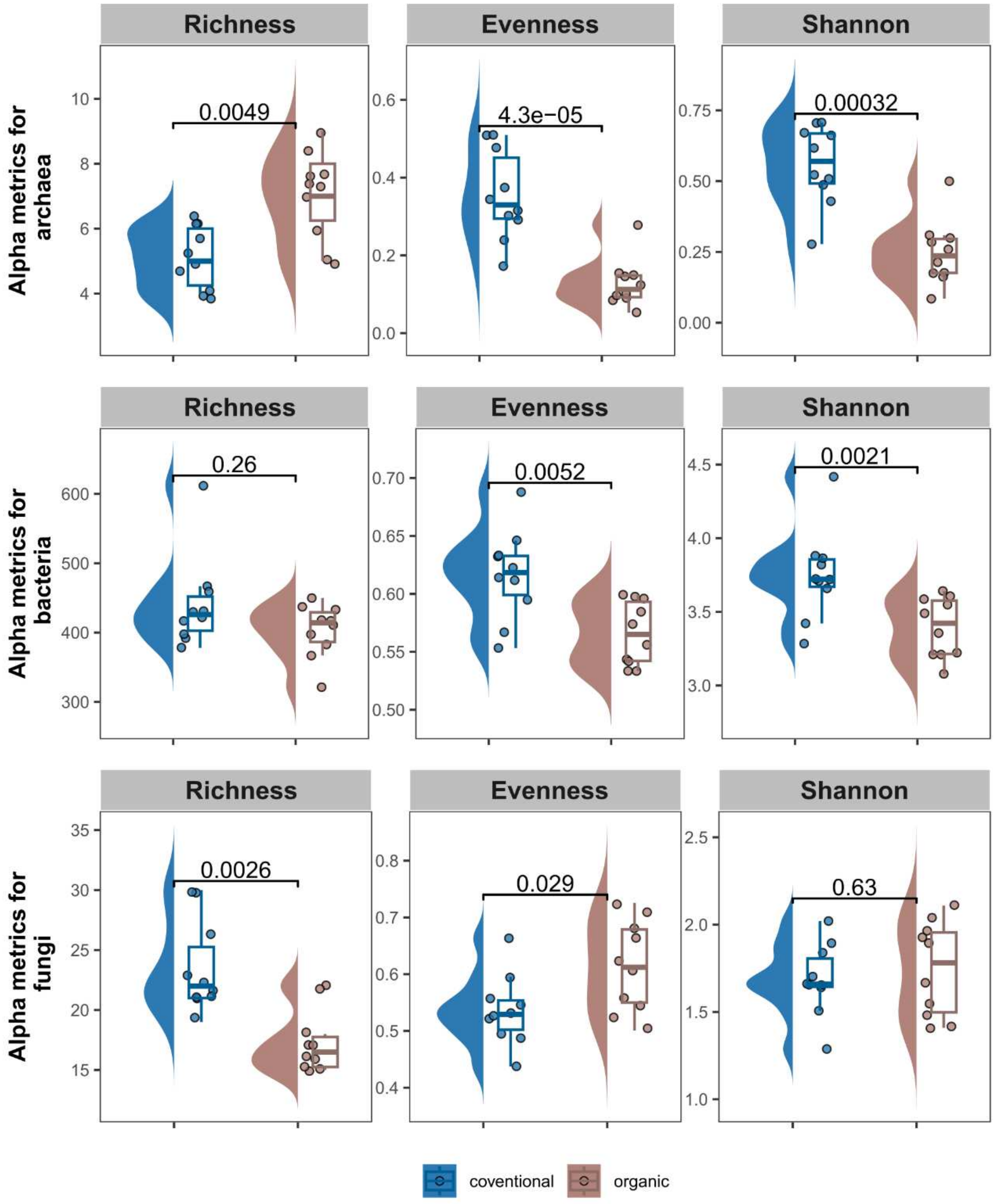


Figure 4 - Pavian plot for organisms identified in the read-based analysis for *Coffea arabica* soil under conventional fertilization. The plot was constructed from the median abundance of each organism detected in each sample.

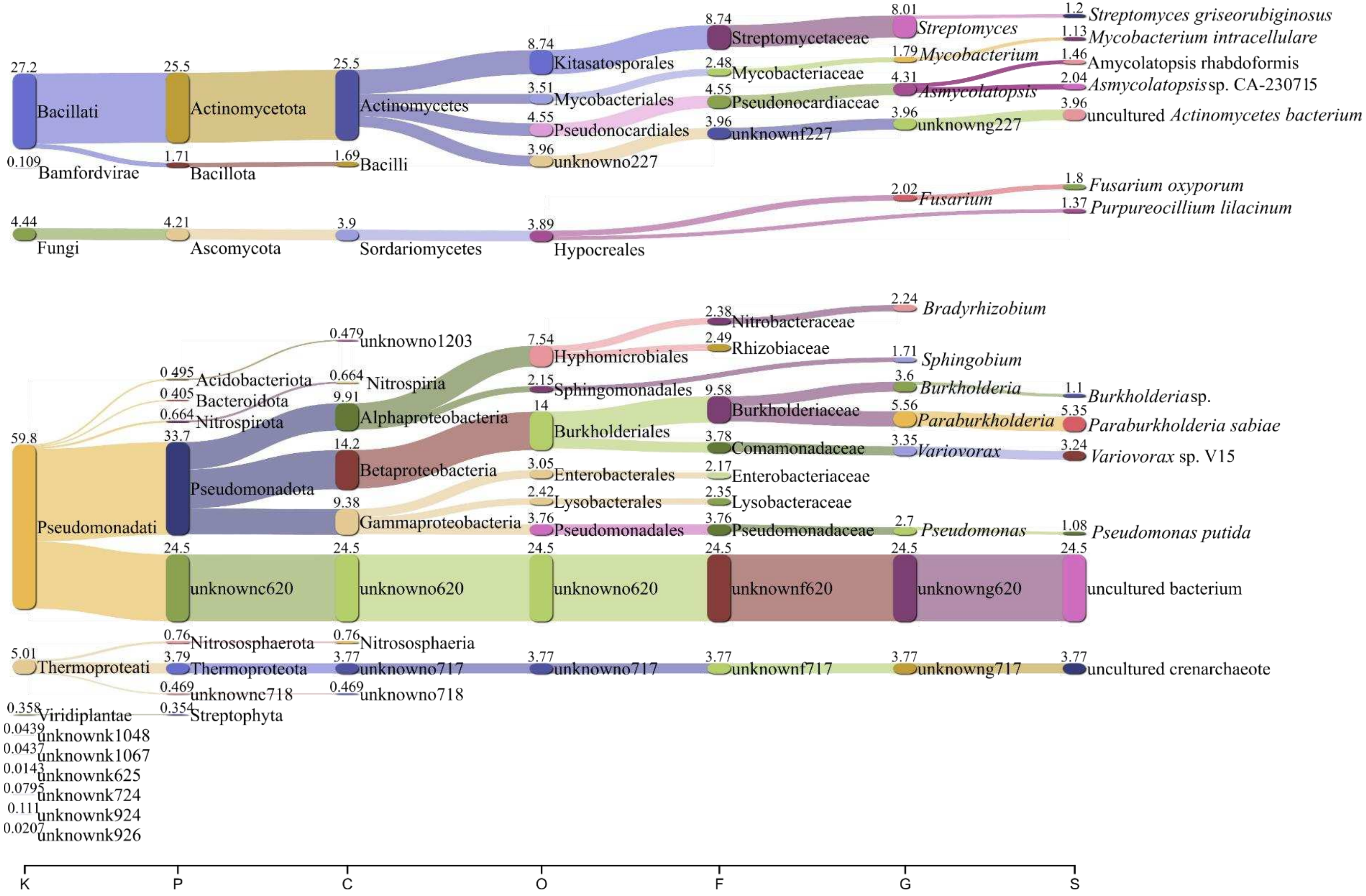
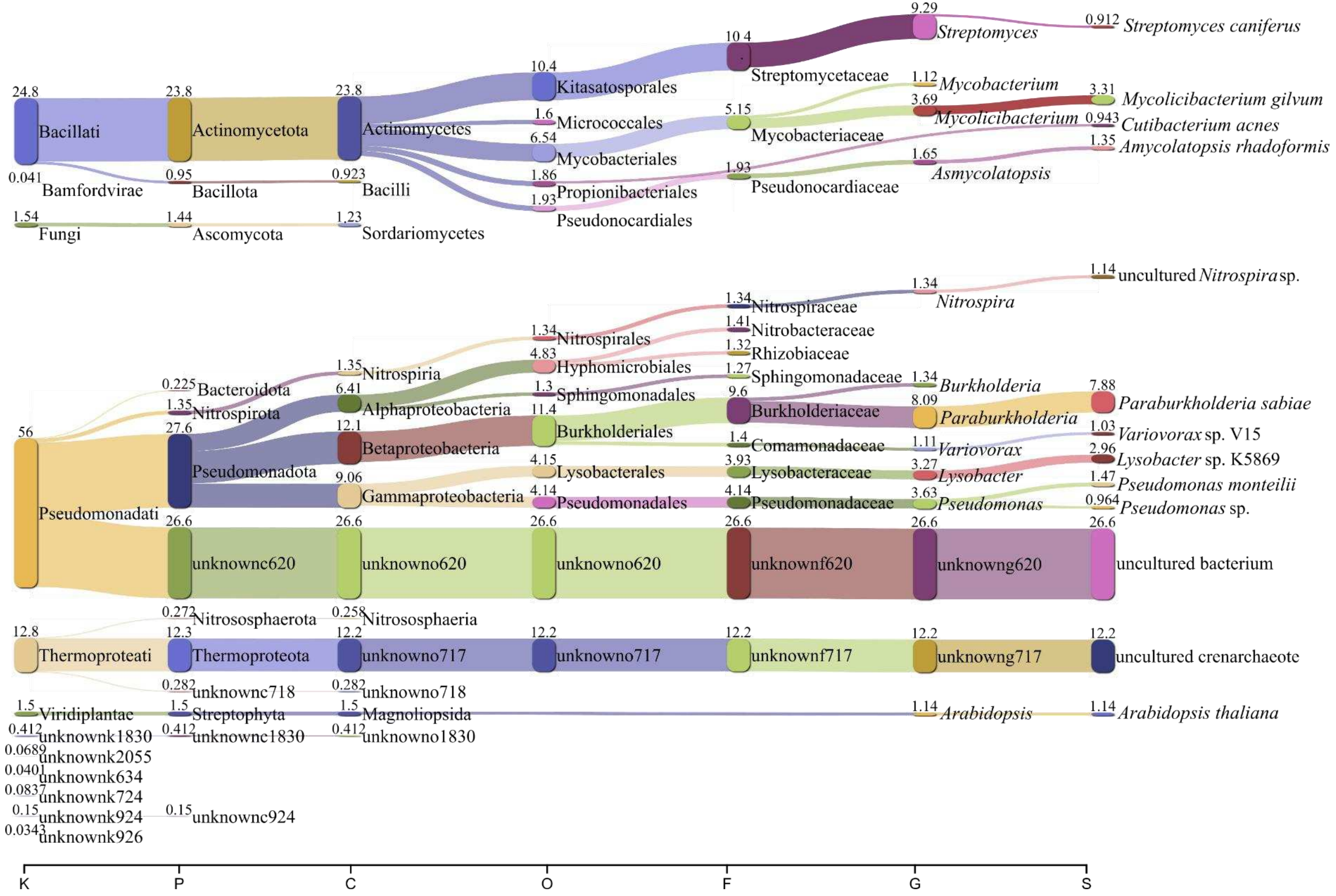


