ANA LUCIA NUNEZ VILLALOBOS

DIVERSITY OF RHIZOBIA AND CARBON SEQUESTRATION IN SOILS UNDER COFFEE CROPPING SYSTEMS AND MATA ATLANTICA BIOME

LAVRAS - MG
2022

ANA LUCIA NUNEZ VILLALOBOS

DIVERSITY OF RHIZOBIA AND CARBON SEQUESTRATION IN SOILS UNDER COFFEE CROPPING SYSTEMS AND MATA ATLÂNTICA BIOME

Dissertação apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-graduação em Ciências do Solo, para a obtenção do título de Mestre.

Dr. Fatima Maria de Souza Moreira

Orientador

Dr. Teotônio Soares de Carvalho

Dr. Marcia Ruffini

Coorientador

LAVRAS-MG

2022

Ficha catalográfica elaborada pelo Sistema de Geração de Ficha Catalográfica da Biblioteca Universitária da UFLA, com dados informados pelo(a) próprio(a) autor(a).

Nunez Villalobos, Ana Lucia.
Diversity of rhizobia and carbon sequestration in soils under coffee cropping systems and Mata Atlantica biome / Ana Lucia Nunez Villalobos. - 2022.
67 p.

Orientador(a): Fatima Maria de Souza Moreira.
Coorientador(a): Teotônio Soares de Carvalho, Marcia Ruffini.
Dissertação (mestrado acadêmico) - Universidade Federal de Lavras, 2022.
Bibliografia.

1. coffee. 2. rhizobia. 3. diversity. I. Moreira, Fatima Maria de Souza. II. de Carvalho, Teotônio Soares. III. Ruffini, Marcia. IV. Título.

ANA LUCIA NUNEZ VILLALOBOS

DIVERSIDADE DE RIZOBIOS E SEQUESTRO DE CARBONO NOS SOLOS SOB AREA DE CAFÉ E BIOMA MATA ATLATANTICA

DIVERSITY OF RHIZOBIA AND CARBON SEQUESTRATION IN SOILS UNDER COFFEE CROPPING SYSTEMS AND MATA ATLANTICA BIOME

Dissertação apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Entomologia, para obtenção do título de Mestre.

Aprovada em 14 de julho de 2022.

Dr. Fatima Maria de Souza Moreira UFLA

Dr. Silvia Maria Longatti UFLA

Dr. Gladyston Rodrigues EPAMIG

Dr. Fatima Maria de Souza Moreira
Orientador

Dr. Teotonio Soares de Carvalho
Dr. Marcia Ruffini
Coorientador

LAVRAS-MG
2022

ACKNOWLEDGEMENTS

To God, because without His favor, I wouldn't have had these opportunities and wouldn't be where I am.

To CAPES, FAPEMIG and CNPq for the financing of this research Project.

To BiosBrasil, for letting me be part of the awesome research team, under the project ¨BIODIVERSIDADE DO SOLO PARA O AUMENTO DA PRODUÇÃO AGRÍCOLA E FLORESTAL SUSTENTÁVEL- Fapemig CAG - RED-00330-16.

To the areas who opened up their doors for us to sample, as well as for sharing their knowledge and giving us their support throughout the investigation: NKG Fazendas Brasileiras Ltda & Patrik Avelar and Fazenda Samambaia. To EPAMIG for their technical support and their help in finding the areas for sampling. All of this wouldn't had happened without the combined support of all of them.

To Dr. Fatima Moreira, Dr. Teotonio Soares, Dr. Amanda Azarias and Dr. Marcia Ruffini for their help and orientation throughout the development of this Project, their patience and their teachings.

To Universidade Federal de Lavras for giving me the opportunity to increase my knowledge in the Soil Sciences through the Post Graduate program.

To my family, for every word, for every inspiration, for all the care they have given me. To my husband who has been the optimal support system and the best companion throughout this time. To my friends and fellow classmates for their insights and help.

ABSTRACT

Coffee is one of the most consumed beverages in the world, making it a global commodity being the main product for export to Minas Gerais and finding plantations in all biomes, replacing or adjacent to natural vegetation. Microorganisms are relevant components of biodiversity promoting plant growth through processes such as biological nitrogen fixation. Considering the importance of rhizobia, the main objective of this work was to study, via cultural characteristics, the biodiversity of nitrogen-fixing bacteria considering the physical and chemical attributes of the soil, as well as carbon sequestration in coffee areas and Atlantic Forest biome. For this, the diversity of isolated rhizobia was evaluated using species of bait plants. The study was carried out in six areas: in each area samples of the first 10 cm of soil were collected in five-point transepts with five meters of separation. The bait plants Macroptilium atropurpureum and Vigna unguiculata were grown in bottles under axenic conditions, with Hoagland solution as a source of nutrition and inoculated with a dilution of the collected soils. Therefore, the presence of nodules and their weight, root weight, SPAD index and dry matter weight were determined. After disinfection, the nodules were macerated in Petri dishes with YMA medium and the colonies that appeared were isolated until reaching the pure strains for logos to characterize and group them. It is concluded that soils under coffee areas have a diverse community of rhizobia. The Atlantic Forest biome presented higher density and diversity of rhizobia when compared to coffee monoculture areas, also having higher total carbon content. There is a relationship between rhizobia density and soil chemical attributes such as pH and copper content. The bait plant Vigna unguiculata was better to capture rhizobia in the studied areas and they were highly variable in terms of physical and chemical attributes. Finally, it can be concluded that the nitrogen fixing bacteria found are highly resilient, since the physical and chemical characteristics and carbon content were very variable and yet, they managed to nodule effectively.

Key Words: Coffee. Mata Atlantica. Rhizobia. Carbon stock. Diversity

RESUMO

O café e uma das bebidas mais consumidas no mundo, fazendo ela um commodity global sendo o principal produto para exportação para Minas Gerais e achando plantações em todos os biomas, substituindo ou adjacente a vegetação natural. Os microrganismos são componentes relevantes da biodiversidade promovendo o crescimento das plantas através de processos como fixação biológica do nitrogênio. Considerando a importância dos rizóbios, o objetivo principal desse trabalho foi estudar via características culturais, a biodiversidade das bactérias fixadoras de nitrogênio considerando os atributos físicos e químicos do solo, assim como o sequestro de carbono nas áreas de café e bioma mata atlântico. Para isso, foi avaliada a diversidade dos rizóbios isolados utilizando espécies de plantas iscas. O estudo foi conduzido em seis áreas: em cada área foram coletadas amostras dos primeiros 10 cms do solo em transeptos de cinco pontos com cinco metros de separação. As plantas isca Macroptilium atropurpureum e Vigna unguiculata foram cultivadas em garrafas sob condições axênicas, com solução Hoagland como fonte de nutrição e inoculadas com uma diluição dos solos coletados. Logo, a presença de nódulos e seus pesos, o peso da raiz, o índice SPAD e o peso da matéria seca foram determinadas. Depois da desinfeção, os nódulos foram macerados em placas Petri com meio YMA e as colônias que foram aparecendo foram isoladas até chegar as estirpes puras para logos caracterizar e agrupar elas. Conclui-se que solos sob áreas de café possuem uma comunidade diversa de rizóbios. O bioma mata atlântico apresentou maior densidade e diversidade de rizóbios quando comparado com as áreas de monocultura de café, tendo também maior conteúdo de carbono total. Existe uma relação entre a densidade de rizobios e os atributos químicos do solo tais como ph e conteúdo de cobre. A planta isca Vigna unguiculata foi melhor para capturar rizóbios nas áreas estudadas sendo que elas foram altamente variáveis quanto aos atributos físicos e químicos. Finalmente, pode se concluir que as bactérias fixadoras de nitrogênio achadas são altamente resilientes, desde que as características físicas e químicas e o conteúdo de carbono foram muito variáveis e ainda assim, elas conseguiram nodular efetivamente.

Palavras chaves: Café. Mata Atlântica. Rizóbios. Estoque de carbono. Diversidade.

TABLE OF CONTENTS

1 INTRODUCTION

Almost every person enjoys a cup of their favorite coffee every morning. Coffee has become one of the most consumed beverages around the world, making it one of the traded and truly a global commodity (KRISHNAN, 2017; FAO). For Minas Gerais, coffee is the main product for exportation. This area of the economy is also one of the most dynamic of the agriculture of the state, considering its big production volume, the capital that is moved by it and also the socioeconomic importance of the crop, since it is a source of employment for many people (EPAMIG, 2010). Coffee plantations can be found in all Biomes of Minas Gerais State: Mata Atlântica, Cerrado and Caatinga, replacing and adjacent to natural vegetation.

Among these biomes the Mata Atlantica. also called the floresta atlantica (GONCALVES, 2015), is mainly in the center south and east parts, with high mountains called mares de morros that generate variation in altitudes (WERNECK *et al.,* 2010). Generally, its soils are dystrophic, low in nutrients, with acid pH and high variation in soil types and availability of water. It is the oldest forest formation in Brazil. It is also considered a hotspot for conservations due to its high biodiversity, endemic species and high vulnerability.

Microorganisms are relevant components of biodiversity because they are able to promote plant growth through several processes: biological nitrogen fixation (OSORIO FILHO *et al.,* 2016), phosphate solubilization (COSTA *et al.,* 2015, GUDIÑO-GOMEZJURADO*et al.,* 2021), decomposing of organic wastes and residues and rehabilitation of mining areas (CASTRO *et al.,* 2017), imparting stress tolerance (ASHWIN *et al.,* 2022), suppression of plant diseases and soil-borne pathogens (MARTINS *et al.,* 2018; JACK *et al.,* 2018) and production of bioactive compounds such as vitamins, hormones and enzymes (ROCHA *et al.,* 2018). These reduce the need for industrialized inputs such as agrochemicals and fertilizers, also reducing their potential impact on the environment (MICHEL *et al.,* 2020; SINGH *et al.,* 2011). Microorganisms perform critical functions related to the resilience and resistance of plant communities through helping to deal with stress and disturbance (JURBUNG *et al.,* 2020). Therefore,

these microorganisms have emerged as an important and promising tool for sustainable agriculture.

Among plant growth promoting microorganisms, bacteria commonly named as rhizobia, are able to fix atmospheric N2 and perform other plant beneficial processes. Rhizobia must exist in the soils as saprophytes and they must be able to efficiently compete against other members of the microbiota to finally reach their goal: infecting the roots and forming nitrogen fixing nodules. Even though the most relevant studies have been done in leguminous crops, recent findings have been performed in crops like maize (LEITE *et al.,* 2020), rice (OSORIO FILHO *et al.,* 2016; COSTA *et al.,* 2015) and Eucalyptus (FONSECA et al., 2018).

Other studies have also shown that the persistence of rhizobia in soils is dependent on the strain and the type of soil (ALBAREDA *et al.,* 2009), other soil properties such as clay and soil carbon content (ZENGENI *et al.,* 2006), rhizodeposition and exudation since they provide the communities with sources of nutrients and carbon (DUCHENE *et al.,* 2017) and plant diversity influences soil microbial diversity as well (STEINAUER *et al.,* 2016). Even though they are susceptible to many environmental factors, rhizobial communities have shown a great resilience under less than ideal conditions.

Types of management that have as a priority the addition of organic components, the favoring of beneficial physical and chemical properties and in general, the looking out for the bacterial communities have a greater survival rate and maintenance of the rhizobia (FIGUEREIDO *et al.,* 2020). It is important for the bacteria to live in conditions where there is enough of the right amount of substrate that it needs and that environmental characteristics are correct. However, rhizobia have developed diverse metabolic pathways to enable them to grow and multiply.

Besides carbon usage, there have been studies that have shown how bacteria perform a key role in the synthesis or soil organic carbon and organic matter. A study by SU *et al.,* 2020 studied the changes in bacterial and fungal community after an input of crop straw in the soil. They linked these changes with the allocation of the straw-C into dissolved organic carbon, microbial biomass carbon, particulate organic carbon and mineral-associated organic carbon. Their findings suggest that denitrifying and nitrogen fixing bacteria Burkholderia-Paraburkholderia, Paraphaeosphaeria and Bradyrhizobium

are correlated with the distribution of the straw-C into the different compartments. They also found that *Bradyrhizobium* was positively correlated with particulate organic carbon derived from straw. This supports the importance of rhizobia in the conversion of plant residues into soil organic carbon. Another study by Stone *et al.*, also reported *Bradyrhizobium*, along with *Acidobacteria* and *Streptomyces* to compose 45-57% of the carbon flow in the four ecosystems under study, through productivity and respiration. Another important finding was that bacteria that used most glucose as a carbon source were also the ones that used most native soil carbon, suggesting that they can highly influence carbon balance in the soils.

CERRI *et al.*, 2016 found that the coffee plantations that adopted good management practices maintained efficiently their stocks, sometimes even the coffee reflecting positively on soil health. Soil properties can influence on the microbial communities present in the soils. Soil organic carbon, as well as organic matter, soil fertility and edaphic parameters are of importance for the survival, reproduction and correct functioning of rhizobial communities. They have also been reported in the processing of soil organic carbon, thereby impacting directly on carbon stocks.

Due to the high importance and multifunctionality of rhizobial communities, it is important to understand their dynamics and the factors that affect their development. A higher diversity of rhizobial communities may favor the symbiosis with legume species and even the development of many other non-legume species, as cited before. The higher the diversity of species in the soil, the higher the resilience of the important biogeochemical processes that they perform (MELLONI *et al.*, 2006).

Given the importance of rhizobia mainly for leguminous, but also, as discussed before, for many other crops of agricultural importance and the environment itself, the main objective of this study was to investigate via cultural characteristics and 16S rRNA gene sequencing, the biodiversity of symbiotic nitrogen fixating bacteria, chemical and physical attributes and carbon sequestration in coffee areas and the Mata Atlantica biome. For this, the specific objectives that were proposed were: evaluate the diversity of rhizobia isolated from the soil in coffee sites and adjacent areas of Atlantic forest by using trap plant species, determine the total organic carbon and other chemical and physical attributes in the areas under coffee sites with high productivity and compared with the

forest areas and evaluate the resilience of rhizobia when changed from Mata Atlantica biome to coffee plantation.

2 THEORETICAL BASIS

2.0 The importance of the coffee crop around the world

Almost every person enjoys a cup of their favorite coffee every morning. Coffee has become one of the most consumed beverages around the world, making it one of the traded and truly a global commodity (KRISHNAN, 2017; FAO). In the last few years, the market has been growing due to increase in consumption in emerging economies. Also, there has been an impact due to the culture of specialty coffees and product innovations in developed countries. Production is concentrated mainly in countries with generally low-income levels, where coffee contributes to a large portion of their earnings. The largest consumers and importers are the European Union and the United States of America. Coffee contributes to sustainable development by generating income, creating rural employment and alleviating poverty (FAO).

In Latin America, Africa and Asia, 125 million people depend on coffee for their livelihood. Depending on the country most of the coffee producers are subsistence farmers that live in countries where there are high indexes of poverty and food insecurity. Added to these social situations, is the fact that there is increasing population pressure, deforestation and land degradation that are constantly threatening production and therefore, a consequence of intensification is the decline of biodiversity (KRISHNAN, 2017).

According to the International Coffee Organization, exports in the first seven months of the coffee year 2021/2022 have increased by 0.6% compared with the year 2020/2021, reaching up to 78.01 million bags (ICO). Even though production is growing and consumption is increasing as well, innovation and new technologies must be sought to make the production of coffee more competitive.

2.1 The coffee crop in Brazil and Minas Gerais

Among the largest coffee producing countries is Brazil in first place by far, followed by Vietnam and Colombia. The total area of planted coffee in Brazil for the year 2021/2022 is approximately 2.48 million hectares, with a growth in area of 0.6% in

comparison with the year before. The expected average productivity for the year 2021/2022 is of 27.4 sc/ha, with the state of Minas Gerais, who is the biggest producing region, expecting 21.7 sc/ha (CONAB, 2021).

Coffee has had importance in Brazilian history since a long time ago. Its cropping consolidated the capitalist system by changing from slavery to paid labor. Also, it stimulated the process of European migration to the Americas, from the other regions to the south center area of the country and from rural areas to the cities. In the mid XIX century, the growth of the coffee cropping system brought a higher development to the areas of Minas Gerais and Sao Paulo. By 2017, there were already 287 thousand producers, distributed in 15 states in an estimated area of 2.3 million hectares and generating more than 8 million jobs. By this time, the image of Brazilian coffee was of "commodity", meaning that it had a lower quality but since then, many efforts have been done to enter new, more demanding markets that can pay higher prices and generate higher revenues (VASCONCELLES, 2017).

For Minas Gerais, coffee is the main product for exportation. This area of the economy is also one of the most dynamic of the agriculture of the state, considering its big production volume, the capital that is moved by it and also the socioeconomic importance of the crop, since it is a source of employment for many people (EPAMIG, 2010). The coffee produced in Minas Gerais stands out thanks to its quality and volume of production. Minas Gerais is responsible for 60% of the country's production, reaching a production of 24,791.1 million coffee bags for the year 2022. The state is also responsible for the 72.8% of the area of Arabica coffee in the country. The state's productivity has been under serious threats in the last years mostly due to adverse climatic conditions, such as long periods of droughts and cold fronts that have reached frosts in some areas (CONAB, 2022).

Coffee plantations are important agroecosystems for evaluating the role of the microbial community and their interactions due to its importance and abundance in the country and the world (JURBUNG *et al*., 2020). Coffee plantations can be found in all Biomes of Minas Gerais State: Mata Atlântica, Cerrado and Caatinga, replacing and adjacent to natural vegetation.

2.2 Mata Atlântica

The Mata Atlântica is also called the floresta atlântica (GONCALVES, 2015). In Minas Gerais it is mainly in the center south and east parts, with high mountains called “mares de morros” that generate variation in altitudes (WERNECK et al., 2010). It is the oldest forest formation in Brazil. It is also considered a hotspot for conservations due to its irreplaceable areas, endemic species and high vulnerability. Generally, its soils are dystrophic, low in nutrients, with acid pH and high variation in soil types and availability of water.

The geological, geomorphological and climatic conditions vary greatly in the Mata Atlântica (PEREIRA, 2019). In the inside of the country, the climate is seasonal, with hot and humid summers and cold and dry winters where the Seasonal (semi decidua or decidual) Forest occurs. A rich tropical flora that loses its leaves during the winter characterizes the semi decidual or decidual forest.

This biome is home to a great diversity of fauna (MACHADO *et al.*, 2015), flora, microorganisms (FRAGA *et al.,* 2012) and ecosystems. It is one of the forests with highest number of animals and plants per unit of area.

2.3 Why are microorganisms important?

Over the past 50 years, microorganisms have been under the spotlight by studying their potential for advancement in medical technology, human and animal health, food processing, food safety and quality, genetic engineering and waste treatments (SINGH *et al.*,2011). The soil environments are complex communities of organisms that influence and are also in part defined by the chemicals and physical parameters of the soils in which they thrive (KENNEDY & SMITH, 1995).

Microorganisms are able to promote plant growth through several processes: biological nitrogen fixation (OSORIO FILHO *et al.*,2016), phosphate solubilization (COSTA *et al.*,, 2015, GUDIÑO-GOMEZJURADO *et al.,* 2021), decomposing of organic wastes (FAN *et al.,* 2014) and residues and rehabilitation of mining areas (CASTRO *et al.,* 2017), imparting stress tolerance (ASHWIN *et al.,* 2022), suppression of plant diseases and soil-borne pathogens (FERREIRA *et al.,* 2020; MARTINS *et al.,* 2018; JACK *et al.,* 2018) and production of bioactive compounds such as vitamins,

hormones and enzymes (ROCHA *et al.,*2018). These reduce the need for industrialized inputs such as agrochemicals and fertilizers, also reducing their potential impact on the environment (MICHEL *et al.,* 2020; WELBAUM *et al.,* 2010).

Microorganisms perform critical functions related to the resilience and resistance of plant communities through helping to deal with stress and disturbance (JURBUNG et al., 2020). Some microbes can also aid in the decomposition of pollutants and degradation of recalcitrant oil residues (CARMO *et al.,* 2011; PITOMBO & REGANHAN-CONGLEIAN, 2013). Studies have also been performed on the capacity of microorganism to help in areas that have been degraded by mining and modify or destroy molecules derived from pollutants or agrochemicals (PEDROSA *et al.,* 2015; MELLONI *et al.,* 2006; CASTRO *et al.,* 2017).

Plant production, both above and below ground, is directly and positively correlated with microbial abundance and diversity (DUCHENE *et al.,* 2017). Plant growth promoting microbes enhance plant growth and development directly or indirectly through mechanisms such as releasing plant growth regulators, solubilization of phosphorus (DA COSTA *et al.,* 2015; MARRA *et al*., 2012), potassium (MEENA *et al.,* 2015), sulfur and zinc (KAMRAN *et al.,* 2017; VERMA *et al.,* 2020, or by producing siderophores (YADAV *et al.,* 2017; CORREIA & OLIVEIRA, 2006; SOARES *et al.,* 2020). Some bacteria that produce siderophores can provide the plant with iron directly by improving the iron nutrition or indirectly by inhibiting the growth of pathogens in the rhizophere that can limit the availability of iron for the plant (KUMAR *et al.,* 2019).

Biological nitrogen fixation currently contributes with the majority of fixated nitrogen in the planet, since the breaking of the triple ligation is done at environmental pressures and temperatures, through enzymatic activities (MOREIRA & SIQUEIRA, 2006). The result of the biological nitrogen fixation is nitrogen that is readily available for the plants and therefore, less prone to losses by lixiviation, volatilization or denitrification, enforcing its importance for environmental sustainability (CASSETARI *et al.,* 2016).