Figure 5 - Pavian plot for organisms identified in the read-based analysis for *Coffea arabica* soil under organic fertilization. The plot was constructed from the median abundance of each organism detected in each sample.



3.3.2 Bin analysis

In the analysis, we recovered 16 dereplicated bins (MAGs) from conventional fertilization soil metagenomic sequences (TC), while the organic system (TO) yielded an impressive 43 bins, nearly three times the number of bins found in the conventional system. The overall characteristic of MAGs is presented in Table 1. Most bins could be identified at the genus and family levels using GTDB-tk taxonomic assignment, comprising members of the phylum Pseudomonadota and class Gammaproteobacteria (Figure S1), phylum Pseudomonadota and class Alphaproteobacteria (Figure S2), phylum Gemmatimonadota (Figure S3), phylum Actinomycetota (Figure S4), phylum Chloroflexota (Figure S5), phylum Acidobacteriota (Figure S6), and phylum Verrucomicrobiota (Figure S7).

For TC, bins represented 1.05-7.6% (median = 3.65%) of the reads sequenced and 7.1-15.5% (median = 10.7%) for TO. This abundance mapping from the alignment was used to build the relative abundance visualization (Figure 6). Both treatments exhibited a similar presence of the phylum Thermoproteota, family Nitrososphaeraceae, genus TA-21, while the family Nitropumilacea, genus *Nitrosotalea* was exclusive for the organic system. The phylum Acidobacteriota was also identified in both treatments, with different main genus and families, but was dominant only in the conventional system. Notably, the phylum Actinomycetota, Zixibacteria, Dormibacterota and Verrucomicrobiota were only detected in the organic fertilization system, and a higher predominance of Pseudomonadota and Gemmatimonadota compared to the conventional fertilization. Interestingly, Methylomirabilota was detected in only one sample from the conventional system. Overall, the taxonomic distribution of MAGs between the soil communities from the conventional and organic fertilization regimens indicated that the organic community was more diversified and more even between the identified taxon, while the conventional one had less taxonomic diversification and was dominated by three main taxa (Acidobacteriota, Pseudomonadota and Thermoproteota).

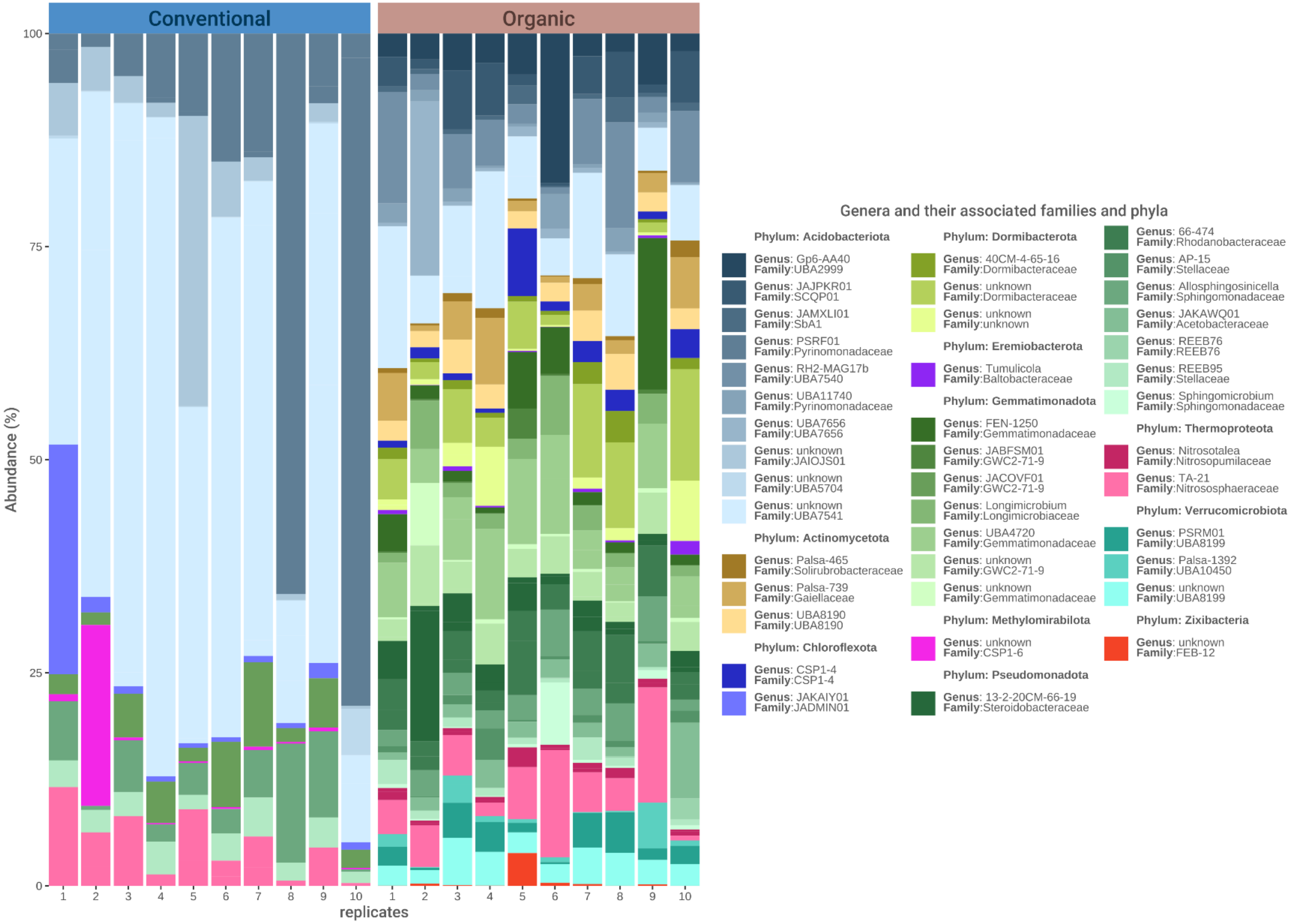
Table 1 – Assembly, taxonomic assignment (GTDB-tk), quality (CheckM 1 and 2) and annotation properties of assembled bins from metagenomic reads from *Coffea arabica* soil samples under conventional treatment (TC) and organic treatment (TO) .

		Assembly				**GTDB-tk taxonomic assignment**		**CheckM (%)**		**CheckM2 (%)**		**Annotation**		
Treatment	**Bin**	**Length (bp)**	**Contigs**	**N50**	**GC content**	**Highest taxon**	**Assignment**	**Completeness**	**Contamination**	**Completeness**	**Contamination**	**CDS**	**tRNA**	**misc_RNA**
TC	TC1_bin_1	2166713	1403	1542	0.66	Genus	JAKAIY01 (o__Ktedonobacterales)	52.41	2.48	42.11	1.97	2264	19	11
	TC2_bin_1	3939909	948	4463	0.73	Family	CSP1-6 (o__Rokubacteriales)	52.59	2.16	58.41	0.43	3954	17	5
	TC2_bin_2	3726122	357	13351	0.57	Family	UBA7541 (o__Acidoferrales)	86.68	2.18	91.19	1.99	3384	33	7
	TC3_bin_1	1141996	738	1516	0.36	Genus	TA-21 (f_Nitrososphaeraceae)	52.96	5.73	47.39	3.28	1134	10	5
	TC4_bin_1	4923686	867	9829	0.6	Family	UBA7541 (o__Acidoferrales)	89	1.86	87.97	1.36	4339	38	12
	TC4_bin_2	3469210	425	9732	0.58	Family	UBA7541 (o__Acidoferrales)	83.62	2.56	86.5	2.39	3184	39	8
	TC4_bin_4	1407316	766	1933	0.66	Genus	REEB95 (f__Stellaceae)	55.68	2.04	52.58	1.51	1593	17	7
	TC5_bin_1	5616986	457	16615	0.71	Family	JAIOJS01 (c__Thermoanaerobaculia)	92.42	0.85	88.85	3.71	4736	52	6
	TC5_bin_3	1899166	568	5221	0.36	Genus	TA-21 (f_Nitrososphaeraceae)	95.4	3.56	87.05	1.03	1936	24	5
	TC5_bin_4	3397640	572	6893	0.6	Family	UBA7541 (o__Acidoferrales)	57.38	1.72	69.51	2.25	3062	20	18
	TC5_bin_5	2611815	529	5316	0.57	Family	UBA7541 (o__Acidoferrales)	56.72	2.74	54.25	2.37	2540	21	3
	TC6_bin_2	1713130	406	4316	0.67	Genus	JACOVF01 (o__Gemmatimonadales)	53.14	1.1	44.1	0.4	1805	21	3
	TC8_bin_1	7396130	612	18374	0.55	Genus	PSRF01 (f__Pyrinomonadaceae)	94.82	2.56	96.85	1.95	6079	57	25
	TC8_bin_2	3135220	534	7668	0.68	Genus	Allosphingosinicella	86.66	1.75	81.42	0.51	3256	43	12
	TC10_bin_1	3395764	1423	2661	0.69	Family	UBA5704 (c__Thermoanaerobaculia)	56.09	0.85	52.77	1.83	3313	21	6
	TC10_bin_2	7137204	73	239997	0.56	Genus	PSRF01 (f__Pyrinomonadaceae)	96.34	1.88	96.14	2.13	5632	42	19
TO	TO1_bin_2	1552704	835	1949	0.65	Genus	REEB95 (f__Stellaceae)	62.44	8.87	66.2	6.5	1772	19	11
	TO1_bin_3	1143420	792	1423	0.39	Genus	Nitrosotalea	52.55	8.96	44.51	8.47	1265	15	1
	TO10_bin_1	2416471	121	27815	0.72	Order	Dormibacterales	95.78	0.19	93.93	2.6	2410	37	7
	TO10_bin_10	1638927	242	7754	0.63	Genus	REEB76 (c__Gammaproteobacteria)	73.27	2.07	77.67	0.64	1610	24	9
	TO10_bin_11	3359788	196	25051	0.67	Family	Dormibacteraceae	88.42	1.85	79.99	0.92	3501	49	3
	TO10_bin_2	1704228	332	5496	0.69	Family	GWC2-71-9 (o__Gemmatimonadales)	51.91	0	55.57	0.25	1676	20	6
	TO10_bin_4	1594482	850	1996	0.66	Genus	Tumulicola	52.42	1.39	53.83	1.13	1741	16	3
	TO10_bin_8	4874905	242	35070	0.66	Genus	JAKAWQ01 (f__Acetobacteraceae)	96.77	0.18	98.15	4.31	4710	44	17

		Assembly				GTDB-tk taxonomic assignment		CheckM (%)		CheckM2 (%)		Annotation		
Treatment	**Bin**	**Length (bp)**	**Contigs**	**N50**	**GC content**	**Highest taxon**	**Assignment**	**Completeness**	**Contamination**	**Completeness**	**Contamination**	**CDS**	**tRNA**	**misc_RNA**
	TO10_bin_9	1976452	533	3598	0.69	Genus	Palsa-465 (f__Solirubrobacteraceae)	51.75	8.62	43.51	4.14	2078	15	9
	TO2_bin_1	4664070	859	6342	0.63	Family	Gemmatimonadaceae	79.34	2.2	80.3	2.04	4298	37	8
	TO2_bin_2	3597127	228	25348	0.69	Genus	13-2-20CM-66-19 (f__Steroidobacteraceae)	92.38	3.27	90.29	0.72	3327	32	16
	TO2_bin_3	1566790	98	29463	0.36	Genus	TA-21 (f_Nitrososphaeraceae)	99.18	0.97	96.45	0	1641	19	4
	TO2_bin_4	2397127	384	7220	0.68	Family	GWC2-71-9 (o__Gemmatimonadales)	70.14	1.1	67.27	0.13	2373	24	8
	TO2_bin_5	2920234	1667	1849	0.73	Genus	Longimicrobium	52.28	3.85	47.47	2.56	2986	8	4
	TO2_bin_6	7638229	531	24265	0.53	Genus	UBA7656 (c__Blastocatellia)	96.58	8.59	95.85	3.17	6115	45	57
	TO3_bin_11	2375751	602	3974	0.68	Genus	13-2-20CM-66-19 (f__Steroidobacteraceae)	50.86	1.72	38.27	0.48	2343	26	8
	TO3_bin_3	4675495	2904	1641	0.62	Family	UBA8199 (o__Pedosphaerales)	54.92	8.42	49.54	7.57	4815	17	4
	TO3_bin_4	3094491	328	12395	0.67	Genus	JAJPKR01 (o__Terriglobales)	89.53	0.85	91.27	1.43	2729	34	5
	TO3_bin_6	2152725	815	2884	0.62	Genus	Palsa-1392 (o_Chthoniobacterales)	65.51	3.72	57.52	2.2	2341	32	3
	TO3_bin_7	2908180	332	10988	0.63	Genus	UBA4720 (f__Gemmatimonadaceae)	83.25	1.75	86.7	0.79	2748	37	8
	TO4_bin_2	2673103	230	15725	0.68	Order	Dormibacterales	98.15	0	90.79	1.97	2720	41	9
	TO4_bin_3	2512832	1023	2765	0.69	Genus	AP-15 (f_Stellaceae)	73.63	3.26	70.41	1.4	2591	28	8
	TO4_bin_4	2765674	138	31591	0.71	Genus	Palsa-739 (f__Gaiellaceae)	91.22	0.99	95.6	1.34	2974	50	6
	TO4_bin_5	3663482	338	15405	0.63	Genus	UBA4720 (f__Gemmatimonadaceae)	91.64	2.75	96.46	2.78	3470	41	10
	TO4_bin_6	4707948	294	23056	0.6	Genus	UBA7541 (o__Acidoferrales)	86.41	0.85	90.68	0.04	3914	44	24
	TO5_bin_2	2383097	821	3524	0.47	Family	FEB-12 (p__Zixibacteria)	87.44	0	84.59	0.98	2070	40	5
	TO5_bin_4	2762176	113	43436	0.72	Genus	CSP1-4 (o__Limnocylindrales)	94.24	2.78	94.64	0.38	2586	49	6
	TO5_bin_5	2211069	1216	1890	0.65	Genus	JABFSM01 (o__Gemmatimonadales)	55.77	5.86	47.54	3.12	2395	17	3
	TO5_bin_8	3362207	895	4127	0.64	Genus	66-474 (f__Rhodanobacteraceae)	52.07	8.62	69.84	15.94	3367	30	8
	TO5_bin_9	948149	611	1522	0.38	Genus	*Nitrosotalea*	54.78	5.33	53.45	3.87	1038	16	1
	TO6_bin_2	1833311	340	5684	0.65	Genus	*Sphingomicrobium*	70.28	0.7	73.2	0.81	1961	28	7
	TO6_bin_3	5472386	1296	4751	0.65	Genus	Gp6-AA40 (o__Vicinamibacterales)	65.52	0	84.72	0.91	5174	28	7
	TO8_bin_1	3064281	514	6561	0.63	Genus	40CM-4-65-16 (f__Dormibacteraceae)	79.85	3.4	71.87	2.08	3344	25	5
	TO8_bin_10	2380514	606	4558	0.65	Genus	66-474 (f__Rhodanobacteraceae)	69.76	6.28	68.16	4.1	2324	22	9