A wide range of microorganisms participate in decomposition, mineralization and nutrient availability, impacting directly on the efficiency of the nutrient cycles (SINGH *et al.,* 2011; FIGUEREIDO *et al.,* 2020). Microbes act as a biological rescue system

capable of solubilizing the insoluble inorganic phosphorus of soil and making it available for the plants (GUDIÑO-GOMEZJURADO *et al.,* 2021). In a study by Costa *et al.,* 2015, they found that inoculations with phosphorus solubilizing bacteria effectively increased shoot dry matter, root dry matter and total dry matter, accumulation of phosphorus, nitrogen, calcium, magnesium, Sulphur and boron in rice. Also, microorganisms play an important role in the transfer of soil nutrients to plants as well as in plant fitness and soil fertility (DUCHENE *et al.,* 2017; AHMAD *et al.,* 2015).

Some organisms have been demonstrated to provide the plants with supplementary dietary nutrients, such as vitamins and phytohormones involved in plant growth and root architecture. A study by Rocha *et al.,* 2019, isolated strains from Cerrado soils that were able to solubilize calcium phosphate and at the same time produce indole acetic acid, which helped increase phosphate availability and increase the efficiency of biological nitrogen fixation, causing a significant impact on plant biomass, growth and nodulation. Some microorganisms present in the rhizosphere have been shown to produce cytokinnins (VAN ZEIJL *et al.,* 2015; PODLESAKOVA *et al.,* 2013), which are involved in cell division, chloroplast differentiation and transport of metabolites. These also retard plant senescence, induces stem morphogenesis and contributes to the functions of other plant organs. Some of the compounds produced by soil microorganisms also help alleviate biotic and abiotic stress factors, such as salinity (NOORI *et al.,* 2018; FUKAMI *et al.,* 2017; SAGHAFI *et al.,* 2018) and water stress (MELO *et al.,* 2017). Ethylene is a stress induced plant hormone that can act contrary to plant growth, whose levels can be lowered by some microorganisms that exhibit ACC deaminase, which is an ethylene precursor (YADAV *et al.,* 2017). Another important produce of microorganisms are some antibiotics that are currently used in areas such as human medicine, veterinary science, animal husbandry and maintenance of livestock, agriculture and aquaculture and that are sometimes easier to isolate from microbes that synthesize chemically (CHANDA & KUMAR, 2017)

Studies have shown how some microorganisms can also protect plants against pathogens and stimulate plant resistance (DUCHENE *et al.,* 2017; YADAV *et al.,* 2017). Nitrogen provided by the rhizobia is used for the production of nitrogen based defense compounds, having counter effects on the performance of chewing herbivores (KEMPEL *et al.,* 2009).

The soil microbial biomass mediates the synthesis of soil organic matter (BEN-LAOUANE *et al.,* 2021) by being responsible for the decomposition of the organic residues deposited by plants and animals (PEDROSA *et al.,* 2015). Nitrogen fixing bacteria associated with legume trees in tropical environments have been shown to increase soil carbon sequestration and stabilization of organic matter (MOURA *et al.*, 2020).

Microorganisms play an active role in aggregation of particles, thereby influencing soil structure and soil water regime (SINGH *et al.,* 2011). These microorganisms are called “natural soil engineers”, since they are involved in processes of mineral weathering and soil formation. Through their metabolism, they can form organic acids that are even able to dissolve rocks (KAVIYA *et al.,* 2019).

The importance of the soil biota for the functioning and sustainability of the ecosystem is such that some parameters that refer to their activities are currently being used as indicators of soil quality and soil health (PEDROSA *et al.,* 2015).

2.4 Rhizobia

Plant growth promoting rhizobacteria are microorganisms that promote plant growth and that can also protect them from pathogens. These bacteria have emerged as an important and promising tool for sustainable agriculture. Even though the most relevant studies have been done in leguminous crops, recent finding have been performed in crops like maize (LEITE *et al.,* 2020) and rice (OSORIO FILHO *et al.*, 2016; COSTA *et al.,* 2015). A variety of nitrogen fixing microbes such as *Arthobacter, Azoarcus, Azospirillum, Azotobacter, Bacillus, Enterobacter, Gluconoacetobacter, Herbaspirillum, Klebsiella, Pseudomonas* and *Serratia* have been isolated from the rhizospheres of various crops and have been shown to contribute with the growth and development of the associated plants (YADAV *et al.,* 2017). These bacteria can have periods in which they grow in the rhizosphere or the rhizoplane and then they move to the internal part of the plant, in hypertrophic structures known as nodules, where they perform important functions in benefit of the plant (CASSETARI *et al.,* 2016). The relationship between the microbes and the plants is bidirectional, meaning that the plants select the organisms and the microorganisms in turn influence plant health (POOLE *et al.,* 2018).

Rhizobia can directly influence plant nutrition through the solubilization of phosphorus by producing low molecular weight organic acids. Also, they can significantly increase the availability and uptake of phosphorus, potassium, calcium and magnesium in different organs of plants (KEBEDE, 2021; KUMAR *et al.*, 2019). In a study by BEN-LOUANE *et al.*, 2021, it was shown that these properties can be further enhanced by using rhizobia along with other tools, such as arbuscular mychorrizae and compost. This strategy significantly improved soil organic matter, nitrogen and phosphorus content, decreased soil ph and increased electrical conductivity. Leite *et al.*, 2020, also studied the use of combinations with rhizobia, in this case with biochar. They found that the co inoculation of biochar-based rock phosphate had a significant effect on maize growth and enhanced phosphorus availability in the soil. Ahmad *et al.,* 2015 conducted a similar experiment, finding that the combination of rhzobial strains with different levels of biochar showed results in improving not only the growth of maize, but also the phosphatase, dehydrogenase and microbial biomass C contents in the rhizosphere.

These bacteria besides bringing benefits to the plants, their development and production, guarantees a reduction in the application of excessive doses of nitrogenous fertilizers and other agrochemical inputs, thereby leading to a better equilibrated, much less aggressive agriculture (MARIN *et al.,* 1999). Through the study of these beneficial bacteria and years of technological development, inoculants have been developed.

These inoculants represent a “practically effective, ecologically safe and economically alternative” tool to achieve the highest production potential by offering not only nitrogen fixation, but also biocontrol o plant diseases, resistance against disease causing pathogens and suppression of diseases. Some mechanism through which rhizobia can perform biocontrol of plant diseases include: antibiosis, parasitism, competition for infection sites and nutrients, activation of induced plant resistance and production of substances such as growth hormones, antibiotics, enzymes, siderophores, hydrogen cyanide and exopolysacharides. They have also been demonstrated to reduce the severity of various diseases, such as those caused by *Macrophomina phaseolina* (ARORA *et al.*, 2001), *Rhizoctonia solani* and *Fusarium* spp (OMAR & ALLA, 1998; SIDDIQUI *et al.*, 2000). Rhizobia has been seen parasitizing and inhibiting the hyphae and reproductive structures of some fungi and also secreting hydrolytic enzymes that are antagonistic to

them. When it comes to other harmful bacteria, rhizobial strains have the capacity to produce antibiotics and cell wall degrading enzymes to fight them off. Inoculation using rhizobia can facilitate a more rapid response of the defense genes in plants (KEBEDE, 2021; KUMAR *et al.,* 2019). Also, the rhizobial symbiosis can also improve plant defense and resistance against herbivores (THAMER *et al.,* 2010). A study by Akhtar *et al.,* 2008 also demonstrated the capacity of rhizobia to reduce galling and nematode multiplication and the biocontrol of root-rot disease complex in chickpea. Also, Martins *et al.,* 2018, examined treatments with rhizobacteria associated with fungicides, and found that they reduced the incidence of web blight in common bean and at the same time improving the crop's yield.

Some microbial strains have been demonstrated to exhibit ACC deaminase activity, which aids in lowering the levels of ethylene in the plants: *Acinetobacter, Achromobacter, Agrobacterium, Alcaligenes, Azospirillum, Bacillus, Burkholderia, Enterobacter and Rhizobium* (YADAV *et al.,* 2017). Rhizobia can also secrete different types of hormones, mainly auxin, to aid in the growth and development of the root system (KUMAR *et al.,* 2019).

Studies have shown that inoculation of legumes with the appropriate rhizobia can increase leaf chlorophyll by increasing photosynthesis and as a result, the plant gets enough carbohydrates for growth and production (NYOKI & NDAKIDEMI, 2014). This is the case for high yielding soybean, who needs high quantities of nitrogen for growth and development and it is estimated that biological nitrogen fixation by rhizobia can cover 60-70% of the plants' needs when inoculated with its main rhizobial partner: *Bradyrhizobium*.

2.5 Bradyrhizobium

Many studies have demonstrated a high diversity of rhizobia in different managements and ecosystems, but most of them have reported major appearance of low growth, alkalinizing isolates, that are classified as *Bradyrhizobium* spp (MOREIRA *et al.,* 1993). In the Cerrado area, after 15 years since the inoculation, *Bradyrhizobium* was still found (TERASAWA *et al.,* 2003). This was also true for *Bradyrhizobium elkanii* and *Bradyrhizobium japonicum* in a study by Zilli *et al.,* 2006. In a study by Gamocho *et al.,* 2014, in which they studied the effect of *B. japonicum* on the non-leguminous plants corn

(*Zea mays*) and Arabidopsis thaliana, they found that the strain increased yield biomass and primed these two plants for drought stress, adding to the benefits of *Bradyrhizobium* for other types of crops, such as those reported for wheat, barley, rice, lettuce and canola (GAMOCHO *et al.,* 2014). In a study done by Cassia *et al.,* 2020 in coffee areas, they found that the genus *Bradyrhizobium* was detected in all soil samples, demonstrating that it is part of the core microbiota in the soil of coffee crops. Also, Sousa *et al.,* 2022 studying the core rhizosphere microbiome of five different *Coffea* species found that in *Coffea stenophylla*, *Bradyrhizobium* dominated. In a study by Jaramillo *et al.,* 2013, they also found the predominance of *Bradyrhizobium* in the Amazon region, once again confirming the importance of the genus.

2.6 Resilience of rhizobia in soils

Rhizobia must exist in the soils and they must be able to efficiently compete against other members of the microbiota to finally reach their goal: infecting the roots and forming nitrogen fixating nodules. To be able to overcome conditions that are under adequate, they become saprophytes.

There are many aspects of the soil environment that can affect the survival of rhizobia in the soils. A study by Chatel & Parker, 1973 studied the effect of depth and temperature on the survival of *R. trifolii* and *R. lupini*. They concluded that both of these conditions affect the growth and development of these bacterial communities. *R. trifolii* demonstrated susceptibility to temperatures as low as 40°C in moist soils. (Also noted by ZENGENI *et al.,* 2006, ZILLI *et al.,* 2013 and BOONKERD & WEAVER, 1982). For both of them, there was a tendency to decline with high soil temperatures and desiccation (CHATEL & PARKER, 1973). Another study by Pena Cabriales & Alexander in 1979 also studied the effects of desiccation on the rhizobial communities and further specified the effects, concluding that the bacteria go through two stages after the drying of soils: the first one is a rapid exponential decline that coincides with the rapid initial water loss and the second is a slower decline that coincides with the time the soil reaches a dry stage. They also observed that cells that created a greater amount of polysaccharides had greater survival rates. Vriezen *et al.,* 2007 later specified on the velocity of soil drying, concluding that when the soils are dried slowly, there is a higher survival of the microbial communities, probably due to the fact that the physiological responses to drying may become activated during that time.