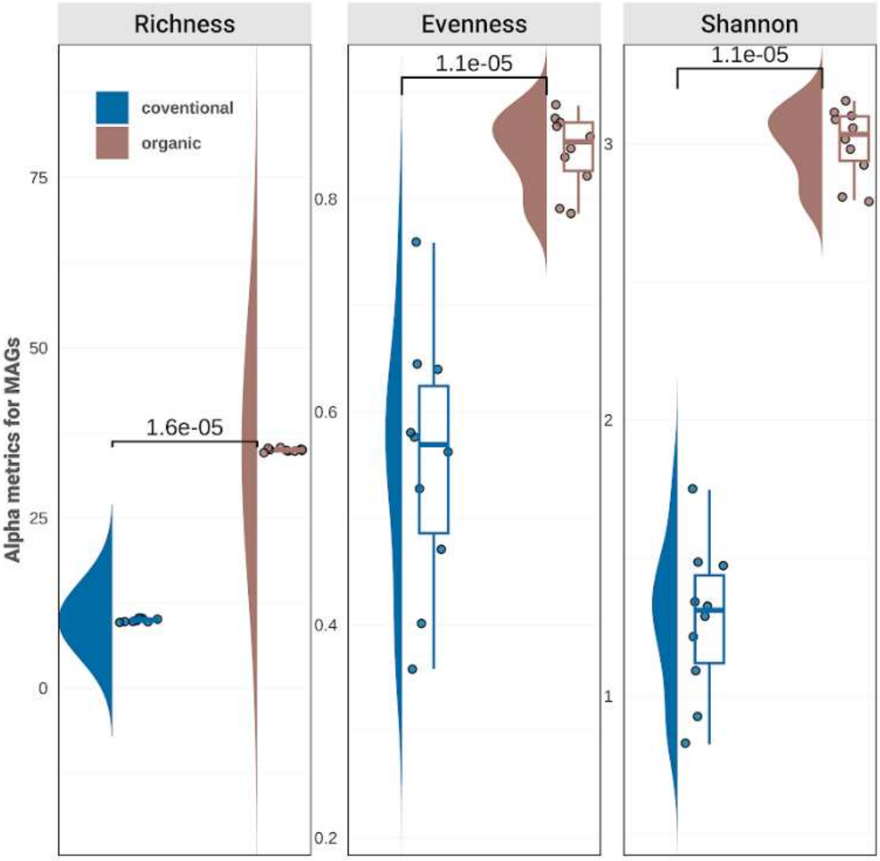
		Assembly				GTDB-tk taxonomic assignment		CheckM (%)		CheckM2 (%)		Annotation		
Treatment	**Bin**	**Length (bp)**	**Contigs**	**N50**	**GC content**	**Highest taxon**	**Assignment**	**Completeness**	**Contamination**	**Completeness**	**Contamination**	**CDS**	**tRNA**	**misc_RNA**
	TO8_bin_11	3327867	1582	2361	0.56	Genus	UBA11740 (f__Pyrinomonadaceae)	54.98	5.97	55.47	6.94	3232	12	38
	TO8_bin_2	4632833	150	60712	0.58	Genus	RH2-MAG17b (c__Terriglobia)	94.23	1.71	97.5	0.99	3756	48	20
	TO8_bin_3	3608766	2106	1768	0.61	Genus	JAMXLI01 (o__Terriglobales)	54.95	7.8	49.39	5.5	3919	27	18
	TO8_bin_6	2372893	306	9536	0.7	Genus	Allosphingosinicella	88.02	0.88	90.92	2.41	2556	44	8
	TO8_bin_7	5211621	2026	2824	0.61	Genus	PSRM01 (o__Pedosphaerales)	73.4	5	71.18	2.25	5085	26	6
	TO8_bin_8	3669005	430	10787	0.58	Family	UBA7541 (o__Acidoferrales)	65.02	6.84	67.15	6.97	3187	33	7
	TO8_bin_9	2410231	302	9714	0.72	Genus	UBA8190 (o__Acidimicrobiales)	83.97	1.45	77.04	1.37	2464	44	8
	TO9_bin_2	3864427	271	23841	0.68	Genus	FEN-1250 (f__Gemmatimonadaceae)	95.75	2.36	97.98	2.75	3533	42	10
	TO9_bin_3	1910017	979	2091	0.62	Genus	Palsa-1392 (o_Chthoniobacterales)	55.13	0	52.31	0.34	2197	19	2

Figure 6 – Relative taxonomic composition of Metagenome-Assembled Genomes (MAGs) from *Coffea arabica* soil under two fertilization regimes (conventional and organic). The genera and their associated families and phyla are presented.



The Alpha metrics from the two coffee fertilization regimens based on MAGs (Fig. 7) revealed that the richness, evenness, and Shannon diversity indices were significantly higher in the organic fertilization treatment, indicating a greater diversity and a more balanced microbial community. Higher richness reflects the presence of a larger number of distinct taxa, while increased evenness suggests a more uniform distribution of these taxa within the community, consistent with the relative taxonomic composition (Figure 6). The elevated Shannon diversity index, which combines both richness and evenness, underscores a more complex and stable microbial ecosystem under these conditions. These findings highlight the potential influence of organic fertilization on promoting microbial diversity and balance.

Figure 7 - Ecological metrics of *Coffea arabica* soil under two fertilization regimes (conventional and organic) based on Metagenome-assembled genomes (MAGs). Each point represents a composite sample; the organic fertilization is presented in brown and the conventional fertilization in blue. The numbers represent significant differences (p < 0.05) according to the F test.



Furthermore, to gain insights into the functional characteristics of the bins identified, we used DRAM to identify the completeness of central metabolic pathways (Figure S8) and the presence of pathways and enzymes related to important microbial roles in the soil (Figure 8). Multiple MAGs, especially those found in the organic fertilization system, presented activity on polymers in the plant cell wall, including cellulose, xyloglucan, and arabinan and fungal cell wall (mixed-linkage glucans), which might indicate a good potential for carbon recycling in the soil. The MAGs from the organic system also presented a greater presence of pathways related to the nitrogen cycle in the soil, especially nitrate and nitrite interconversion and conversion of nitric oxide to nitrous oxide.

Regarding the presence of PGPGs, MAGs from both fertilization regimens presented a great abundance of genes related to nodulation, reactive oxygen species (ROS) scavenging, iron acquisition, salinity stress-potassium transport, plant embryogenesis-spermidine, IAA-related tryptophan metabolism, and phosphorus solubilization; however, few genes related to nitrogen fixation were found, indicating that this function might be carried out by less abundant nitrogen-fixing bacteria, as those found in the read-based analysis.

Figure 8 - DRAM functional annotation: carbohydrate-active enzymes, nitrogen metabolism, sulfur metabolism, reductases, methanogenesis and methanotrophy from Metagenome-Assembled Genomes (MAGs) from *Coffea arabica* soil under two fertilization regimes (conventional and organic).