Another condition that may affect the performance of rhizobia in the soils is the presence of metals. Broos *et al.,* 2005, concluded that metals do not only affect the size of the community, but also the genetic diversity present. In a study with metal-saturated soils, they found that there was a significant decrease in cells after only one month of incubation. They also compared Cadmium and Zinc as contaminants and found that it was zinc and not cadmium the metal that was most toxic for the rhizobia in the absence of the host plant (BROOS *et al.,* 2005).

Other studies have also shown that the persistence of rhizobia in soils is dependent on the strain and the type of soil (ALBAREDA *et al.,* 2009), other soil properties such as clay and soil carbon content (ZENGENI *et al.,* 2006), rhizodeposition and exudation since they provide the communities with sources of nutrients and carbon (DUCHENE *et al.,* 2017) and also soil moisture, pH and factors inherent to the plants, such as age and health conditions (YADAV *et al.,* 2017) and plant diversity influences soil microbial diversity as well (STEINAUER *et al.,* 2016).

Studies have demonstrated that the populations of rhizobia are able to maintain their viability and be able to nodulate and efficiently fixate nitrogen once the conditions are favorable again even if they reach arid conditions, by developing adaptation mechanisms that are still not yet completely understood (MELLONI *et al.,* 2006). In a study done in Cerrado soils, Zilli *et al.,* 2013, found that after the harvesting of soybean, one of the factors that affected the density of the rhizobial communities was the lack of rain but, if these areas are later irrigated and the communities reinforced through inoculation, they can reach optimum levels again. Another mechanism of survival, by Zilli *et al.,* 2006, whose study found nodulating species that were inoculated nowhere near the area of study, is that there might be dispersion through air currents.

Hirsh, 1996 says that rhizobia genus: *Rhizobium, Bradyrhizobium, Sinorhizobium, Mesorhizobium, Azorhizobium* and *Allorhizobium* possess granules of poli-b-hydroxibutirate. These can further be catabolized and used as carbon sources to increase survival and energy, contributing to the activity of these bacteria under unfavorable conditions. In another more recent hypothesis, they theorize that non spore formers such as rhizobia can survive in the soils through the formation of biofilms on either biotic or abiotic surfaces (FUJISHIGE *et al.,* 2008). Some estimates indicate that rhizobial species can survive in soils for four to five years without their plant host, but an estimate cited by

Hirsh, 2010 also said that in some cases, rhizobial communities could also survive up to 15 years.

Even though they are susceptible to many environmental factors, rhizobial communities have shown a great resilience under less than ideal conditions. A study by Lima *et al.,* 2009 found that there was a higher diversity and nodulation in managed systems (agriculture and agroforestry). This study supported the information that rhizobia are highly resilient to changes in land use, such as after deforestation. They concluded that the management system can influence the density, diversity and occurrence of the rhizobial microbes and their functionality. A study done by Jaramillo *et al.,* 2013 in the same region concluded that specifically *Bradyrhizobium* reflected a high adaptation with different plant communities, soil characteristics and land uses. This can also be supported by the fact that many studies have found *Bradyrhizobium* as the most highly ocurring rhizobial strain in many areas, as cited before in this literature revision.

Types of management that have as a priority the addition of organic components, the favoring of beneficial physical and chemical properties and in general, the looking out for the bacterial communities have a greater survival rate and maintenance of the rhizobia (FIGUEREIDO *et al.,* 2020). To be successful, microbial communities must be able to respond rapidly and efficiently to plant cues, signals and chemoattractants and for them to be reach their competitiveness potential, conditions must be as ideal as possible (POOLE *et al.,* 2018).

2.7 Relation carbon and soil fertility with rhizobia

Rhizobia, like any other living organism require “fuel” to be able to undergo all its processes and perform in all of the services that were mentioned in chapters before. Therefore, it is important for the bacteria to live in conditions where there is enough of the right amount of substrate that it needs and that environmental characteristics are correct. However, rhizobia have developed diverse metabolic pathways to enable them to grow and multiply. They can survive using different carbon compounds, such as sugars, organic acids, amino acids and phenolic (KAHN *et al.,* 1998). Rhizobia are very diverse, so the diversity of carbon sources that they use for living is also important to understand.

A study by Elskeikh & Wood, 1989, showed that tolerance to salt by rhizobia depends on pH, temperature and carbon source that each strain uses. In a study done in

Argentina, with different rhizobial strains isolated from the root nodules of several legumes, where the action of five herbicides on their growth was tested, the authors concluded that there is a potential of bacterial strains to also use xenobiotic C sources (ZABALOY & GOMEZ, 2005).

Besides carbon usage, there have been studies that have shown how bacteria perform a key role in the synthesis or soil organic carbon and organic matter. A study by Su *et al.,* 2020 studied the changes in bacterial and fungal community after an input of crop straw in the soil. They linked these changes with the allocation of the straw-C into dissolved organic carbon, microbial biomass carbon, particulate organic carbon and mineral-associated organic carbon. Their findings suggest that denitrifying and nitrogen fixing bacteria *Burkholderia-Paraburkholderia*, *Paraphaeosphaeria* and *Bradyrhizobium* are correlated with the distribution of the straw-C into the different compartments. They also found that *Bradyrhizobium* was positively correlated with particulate organic carbon derived from straw. This supports the importance of rhizobia in the conversion of plant residues into soil organic carbon. Another study by Stone *et al.*, also reported *Bradyrhizobium*, along with *Acidobacteria* and *Streptomyces* to compose 45-57% of the carbon flow in the four ecosystems under study, through productivity and respiration. Another important finding was that bacteria that used most glucose as a carbon source were also the ones that used most native soil carbon, suggesting that they can highly influence carbon balance in the soils.

A study by Cerri *et al.,* 2016 studied carbon and nitrogen stocks in coffee areas where there was previously pasture. They found that the coffee plantations that adopted good management practices maintained efficiently their stocks, sometimes even the coffee reflecting positively on soil health. Soil properties can influence on the microbial communities present in the soils. Castro *et al.,* 2017 found that the aluminum content, organic matter and pH had a great influence on the microbial in soils from iron mining areas. The pH was also identified by Pires *et al.,* 2017 as an edaphic factor with influence on the microbial community, focusing especially on the occurrence of unrelated rhizobial types in the nodules of *Mimosa* spp. They concluded that fertility was also related with these communities, in a way that the most acidic and less fertile soils favored the association with *Paraburkholderia*, while more neutral and highly fertile soils favored the association with *Rhizobium*. Cao *et al.,* 2021 also supported that soil pH, contents of

total phosphorus, total potassium and total organic carbon were the main determinants for different communities in the Loess Plateau in China. Zengeni *et al.,* 2006 also studied the influence of soil carbon and organic matter on the survival of rhizobia in soils without a host. They found that soil carbon is important during crop rotations in which rhizobia must survive saprophitically.

Since organic production and the use of natural amendments is also becoming very important in the search for sustainability, studies have also been done studying the response of rhizobial communities to these. Kimitri & Odee, 2010 found that integrated soil fertility, with the use of manure as soil amendment led to an increase in indigenous rhizobia, concluding in higher shoot biomass in cowpea. Zengeni *et al.,* 2006 also found this effect in soils in Zimbabwe, where there was inoculation 1-4 or 6 years before. The application of manure increased indigenous rhizobia. Soil carbon and organic matter were vital on the survival of rhizobia in soils without a host. They found that soil carbon is important during crop rotations in which rhizobia must survive saprophitically.

Soil organic carbon, as well as organic matter, soil fertility and edaphic parameters are of importance for the survival, reproduction and correct functioning of rhizobial communities. They have also been reported in the processing of soil organic carbon, thereby impacting directly on carbon stocks.

2.8 Importance of diversity studies

Due to the high importance and multifunctionality of rhizobial communities, it is important to understand their dynamics and the factors that affect their development. A higher diversity of rhizobial communities may favor the symbiosis with legume species and even the development of many other non-legume species, as cited before. The higher the diversity of species in the soil, the higher the resilience of the important biogeochemical processes that they perform (MELLONI *et al.,* 2006). Today's agriculture is demanding for new practices that ensure sustainability through soil biological activity and long term production with plant health (SINGH *et al.,* 2011). Microbial diversity and the study of the plant microbiome are becoming an important tool for maintaining the sustainability of agricultural production systems (YADAV *et al.,* 2017).

By 1995, as written by Kennedy & Smith, we were “unaware of the true extent or dimension of the diversity of soil microbes” and also “the actual contribution of diversity to system functioning is unknown”. By 1996, Pankhurst *et al.,* also acknowledged that very little was known about the biodiversity of the soil microbial communities and that taxonomic approaches were limited by the apparent non-culturability of the majority of the species in the soil. Now, however, thanks to new molecular techniques and a vast variety of studies, we have been able to discover new functionalities and understand the true importance of the rhizobial community, but there is still a long way to go. It is important to study diversity to understand the distribution of these communities, their functional roles and identify their changes as affected by disturbance or management.

Because of their many areas of action, not only agriculture will be benefited with the understanding of the rhizobial communities, but also vital endeavors such as the recuperation of degraded areas and conservation. A higher diversity may favor and maximize the nitrogen fixation in these places (MELLONI *et al.,* 2006).

Besides nitrogen fixation, other benefits such as biocontrol against a broader range of pathogens, finding of new mechanisms and improving the efficiency of the strains can be deepened through the study of diversity of rhizobial communities (KEBEDE, 2021).

Soils can be the source for species with potential for inoculants that can later be used in agriculture. Not only can different ecosystems and biogeographical areas be a source of new species, but also novel modes of action of the rhizobial communities (CHANDRA & KUMAR, 2017). It is important to select strains that are competitive as saprophytes as well, to ensure that they will survive under harsh conditions and persist in the absence of their host (HOWIESON, 1995). Since these strains may be found in the most different ecosystems, it is imperative to know the biodiversity of rhizobia and to study local populations (LINDSTROM *et al.,* 2010). The proper management to ensure sustainability will only be fully developed by understanding how land uses and soil attributes affect the edaphic biodiversity and their varied functions (LIMA *et al.,* 2009).

3 HYPOTHESIS

- There is a diverse rhizobia community in the soils under coffee cropping.
- There is more rhizobia diversity in the Mata Atlantica biome than in the coffee monoculture.
- There is higher organic carbon in the Mata Atlantica biome areas that in the coffee monoculture areas.
- There is a relationship between *Bradyrhizobium* (or rhizobia) diversity and soil organic matter and chemical and physical attributes.

4 OBJECTIVES

GENERAL

- Investigate via cultural characteristics and 16S rRNA gene sequencing, the biodiversity of symbiotic nitrogen fixating bacteria, chemical and physical attributes and carbon sequestration in coffee areas and the Mata Atlantica biome.

SPECIFIC

- Evaluate the diversity of rhizobia isolated from the soil in coffee sites and adjacent areas of Atlantic forest by using trap plant species.
- Determine the total organic carbon and other chemical and physical attributes in the areas under coffee sites with high productivity and compared with the forest areas.
- Evaluate the resilience of rhizobia when changed from Mata Atlantica biome to coffee plantation.

5 METHODOLOGY

The present work was done in conjunction with other study groups, investigating other groups of soil organisms and chemical and physical soil characteristics within the framework of the Bios Brasil macro project.

5.0 Localization and climate

The study was conducted between the months of December 2021 and July 2022. The samples were collected in the following areas:

1. Fazenda NKG/Santo Antônio do Amparo – One transect in each of the two plots and two transects in the adjacent forest.

2. Fazenda Grupo Cambraia/Santo Antônio do Amparo - One transect in each of the two plots and two transects in the adjacent forest.
3. Fazenda Cafua/Ijaci – One transect.
4. Fazenda Ouro Verde/Lavras- One transect.
5. Matinha/UFLA- One transect
6. Subestacao/UFLA- One transect

Each transect had five points, each 50 meters apart was marked for sample collection.

Sampling process in the coffee plantation and Atlantics forests

In the coffee plantations' points, a subsample was collected from the projection of the plant canopy of the tree directly on the sampling point, the two trees on the sides in the same line and the two trees across from the streets, making a circle (for reference, see Annex 1). The subsamples were taken from the first 10 cms of soil. The five subsamples were mixed together to make one sample. The samples were placed in sterile bags and prepared for transport. They were kept in cold storage at 4 C.

In the Mata Atlantica points, each subsample was collected in a circle around the sampling point, with a total of five subsamples per sample (for reference, see Annex 2). The samples were taken from the first 10 cms of soil. The samples were placed in sterile bags and prepared for transport in the same way as the coffee samples.

For the chemical analyses, the following protocols were followed (according to the laboratory):

- pH in water with a relation 1:2.5
- Ca, Mg and Al: Extractor KCl, 1 mol/L
- S: Extractor monocalcic fosfate in acetic acid
- P, Na, K, Fe, Zn, Mn and Cu: Extractor Mehlich 1
- H + Al: Eaxtractor SMP
- Organic matter: Oxidation Na2Cr2O74N + H2SO4 10N
- B: Extractor hot water

5.1 Rhizobia isolation using siratro and cowpea as trap plants

For trapping rhizobia from soil samples, promiscuous plant species cultivated in axenic conditions were used. The seeds used for trap plant were the legume siratro (*Macroptolium atropurpureum)* and the legume cowpea (*Vigna unguiculata*). The siratro seeds first underwent a scarification and superficial disinfection using sulfuric acid (H2SO4) for 15 minutes. For superficial disinfection of the cowpea seeds, NaClO was used. After that, they were washed will sterile distilled water six times. Then, they were immersed in sterile water for two hours for imbibition. For germination, they were placed in Petri dished lined with moistened cotton and filter paper. The dishes were kept at 28C for 24 hours for germination. Meanwhile glass bottles with nutrient solution were prepared. Two strips of filter paper were put inside each glass bottle to pull the nutritive solution and serve as support for the plant. The nutritive solution with little nitrogen contained (per liter of solution) 0.025 mL $NH_4H_2PO_4$, 0.6 mL KNO_3, 0.4 mL $Ca(NO_3)_2*4H_2O$, 2 mL $MgSO_4*7H_2O$, 3 mL K_2SO_4, 10 mL $Ca(H_2PO_4)_2*H_2O$, 0.344 gr $CaSO_4*2H_2O$, 1 mLmicronutrients and 1 mL FeEDTA. The complete nutritive solution contained (per liter of solution) 0.25 mL $NH_4H_2PO_4$, 1.5 mL KNO_3, 1 mL $Ca(NO_3)_2*4H_2O$, 0.5 mL $MgSO_4*7H_2O$, 0.25 mL micronutrientes and 0.25 mL FeEDTA.

The bottles completely prepared, with the paper strips, nutritive solution and covered with aluminum paper were autoclaved twice for 40 minutes. After germination of the seeds, one seedling was placed in each bottle in a vertical flux camera to avoid contamination.

The soil samples were subjected to a dilution. First, 20 g of soil were weighed and diluted in 20 mL of autoclaved saline solution (NaCl 0,85%). This was taken to a stirrer at 125 rpm for 10 minutes. From this dilution, 1 mL was taken and placed in the bottles with the previously planted siratro seedlings. The controls used were (1) low nitrogen concentration (5.25 mg L^{-1}), (2) high nitrogen concentration (52.5 mg L^{-1}) and (3) efficient strains UFLA4-212 for the siratro experiment and 03-11B and 03-84 for the cowpea experiments. The inoculated plants were then taken to a closed room and checked for nodulation daily.

After 35 days of growth, the presence or absence of nodules was determined and a SPAD (Soil Plant Analysis Development), was used to measure the chlorophyll content of the leaves of each plant, taking 10 measures from the third leaf and calculating an average. The plants were then taken out of the nutritive solution and the nodules and roots dried up, counted and weighed. The nodules were placed in Falcon tubes with Silica Gel in the bottom and cotton as a separation for storage. The aerial portion of the plant was placed in paper bags for drying.

5.2 Isolation of rhizobia from nodules

After the plants had been confirmed for nodulation, three nodules from each plant were taken. For disinfection, they were placed in alcohol 92% for 20 seconds, then for two minutes in NaClO and then washed with sterile distilled water six times to wash out the excess. They were then macerated on a plaque with Medium 79 (FRED & WAKSMAN, 1928), also known as YMA, with Bromothymol blue as a pH indicator. They were examined daily for growth. The colonies that appeared were then purified until pure strain.

The bacterial cultures were characterized under:

- Time to the appearance of isolated colonies
- Size of colony
- Changes in medium pH
- Shape of the colony
- Colony elevation
- Border appearance
- Surface appearance

- Quantity of exopolysaccharides
- Consistency of exopolysaccharides produced
- Optical appearance
- Color
- Absorption of indicator

After characterization of stirpes, they were grouped using as main parameters the time of appearance of isolated colony, the changes in medium pH and the quantity of exopolysaccharides produced. For sequencing a representative of each cultural group in each sampling point was selected by using software R, ensuring that these conditions were covered: at least one representative of each sampling point, avoid representatives from the same nodule and that they presented typical rhizobial characteristics.

Gram coloration

The isolates were first reproduced in liquid YMA medium and placed for agitation until they reached their peak growth, according to characterization. With the inoculating loop, approximately two drops of the medium with the colonies was placed on a microscope slide and then distributed throughout the surface. Then, they were left to dry. After drying, they were fixated using the Bunsen burner. The process for coloration was as follows: first, a drop of violet crystal was placed on the slide and distributed and left for one minute to dry, after washing with abundant water, a drop of lugol enough to cover the surface of the slide was placed and left for three minutes. After the time passed, the slide was washed with abundant water. Imediately, the slide was washed with pure acetone for 35 seconds and with abundant water afterwards and left to dry. The final step was placing a drop of safranine enough to cover the surface of the slide and left for two minutes. After this time, the safranine was washed with abundant water and the slide left to dry for posterior visualization under a 40x microscope to determine the coloration.

Alkaline lyses

For the extraction of DNA, alkaline lyses was used. First, a portion of the cultures in 79 (YMA) medium, incubated depending on their specific characterization were taken. Colonies were placed in 20 microliters of SDH tampon, then placed at 94°C during 15 minutes to boil. Immediately after, they were placed in ice for 5 minutes, to provide a

temperature shock. The samples were then centrifuged for 12 seconds. After this, 100 microliters of Milli Q ultrapure water were added and the eppendorfs agitated manually until suspension of the pellet. The samples were then centrifuged for 5 minutes at 4°C, at a velocity of 13,200 rpm. Finally, 50 microliters of the supernatant were taken and placed in a new sterile Eppendorf, having the care not to touch the pellet. The DNA samples were then quantified and an electrophorese gel was done to certify quantity and quality of the extraction.

5.3 16S rRNA sequencing and identification

The strains were identified through sequencing of the 16S rRNA gene. A volume of the extracted DNA was used as a template for the PCR. For the amplification, the primers 27F (5' - AGAGTTTGATCCTGGCTCAG - 3') and 1492R (5' - GGTTACCTTGTTACGACTT - 3') were used. After verification of the amplication through electrophoresis, the PCR products were packed and sent to the laboratory ACTGene Analises Moleculares for sequencing.

Once the sequences were sent back, they were edited using the BioNumerics (Applied Maths, A Bionumerieux Company), version 7.6.3., with sequences with at least 200 bp considered good for blasting. Sequences with less than 200 bp or with low quality were not used. The sequences were then taken to the Basic Local Alignment Search Tool (BLAST) for identification and the results were recorded.

5.4 Statistical analysis

The five points in each area were grouped into one mean for each area to use for the analyses. To evaluate the effect of management on each variable, a model was adjusted for each (function lm). For the effect of number of nodules on SPAD, a general least squares (GLS) model better adjusted to the data. To evaluate differences between the two plants, a t-test was used.

A PCA was performed to evaluate differences in all fertility variables between the areas and the type of management.

6 RESULTS AND DISCUSSION

Sampling areas

The characteristics of the coffee sampling areas were varied, as shown in Table…The sampled areas were distributed in the cities of Lavras, Ijaci and Santo Antonio de Amparo, with the forest areas immediately adjacent to the coffee plantations.

Chemical and physical analyses

In general, the soils of the sampled areas were moderately acidic, with mean pH of 4.6 for the coffee areas, ranging from 3.6 to 5.5 and with a mean of 4.8 for the forest areas, ranging from 3.9 to 6.1, with no significant differences between the two types of management. In terms of nutrient content, the highest difference between the two types land use were found in the P ($p=0.04315$) content (Table 1) and Mn ($p=0.03147$) content (Table 2).

Considering the nodule number is a semi quantitative measurement of the number of rhizobia cells extant in the soils, the effect of soil chemical attributes was related to the saprophytic phase of rhizobia populations. Because small amounts of soil were inoculated in the plants cultivated in nutrient solution with optimal chemical conditions, chemical attributes of soil samples were diluted and would have negligible effects on nodulation. Thus, the following interpretations must consider that when mentioning nodulation, we are referring indirectly to the saprophytic stage of rhizobia cells in soils samples.