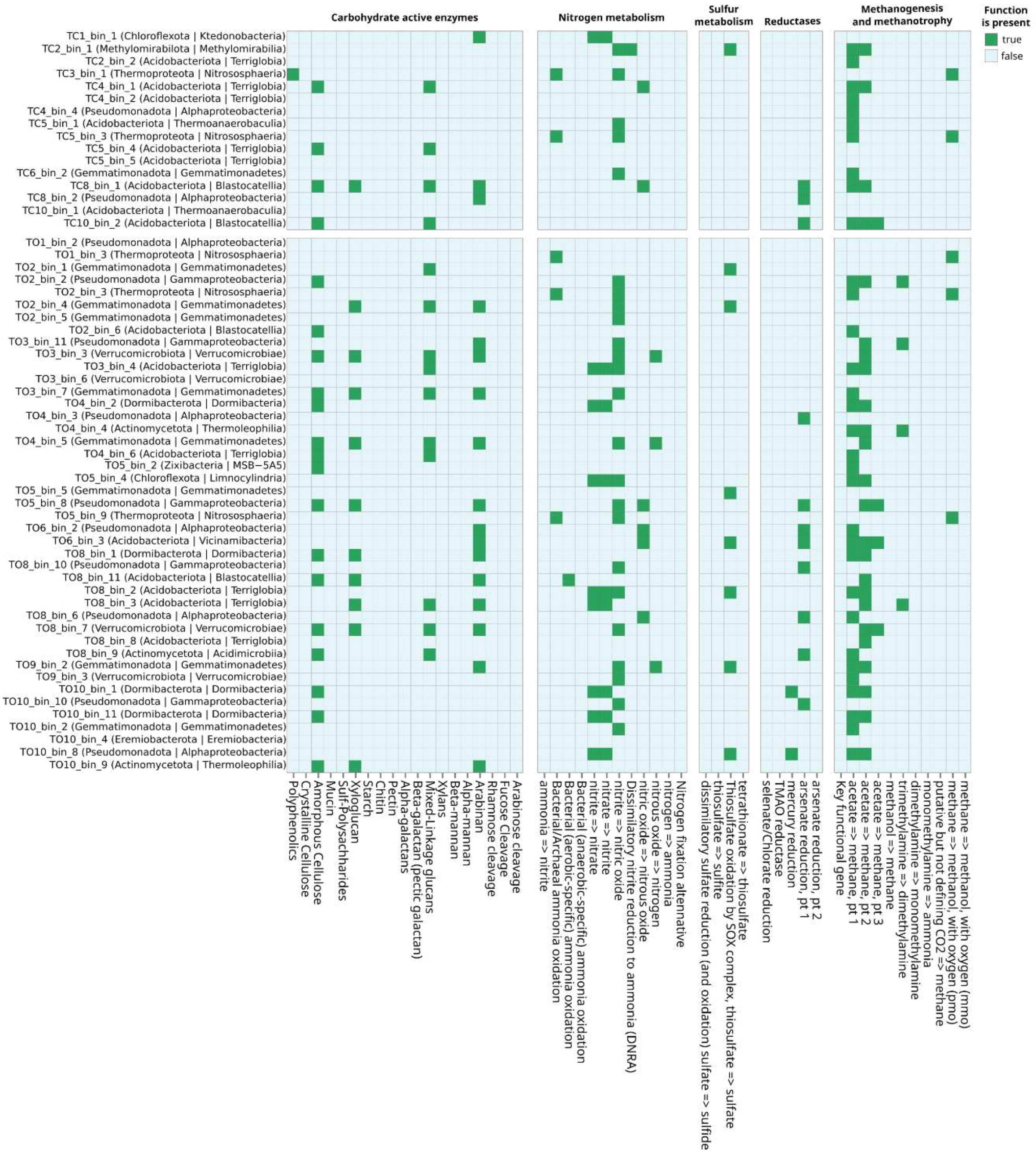
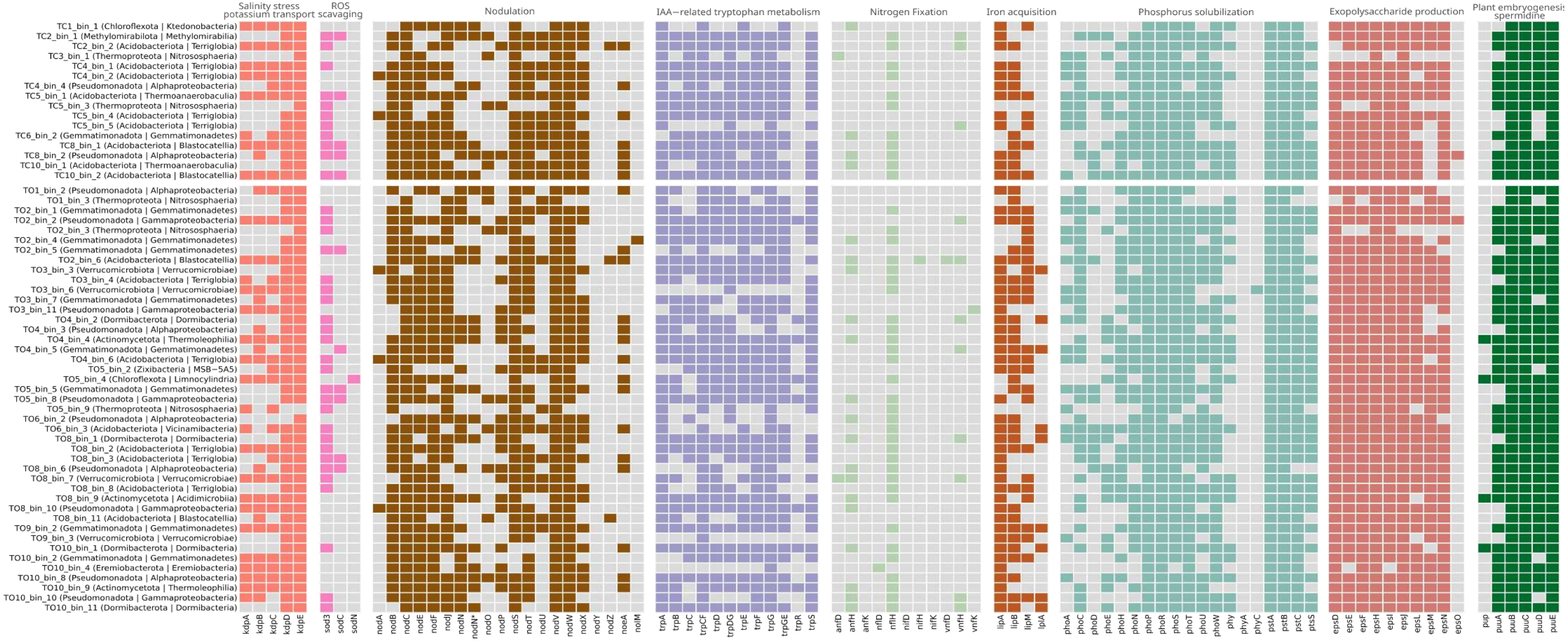


Figure 9 – Prediction of plant growth-promoting genes (PGPGs) in the Metagenome-Assembled Genomes (MAGs) from *Coffea arabica* soil under two fertilization regimes (conventional and organic).: nitrogen fixation (*nif*H, *nif*D, *nfl*H, *nfl*D, *nif*K, *vnf*K, *vnf*H, *vnf*D, *anf*D, *anf*H, *anf*K), exopolysaccharide (EPS) (*eps*D, *eps*E, *eps*F, *eps*H, *eps*I, *eps*J, *eps*L, *eps*M, *eps*N, *eps*O), nodulation (*nod*A, *nod*B, *nod*C, *nod*E, *nod*F, *nod*J, *nod*N, *nod*N_like, *nod*O, *nod*P, *nod*S, *nod*T, *nod*U, *nod*V, *nod*W, *nod*X, *nod*Y, *nod*Z, *noe*A, *nol*M), reactive oxygen species (ROS) scavenging (*sod*3, *sod*C, *sod*N), iron acquisition (*lip*A, *lip*B, *lip*M, *lpl*A), salinity stress-potassium transport (*kdp*A, *kdp*B, *kdp*C, *kdp*D, *kdp*E), plant embryogenesis-spermidine (*pup*, *puu*A, *puu*B, *puu*C, *puu*D, *puu*E), IAA-related tryptophan metabolism (*trp*A, *trp*B, *trp*C, *trp*CF, *trp*D, *trp*DG, *trp*E, *trp*F, *trp*G, *trp*GE, *trp*R, *trp*S), and phosphorus solubilization (*pho*A, *pho*C, *pho*D, *pho*E, *pho*H, *pho*N, *pho*P, *pho*R, *pho*S, *pho*T, *pho*U, *pho*W, *phy*, *phy*A, *phy*C, *pst*A, *pst*B, *pst*C, *pts*S). Gray boxes indicate absence of genes, while non-gray boxes indicate presence.



3.4 Discussion

The microbiomes interact both in the soil (rhizosphere) and above it (phyllosphere) and are collectively called the holobiome. The rhizosphere is directly influenced by the roots presenting high microbial activity due to root exudates that serve as a source of energy (JAT et al., 2021). This community affects plant growth, nutrition and health and can be manipulated by introducing biofertilizers, biostimulants and biopesticides (SANTOS; OLIVARES, 2021). Studies on the soil-associated microbiomes of various food crops, such as rice (KIM; LEE, 2020), corn, soybeans, citrus (LIMA et al., 2024) and wheat, have revealed that each plant has a core group of microorganisms and that microbial communities vary according to host characteristics and environmental factors, such as altitude, soil type and rainfall. These microorganisms directly influence plant health, promoting growth, suppressing pathogens and mitigating the negative effects of salinity, water stress and xenobiotics (KIM; LEE, 2020; LIMA et al., 2024). Coffee, one of the most important commodities on the global market. Although the genomes of coffee trees, especially *C. arabica*, have been widely studied, there are still significant gaps in plant-microorganism interactions, including research into the microbiome. Therefore, in the current study, we conducted the first metagenomic analysis of coffee soil *in situ*. First, we performed read-based analysis to provide an initial view of the soil community under two fertilization regimens, conventional and organic. Then, we generated MAGs to further gain insights into the main microorganisms of the community and their functional roles.

The soil from the organic treatment presented increased levels of different soil components (Figure 1). Composted organic waste, such as the one composed to be used as a fertilizer in the property related to present study, increases the mineral organic matter associated with the soil (a more stable carbon pool) and the availability of phosphorus, which favors the synergy between organic waste and mineral fertilizers, with the aim of increasing soil productivity and agricultural sustainability (MARTINS et al., 2024). In the multidimensional analysis at the read level, the archaeal community of the organic fertilized soil was correlated to sulfur, phosphorus, calcium, organic matter, magnesium, manganese, zinc, and boron contents, the sum of exchangeable bases, and the cation exchange capacity; meanwhile, in the conventionally fertilized soil, the community was related to remaining content, pH, iron content and base saturation. A similar trend was observed for the bacterial and fungal communities

(Figure 2). These results highlighted how the inclusion of the organic compound as a fertilizer influences the microbial composition of the soil which in turn also affects these parameters (SHU et al., 2022). Additionally, the increase in organic matter through the incorporation of organic compounds enhances soil conditioning, increasing cation exchange capacity and enabling the organic soil to function as a substantial nutrient reservoir for plants (LAZCANO et al., 2021).

According to the Pavian plots (Figures 4 and 5), although the communities exhibited structural similarities, certain differences highlight the impact of the fertilization regimes. Conventional fertilization was associated with a higher prevalence of species linked to pathogenic interactions, such as *Fusarium oxysporum*, suggesting that this regime may promote the presence of potentially harmful microorganisms (SUN et al. 2022). In contrast, organic fertilization favored the presence of beneficial microorganisms, such as *Nitrospira* and *Lysobacter sp.*, which are involved in nutrient cycling and plant growth promotion (LI et al. 2021; SHU et al. 2023). These results demonstrate that, even in soils from the same region, microbial composition can be significantly influenced by different fertilization practices.

These results were also reflected in the taxonomic composition identified in the MAGs. In the conventional system, the main phylum identified was Acidobacteriota. Acidobacteria comprise acidophilic bacteria with most of the known strains being uncultured. However, these bacteria can make an important contribution to the ecosystem, as they are particularly abundant in the soil. These microorganisms can participate in nitrogen fixation, phosphorus solubilization, EPS and plant hormone production (GONÇALVEZ et al., 2024). They represent a group with great genetic diversity, and the MAGs in the current study represent yet uncharacterized genera and families. Nevertheless, plant-growth promotion traits were identified in the present MAGs, except for nitrogen fixation, indicating how they might act in the soil community described here.

The phyla Actinomycetota, Zixibacteria, Dormibacterota, and Verrucomicrobiota were detected exclusively in the organic fertilization system. The Actinomycetota phylum includes bacteria with filamentous organization and metabolic versatility, primarily due to their extensive production of extracellular enzymes, which play a key role in decomposing organic matter and improving soil structure (PALANIYANDI et al., 2013). Recent studies have also highlighted the role of these bacteria in the biodegradation of pesticides (JEON et al., 2024). Actinomycetes are also known to produce various bioactive secondary metabolites, including some active against phytopathogenic microorganisms (YANG et al., 2023). Somes species can also present a predatory activity promoting the lysis of their prey. This behavior has been recently proposed as a biocontrol strategy against plant pathogens (IBRAHIMI et al., 2023). The phylum *Candidatus* Zixibacteria (CASTELLE et al., 2013) includes uncultivated bacteria identified in subsurface sediments, estuarine waters, cave deposits, and mine fluids, demonstrating its broad environmental distribution (CASAR et al., 2021). The uncultivated bacterial phylum *Candidatus* Dormibacterota has been identified in Antarctic desert soils (MONTGOMERY et al., 2021; ZHANG et al., 2024) and have metabolic adaptations that enable survival under different environmental conditions, such as the ability to fix atmospheric CO_2 through the Calvin-Bassham-Benson cycle (MONTGOMERY et al., 2021). The phylum Verrucomicrobiota comprises three validated classes and four validated orders (HEDLUND et al., 1997; OREN & GARRITY, 2021). There is also a candidate order, Methylacidiphilales, formed by microorganisms found in extremely acidic geothermal environments (pH as low as 0.8) and high temperatures (RATNADEVI et al., 2023).

In both systems, Pseudomonadota and Thermoproteota presented a significant abundance. The phylum Pseudomonadota (OREN et al., 2024), formerly known as Proteobacteria, is a phylum of gram-negative bacteria. It is one of the largest and most versatile phyla in the Bacteria domain, including more than 460 genera and 1,600 species, encompassing phototrophic, chemolithotrophic and heterotrophic bacteria. Pseudomonadota are reported to be one of the predominant phyla in natural environments, often impacted by human activities (LENNERT et al., 2024). The genera detected here has been recently identified and are still poorly described and their role in the soil environment of coffee plants remains to be further explored. Moreover, Thermoproteota are a phylum of Archaea essentially comprising thermophiles and hyperthermophiles and are methyl-reducing hydrogenotrophic methanogens. These archaea contribute to carbon fixation in soil, contributing to anaerobic carbon-cycling dynamics (KOHTZ et al., 2024).

Furthermore, the functional analysis of the genes identified in the MAGs highlighted the presence of PGPGs in genomes from both systems. Meanwhile, the organic system presented MAGs with a higher abundance of genes related to carbon recycling in the soil, specifically the degradation of plant cell wall components and nitrification. Additionally, in the organic system, more MAGs were identified with these functions, indicative of greater functional redundancy. This functional redundancy indicates greater potential resilience of the microbial community from organic treatment to climatic events, such as prolonged periods of drought and high temperatures. This response can be related to a larger number of microorganisms capable of performing essential functions for plant health, ensuring the maintenance of ecosystem functionality, even if some microorganisms are eliminated (CHEN et al., 2022).

This study also has some limitations. The low classification of reads in the Kraken2 analysis may be due to the low representation in the databases of microorganisms that make up the soil microbiome, compared to the microbiome of mammals and marine sediments. Kraken2 itself already identifies non-cultivable and unidentified microorganisms as its main taxon in the current work. Given this low ranking, diversity may have been poorly represented, and the results of the reads should be considered with caution. Meanwhile, the analysis with MAGs has a greater potential to represent - truly - the main members of the community. Considering that MAGs need a good coverage of reads to be assembled assertively; well-identified MAGs have great potential to represent important members of a given community (LIU et al. 2022). Consistently, the mapping of the reads to the described MAGs resulted in more coverage of the sequencing data for both conventional and organic treatments. Nevertheless, many reads remained unidentified and unmapped. This lack of mapping of reads originating from soil compared to samples from mammals, for example, is discussed in greater detail by ANTHONY et al. (2024). This might indicate a fragmented nature of part of the sequencing data. Overall, this highlights the importance of generating novel datasets of soil microbiomes and of new endeavors to explore the coffee-associated microbiome. Therefore, future research should focus on expanding the databases used for classification and further investigating the diverse microbial communities within coffee and soil ecosystems.