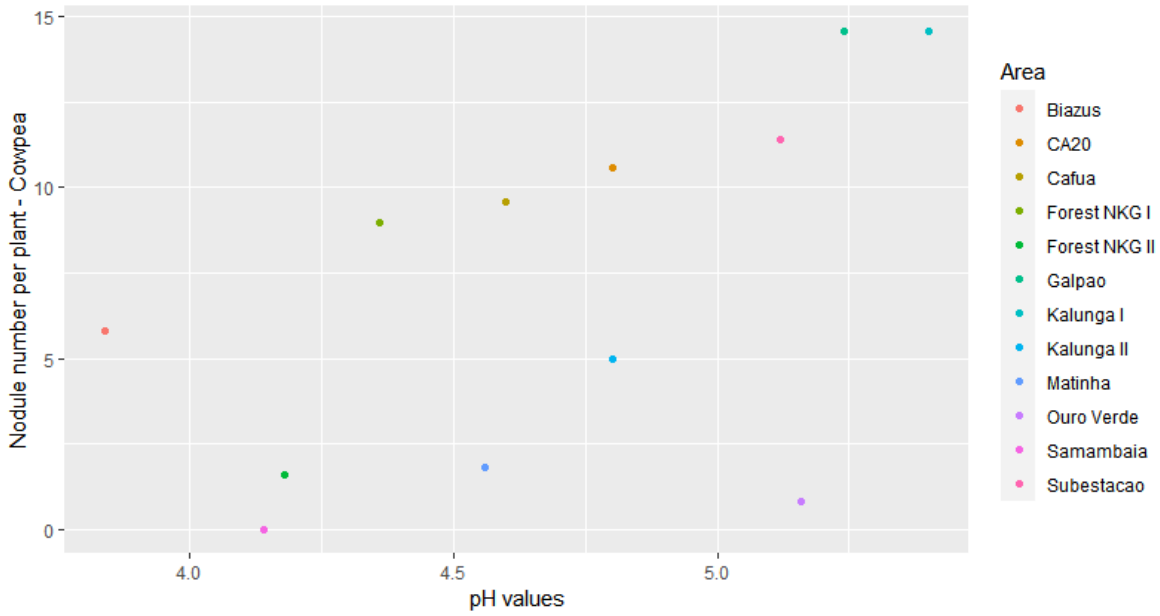
There was no effect of organic matter on the nodulation of either cowpea nor siratro for this study. These data are consistent with the conclusions of Melloni *et al.*, 2006, in which they did not find any significant correlation between organic matter and nodulation variables (number and fresh matter) for bean and cowpea.

In soil conditions, studies have been done to investigate the effects of soil organic matter on nodulation and saprophyitic activity. In a study by Del Valle *et al.*, 2020, they explained how soil organic matter actually attenuates nodulation, since plant-microbe interactions are mediated by signaling. The research showed that higher organic carbon

contents in soils repress flavonoid signaling up to 70%. In their plant experiments, they studied the signaling between a legume and a nitrogen fixing symbiont, finding that there was a 75% decrease in nodulation in the plants, concluding that soil organic carbon might actually be decreasing the lifetime of the flavonoids responsible for signaling between plant hosts and nitrogen fixing microbes. Many studies have been done with incorporation of different organic amendments and composts, showing the opposite of the latter results (INNOCENT *et al.*, 2012; OLAYINKA *et al.*, 2008; MASEFIELD, 2008). These positive results over the microbe populations may be due to the decomposition process of the newly added organic matter, in which the microorganism communities are stimulated to grow, compared to already processed soil organic matter, in which populations have already reached stability.

For cowpea, there was a significant effect of pH on nodule number (Figure 1). This fact was supported by Castro *et al.*, 2017, who found that the soil properties with greatest influence on the microbial communities were done by Al content, organic matter and pH. A study by Cao *et al.*, 2021 also supported that soil pH and also contents of total phosphorus, total potassium and total organic carbon were the main determinants for rhizobial communities. The nodule number is a semi quantitative measurement of the number of rhizobia cells in the soils. This means that pH has an effect on the density of rhizobia in the soils.

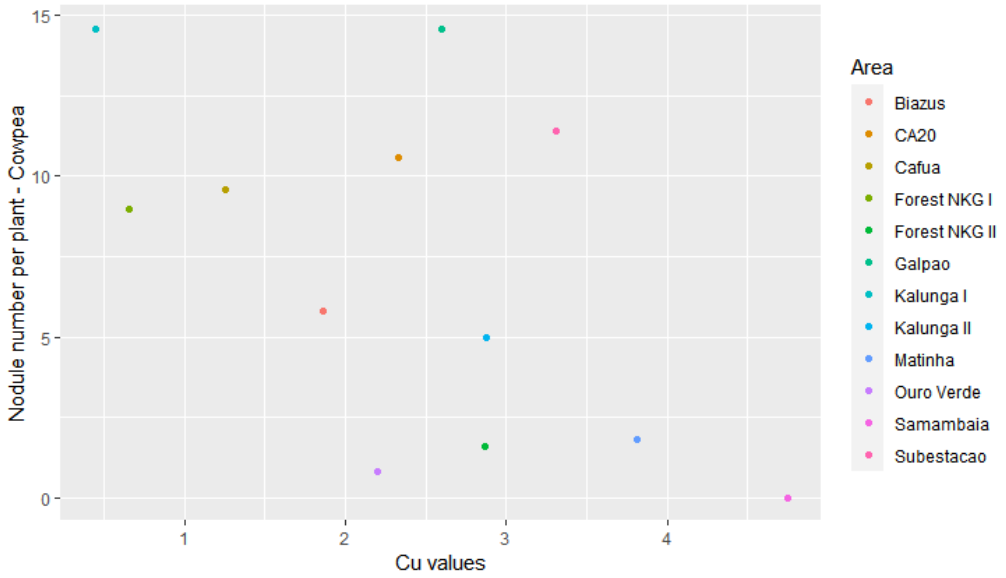
Figure 1. Relationship between pH values of original soil and rhizobia density in soils for cowpea trial, with a p value of 0.05571, multiple R-Squared of 0.319 and Adjusted R-Squared of 0.2509



Fonte: Do autor, 2022.

For cowpea, there was also a negative significant effect of Cu on nodule number (Figure 2). These data contrast with a study by Wahab *et al.*, 1996, in which they studied nodulation, nitrogen fixation and plant growth of faba bean through the use of cobalt and copper additions, they found that copper promoted nodule mass by 44.7%, demonstrating that there is a positive effect of the element on nodulation. Also, they found that leghaemoglobin content, dry matter and total nitrogen content of shoots and roots was also significantly increased. Another research studied the effects of Cu on cowpea's numbers of nodules per plant and plant yield. Their results found that both variables were dependent upon the solution of Cu activity. The mean number of nodules per plant started to decrease at lower Cu activities.

Figure 2. Decreasing tendency of nodule number per plant in cowpea in relation to Cu values, with p value 0.05419, multiple R-Squared of 0.3223 and Adjusted R-Squared of 0.2545.

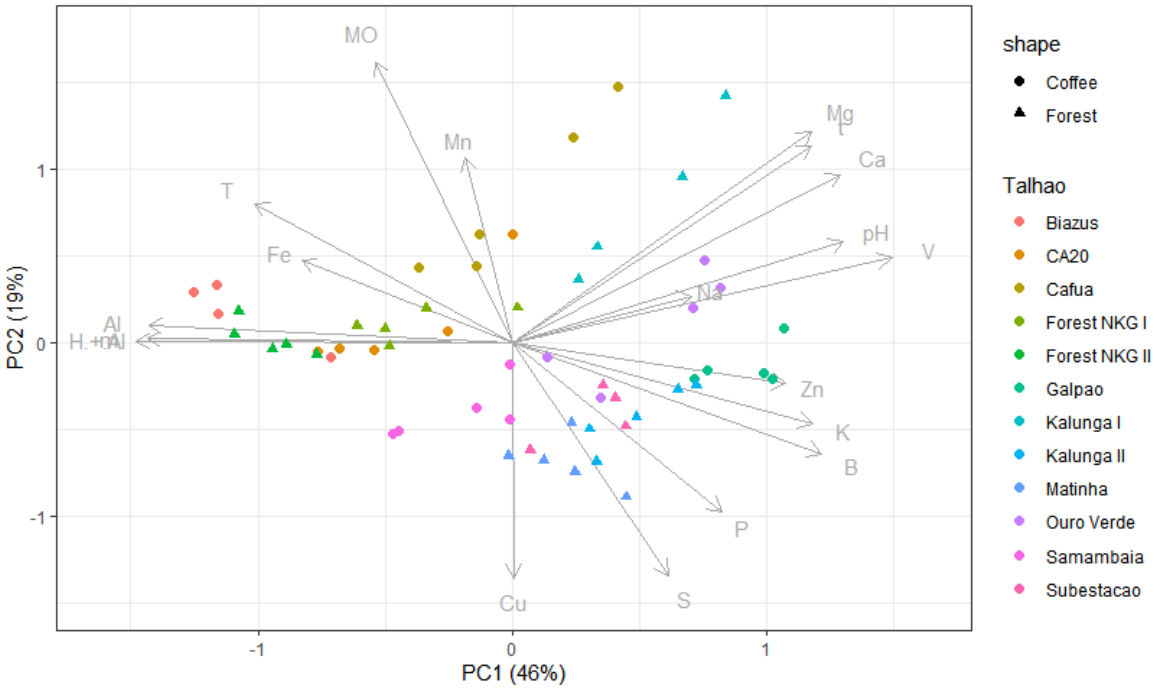


Fonte: Do autor, 2022.

For siratro, there was no significant effect from any of the fertility variables, although it is worthy to mention that there was a small effect of Cu on nodule number as well.

The principal components analysis (PCA) between the chemical properties of the soils and the areas under study (Figure 3) explained 65% of the total variance (PCA 1: 46% and PCA 2: 19%). The results show that the areas were highly variable in terms of fertility. The forests in NKG farm (Forest NKG I and NKG II showed lower fertility properties and higher contents of Al, as well as the CA20 and Biazus coffee areas. The other forests (Kalunga I, Kalunga II, Subestacao and Matinha) were clustered in the same areas, in which generally high fertility properties are shown. The other coffee areas show variable degrees of soil properties, probably due to their differing fertilization programs.

Figure 3. Principal components analysis (PCA), relating soil chemical properties with the studied areas and their land use (coffee or forest).



Fonte: Do autor, 2022.

Table 1. Chemical characteristics of the sampled soils in the city of Santo Antonio de Amparo and Lavras, Minas Gerais.