3.5 Conclusion

Organic fertilization enhances soil quality by increasing organic matter and nutrient availability. Acidobacteriota dominates in the conventional fertilization regimen, while organic fertilization favors Actinomycetota. The organic system has a greater abundance of genes involved in carbon recycling and plant-growth promotion, which might represent a higher resilience of the community. Overall, the current results provide insights into the functional dynamics of microorganisms and highlight the relevance of soils managed with more sustainable and resilient agricultural practices in the face of climate change. Future studies can explore the uncharacterized taxa identified here and elucidate their specific roles in coffee soil microbiomes.

3.6 Data availability

All the sequencing data is deposited on the BioProject PRJNA1248875.

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3.8 Supplementary material

Figure S1 - Phylogenetic classification of MAGs according to the GTDBtk of the phylum Pseudomonadota and class Gammaproteobacteria from *Coffea arabica* soil under two fertilization regimes (conventional and organic).

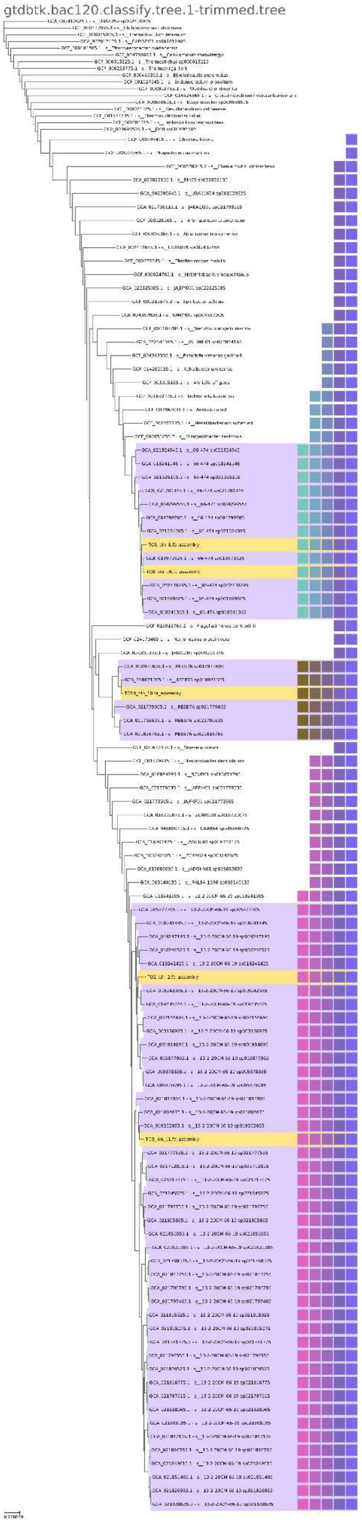

Figure S2 - Phylogenetic classification of MAGs according to the GTDBtk of the phylum Pseudomonadota and class Alphaproteobacteria from *Coffea arabica* soil under two fertilization regimes (conventional and organic).

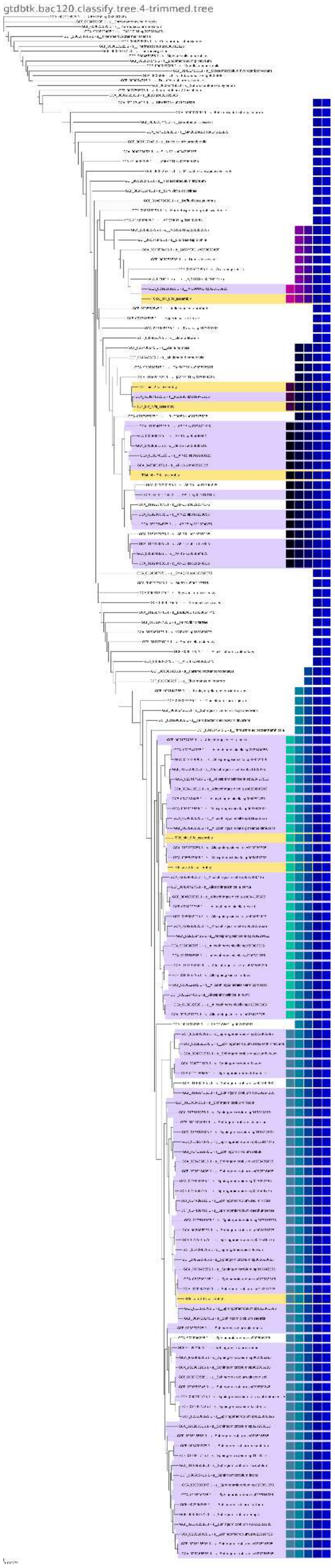

Figure S3 - Phylogenetic classification of MAGs according to the GTDBtk of the phylum Gemmatimonadota from *Coffea arabica* soil under two fertilization regimes (conventional and organic).

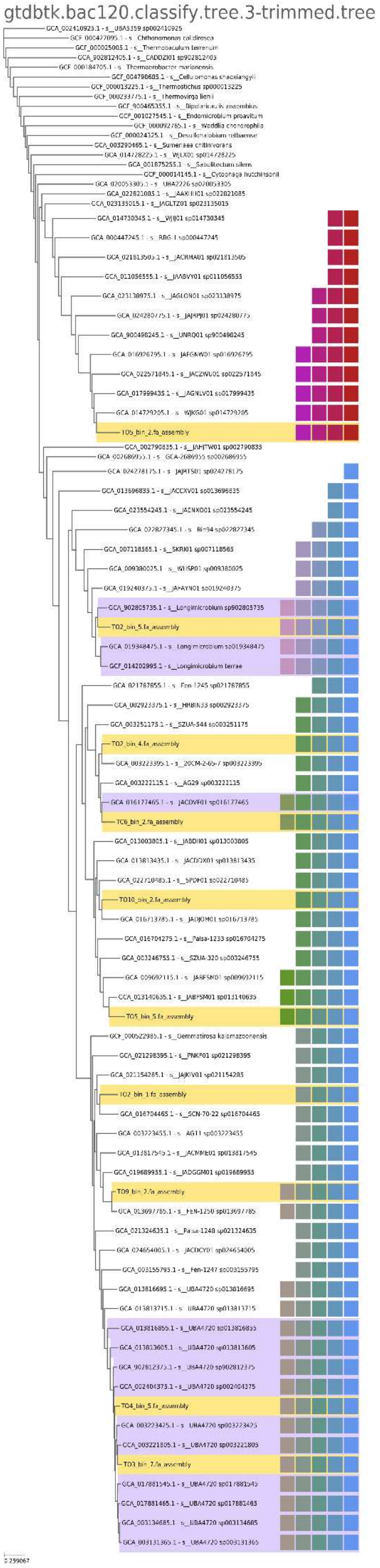

Figure S4 - Phylogenetic classification of MAGs according to the GTDBtk of the phylum Actinomycetota from *Coffea arabica* soil under two fertilization regimes (conventional and organic).

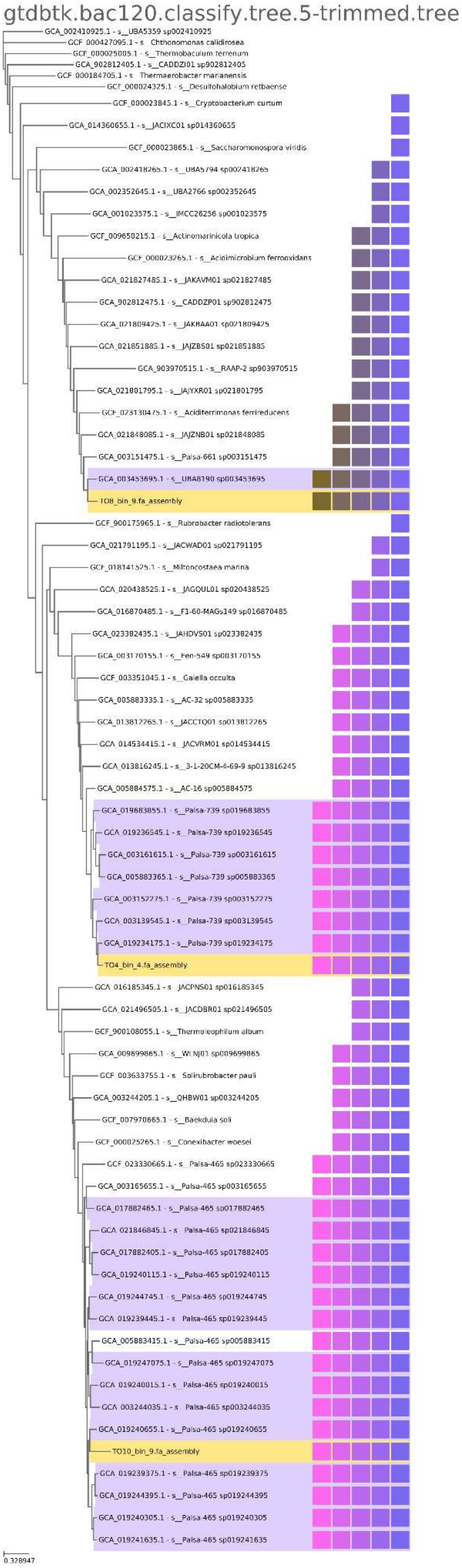

Figure S5 - Phylogenetic classification of MAGs according to the GTDBtk of the phylum Chloroflexota from *Coffea arabica* soil under two fertilization regimes (conventional and organic).