Area	Land Use	pH			MO			K			P		
			Int		dag/kg	Int		mg/dm3	Int		mg/dm3	Int	
Cafua	Coffee	4,6	Low	ab	6,3	Good	d	78,6	Good	ab	3,0	Very low	a
Kalunga I	Forest	5,4	Low	cd	5,9	Good	cd	188,3	Very good	cd	3,7	Very low	ab
CA20	Coffee	4,8	Low	ab	4,3	Good	abc	57,6	Medium	ab	1,6	Very low	a
Forest NKG II	Forest	4,2	Very low	a	5,3	Good	bcd	46,9	Medium	a	1,4	Very low	a
Matinha	Forest	4,6	Low	bcd	3,2	Medium	a	130,4	Very good	bcd	54,2	Very good	d
Galpao	Coffee	5,2	Low	e	3,3	Medium	a	356,7	Very good	e	27,7	Very good	abcd
Kalunga II	Forest	4,8	Low	d	2,7	Medium	a	195,6	Very good	d	32,1	Very good	abcd
Ouro Verde	Coffee	5,2	Low	abc	4,4	Good	abc	112,8	Good	abc	39,0	Very good	bcd
Subestacao	Forest	5,1	Low	e	3,3	Medium	a	281,1	Very good	e	40,7	Very good	cd
Samambaia	Coffee	4,1	Very low	cd	3,5	Medium	ab	181,6	Very good	cd	13,1	Medium	abc
Biazus	Coffee	3,8	Very low	ab	6,8	Good	d	53,4	Medium	ab	1,5	Very low	a
Forest NKG I	Forest	4,4	Very low	ab	3,9	Medium	ab	74,0	Good	ab	2,1	Very low	a

Means followed by the same letter in the line do not differ by the Tukey HSD test at 5% probability. The interpretation was done based on the recommendations of the Soil Fertility Commission of the State of Minas Gerais (ALAVAREZ *et al.,* 1999).

Table 2. Continued chemical characteristics of the sampled soils in the city of Santo Antonio de Amparo and Lavras, Minas Gerais.

Area	Land Use	Ca			Mg			Al			SB		
		cmolc/dm3	Int		cmolc/dm3	Int		cmolc/dm3	Int		cmolc/dm3	Int	
Cafua	Coffee	4,5	Very good	bc	2,2	Very good	de	0,4	Low	ab	6,9	Very good	de
Kalunga I	Forest	4,9	Very good	c	2,4	Very good	e	0,1	Very low	a	7,7	Very good	e
CA20	Coffee	1,0	Low	a	0,8	Medium	ab	0,8	Medium	b	1,9	Medium	abc
Forest NKG II	Forest	0,4	Very low	a	0,3	Low	a	1,5	Good	c	0,7	Low	a
Matinha	Forest	2,3	Medium	abc	0,8	Medium	ab	0,3	Low	ab	3,4	Medium	abcd
Galpao	Coffee	4,7	Very good	c	1,9	Very good	bcde	0,1	Very low	a	7,5	Very good	e
Kalunga II	Forest	2,8	Good	abc	1,2	Good	abcd	0,1	Very low	a	4,5	Good	bcde
Ouro Verde	Coffee	4,5	Very good	bc	2,1	Very good	cde	0,2	Very low	a	6,8	Very good	de
Subestacao	Forest	2,9	Good	abc	1,0	Good	abc	0,1	Very low	a	4,7	Good	cde
Samambaia	Coffee	1,9	Medium	ab	0,7	Medium	a	0,8	Medium	b	3,1	Medium	abc
Biazus	Coffee	0,5	Low	a	0,2	Low	a	1,9	Good	c	0,9	Low	ab
Forest NKG I	Forest	1,4	Medium	a	0,7	Medium	a	0,7	Medium	ab	2,3	Medium	abc

Means followed by the same letter in the line do not differ by the Tukey HSD test at 5% probability. The interpretation was done based on the recommendations of the Soil Fertility Commission of the State of Minas Gerais (ALVAREZ *et al.,* 1999).

Table 3. Continued chemical characteristics of the sampled soils in the city of Santo Antonio de Amparo and Lavras, Minas Gerais.

Area	Land Use	Zn			Fe			Mn		
		mg/dm3	Int		mg/dm3	Int		mg/dm3	Int	
Cafua	Coffee	1,9	Good	ab	48,88	High	a	74,94	High	d
Kalunga I	Forest	2,8	High	abcd	47,42	High	a	43,88	High	bcd
CA20	Coffee	1,4	Medium	ab	166	High	c	28,72	High	abc
Forest NKG II	Forest	0,62	Low	a	98,64	High	abc	22,58	High	abc
Matinha	Forest	3,3	High	bcd	33,84	Good	a	4,98	Low	ab
Galpao	Coffee	2,26	High	abc	28,68	Medium	a	6,24	Medium	ab
Kalunga II	Forest	3,18	High	abcd	43,12	Good	a	4,82	Low	a
Ouro Verde	Coffee	4,66	High	cd	38,46	Good	a	8,16	Medium	ab
Subestacao	Forest	5,18	High	d	26,4	Medium	a	3,92	Low	a
Samambaia	Coffee	2,02	Good	ab	38,84	Good	a	61,24	High	cd
Biazus	Coffee	0,74	Low	ab	130,96	High	bc	8,04	Medium	ab
Forest NKG I	Forest	1,18	Medium	ab	80,62	High	ab	56,22	High	cd

Means followed by the same letter in the line do not differ by the Tukey HSD test at 5% probability. The interpretation was done based on the recommendations of the Soil Fertility Commission of the State of Minas Gerais (ALVAREZ *et al.,* 1999).

Table 4. Continued chemical characteristics of the sampled soils in the city of Santo Antonio de Amparo and Lavras, Minas Gerais.

Area	Land Use	Cu			B			S		
		mg/dm3	Int		mg/dm3	Int		mg/dm3	Int	
Cafua	Coffee	1,256	Medium	ab	0,112	Very low	ab	9,1	Medium	a
Kalunga I	Forest	0,446	Low	a	0,124	Very low	ab	2,98	Very low	a
CA20	Coffee	2,332	High	bcd	0,102	Very low	ab	5,8	Low	a
Forest NKG II	Forest	2,868	High	cd	0,068	Very low	a	5,96	Very low	a
Matinha	Forest	3,81	High	de	0,26	Low	cde	155,82	Very good	d
Galpao	Coffee	2,596	High	bcd	0,496	Medium	f	108,14	Very good	bcd
Kalunga II	Forest	2,872	High	cd	0,378	Medium	ef	93,34	Very good	bc
Ouro Verde	Coffee	2,202	High	bc	0,364	Medium	ef	50,42	Very good	ab
Subestacao	Forest	3,31	High	cde	0,266	Low	de	52,72	Very good	ab
Samambaia	Coffee	4,744	High	e	0,21	Low	bcd	122,98	Very good	cd
Biazus	Coffee	1,86	High	abc	0,072	Very low	a	9,04		a
Forest NKG I	Forest	0,654	Low	a	0,13	Very low	abc	10,54		a

Means followed by the same letter in the line do not differ by the Tukey HSD test at 5% probability. The interpretation was done based on the recommendations of the Soil Fertility Commission of the State of Minas Gerais (ALVAREZ *et al.,* 1999).

Table 5. Continued chemical characteristics of the sampled soils in the city of Santo Antonio de Amparo and Lavras, Minas Gerais.

Area	Land Use	t			T			V			m		
		cmolc/dm3						%					
Cafua	Coffee	7,3	Good	cd	17,0	Very good	cd	40,1	Medium	bcde	6,5	Very low	ab
Kalunga I	Forest	7,9	Good	d	11,5	Good	ab	65,3	Good	ef	1,8	Very low	a
CA20	Coffee	2,7	Medium	a	12,8	Good	abc	16,1	Very low	ab	35,0	Medium	c
Forest NKG II	Forest	2,3	Low	a	17,0	Very good	cd	4,4	Very low	a	67,5	Good	d
Matinha	Forest	3,7	Medium	ab	11,3	Very good	ab	30,5	Low	bcd	7,6	Very low	ab
Galpao	Coffee	7,6	Good	d	9,8	Good	ab	76,4	Good	f	1,5	Very low	a
Kalunga II	Forest	4,6	Good	abcd	8,9	Good	a	50,3	Medium	de	2,8	Very low	a
Ouro Verde	Coffee	7,0	Good	bcd	13,1	Good	abc	52,4	Medium	def	2,9	Very low	a
Subestacao	Forest	4,8	Good	abcd	10,6	Good	ab	44,4	Medium	cde	3,1	Very low	a
Samambaia	Coffee	3,9	Medium	abc	14,8	Good	bc	22,0	Low	abc	21,2	Low	abc
Biazus	Coffee	2,7	Medium	a	20,2	Very good	d	4,5	Very low	a	67,2	Good	d
Forest NKG I	Forest	3,0	Medium	a	11,1	Good	ab	21,4	Low	abc	24,8	Low	bc

Means followed by the same letter in the line do not differ by the Tukey HSD test at 5% probability. The interpretation was done based on the recommendations of the Soil Fertility Commission of the State of Minas Gerais (ALVAREZ *et al.*, 1999).

Nodule number, root and nodule weight, dry matter

The siratro plants nodulated with soil samples from all of the areas studied (Table 3). The highest number of nodules per plant was found in the Kalunga I forest and the lowest number of nodules per plant was found in the Ouro Verde coffee for this experiment. There was no nodulation from the non-inoculated controls, verifying that there was no contamination of the experiment. The reference strain UFLA 04-212 nodulated , indicating that the conditions were favorable for symbiosis with the host plant.

Table 6. Average SPAD, root weight, nodule number, shoot dry matter for each area for siratro experiment, means of 10 replicates (two plants per five sample points).

Area	Land Use	SPAD index		Root weight g		Nodule number number		Shoot dry matter g	
Cafua	Coffee	13,8	a	0,167	a	1,2	a	0,049	a
CA20	Coffee	14,7	a	0,138	a	0,7	a	0,059	a
Galpao	Coffee	20,1	a	0,171	a	3,7	a	0,086	a
Ouro Verde	Coffee	10,9	a	0,148	a	0,1	a	0,043	a
Samambaia	Coffee	13,2	a	0,191	a	0,5	a	0,067	a
Biazus	Coffee	19,9	a	0,237	a	4,1	a	0,076	a
Kalunga I	Forest	21,9	a	0,152	a	4,8	a	0,054	a
Forest NKG II	Forest	19,7	a	0,147	a	3,2	a	0,064	a
Matinha	Forest	13,2	a	0,166	a	0,8	a	0,046	a
Kalunga II	Forest	20,0	a	0,087	a	3,0	a	0,047	a
Subestacao	Forest	15,4	a	0,146	a	1,6	a	0,052	a
Forest NKG I	Forest	22,5	a	0,153	a	3,0	a	0,066	a
High N	Control	22.2	a	0.3875	a	0,0	a	0.1205	a
UFLA 04-212	Control	28.3	a	0.1245	a	1,0	a	0.0830	a
Low N	Control	8.2	a	0,230	a	0,0	a	0,040	a

Means followed by the same letter in the line do not differ by the Tukey HSD test at 5% probability.

For the case of the cowpea plants, there was one area (Samambaia coffee) that did not present nodulation. The highest number of nodules per plant was also found in the Kalunga I forest and the lowest number of nodules per plant (except for Samambaia), was again Ouro Verde coffee for this experiment too. There was also no nodulation in the

controls. There was a normal nodulation in the two reference strains 03-11B and 03-84 for the cowpea experiment.