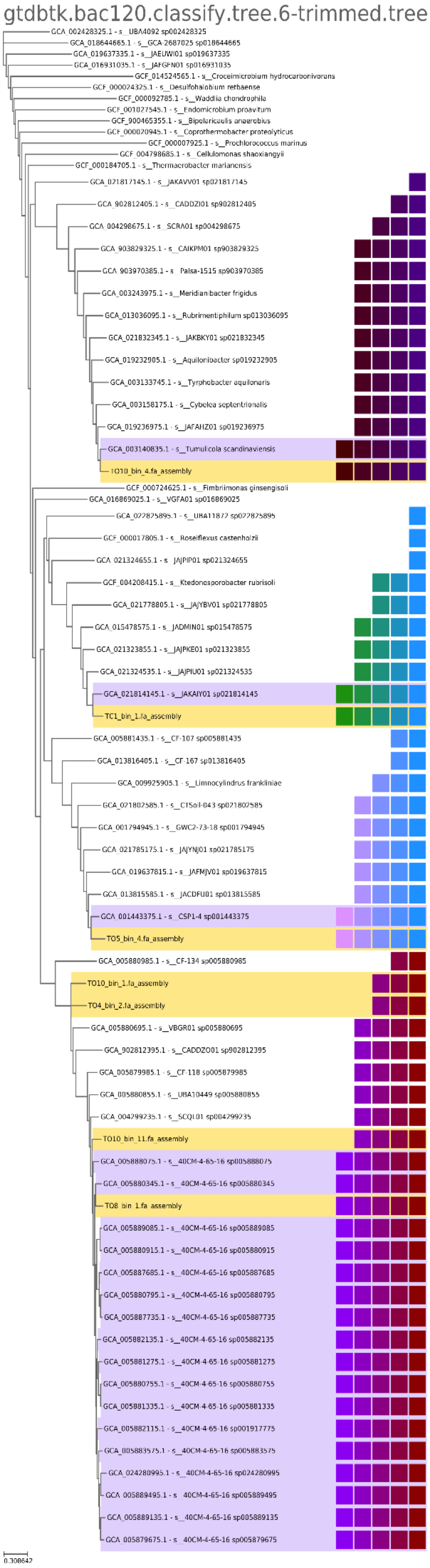

Figure S6 - Phylogenetic classification of MAGs according to the GTDBtk of the phylum Acidobacteriota from *Coffea arabica* soil under two fertilization regimes (conventional and organic).

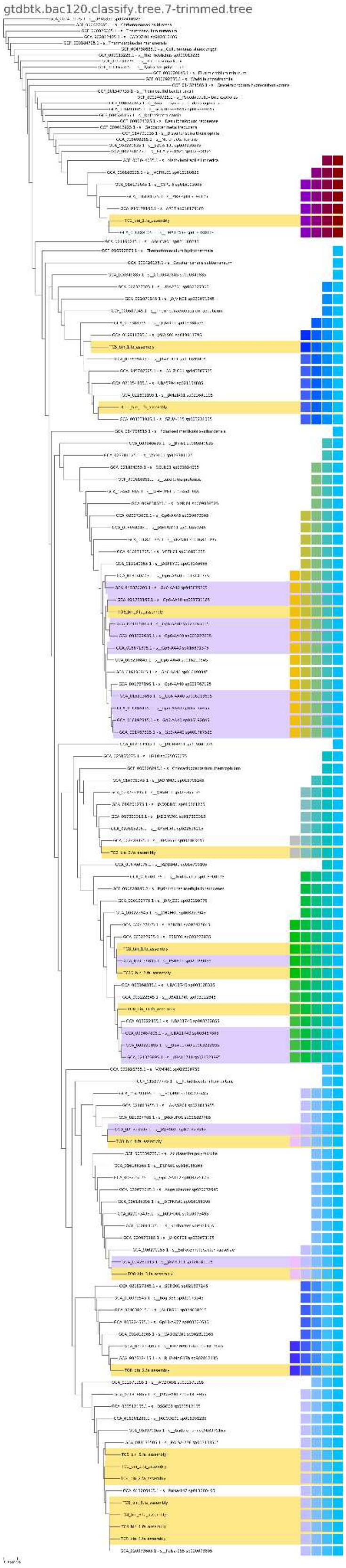

Figure S7 - Phylogenetic classification of MAGs according to the GTDBtk of the phylum Verrucomicrobiota from *Coffea arabica* soil under two fertilization regimes (conventional and organic).

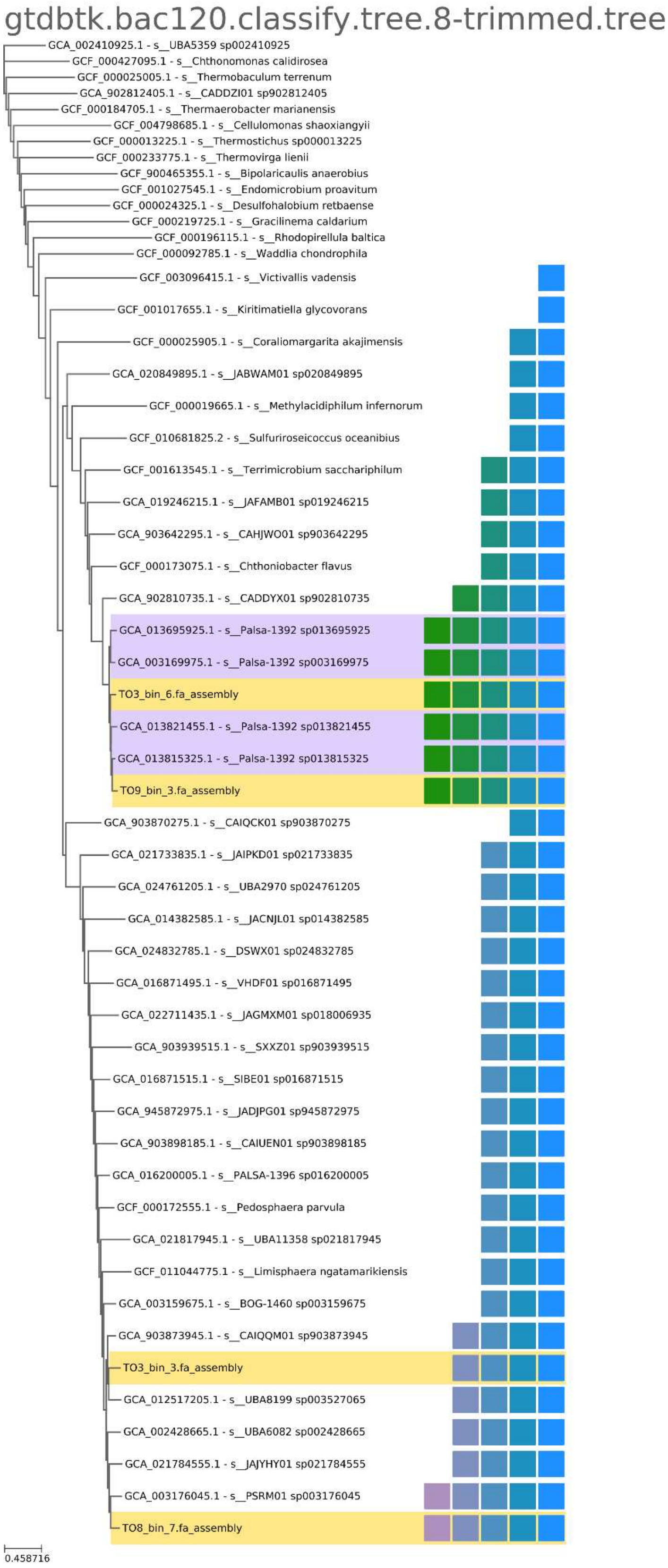

Figure S8 - DRAM functional annotation of central metabolic pathways from Metagenome-Assembled Genomes (MAGs) from two coffee fertilization regimens from *Coffea arabica* soil under two fertilization regimes (conventional and organic).