Table 7. Average SPAD, root weight, nodule number and shoot dry matter for each area studied, for the cowpea experiment.

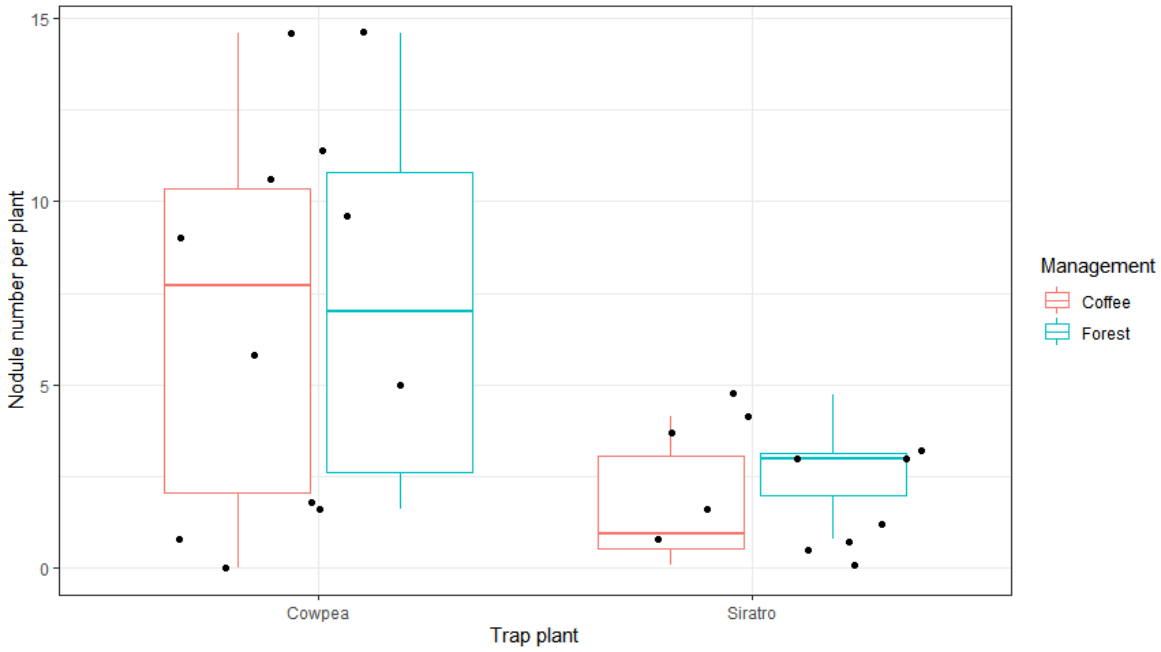
Area	Land Use	SPAD		Root weight		Nodule number		Shoot dry matter	
		index		g		number		g	
Cafua	Coffee	23,78	abc	1,728	abcd	9,6	a	0,56	ab
Kalunga I	Forest	26,6967	abcd	1,889	abcd	14,6	ab	0,82	ab
CA20	Coffee	21,2133	ab	2,754	d	10,6	a	0,88	ab
Forest NKG II	Forest	27,7933	abcd	1,732	abcd	1,6	a	0,73	ab
Matinha	Forest	23,69	abc	2,586	cd	1,8	a	1,01	ab
Galpao	Coffee	22,95	abc	2,238	bcd	14,6	ab	0,7	ab
Kalunga II	Forest	21,88	ab	2,236	bcd	5	a	0,93	ab
Ouro Verde	Coffee	19,09	a	1,632	abcd	0,8	a	0,63	a
Subestacao	Forest	20,73	ab	1,706	abcd	11,4	a	0,71	ab
Samambaia	Coffee	21,31	ab	1,565	abcd	0	a	0,62	ab
Biazus	Coffee	24,56	abcd	1,599	abcd	5,8	a	0,68	ab
Forest NKG I	Forest	24,8	abcd	1,541	abcd	9	a	0,58	ab
High N	Control	35	d	1	ab	0	a	1,062	b
03-11B	Control	28	abcd	1,078	ab	14	b	0,582	ab
03-84	Control	32.2	cd	0,786	a	7,75	ab	0,451	a
Low N	Control	30.5	bcd	1,251	abcd	0	a	0,394	a

Means followed by the same letter in the line do not differ by the Tukey HSD test at 5% probability.

There was a significant difference in nodulation between the two plant species, with cowpea having the highest mean nodule number (Figure 4). In a study by Pueppke,1986, he found that a difference in nodulation between siratro and cowpea may be due to the strains present. With a strain called 191, the siratro plants nodulated more than cowpea in the primary and lateral roots, while with a strain called 3G4b16, the cowpea plants nodulated more than the siratro in both primary and lateral roots. The result obtained differs from the data obtained by Thies *et al.*, 1991, in which they compared nodulation in four legume species, including cowpea and siratro and found a consistently higher population counts in siratro than in the other three, although they did not report

nodule number. Other studies also report on population counts rather than nodule number, so further information will be used to complete this assessment.

Figure 4. Comparison between nodule number, land use and the trap plant used.

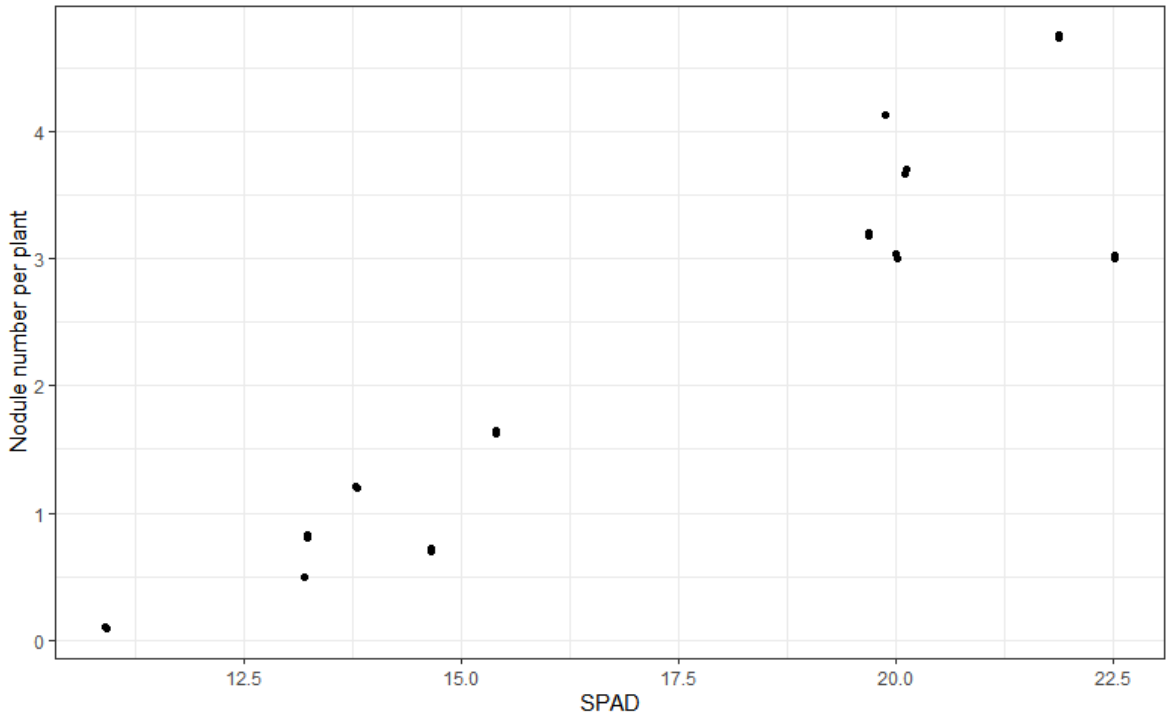


Fonte: Do autor, 2022.

There was no effect of the type of land use (coffee or forest) on the nodulation of either cowpea nor siratro plants for this study, being consistent with the study of Melloni *et al.*, 2006, in which they reported that they found no differences in nodule number for the field and mountain areas for cowpea (Figure 4).

There was a significant effect of nodule number on the SPAD index for siratro, indicating the efficiency of the bacterial populations on fixating nitrogen (Figure 5). There was no effect of nodule number on dry matter. This fact was also observed by Lima *et al.*, 2009, in which they observed that the means on nodule numbers did not affect the shoot dry matter weight was well. They considered all communities efficient.

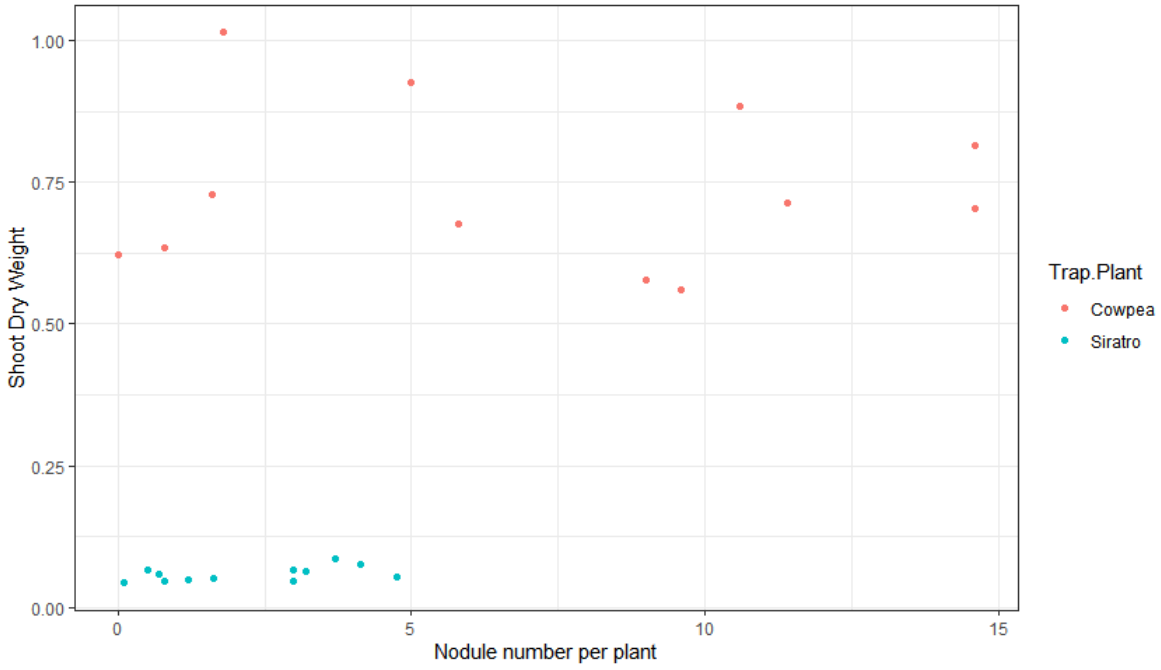
Figure 5. Relation between SPAD index and nodule number per plant for the siratro experiment.



Fonte: Do autor, 2022.

For cowpea, there was a significant effect of root weight on dry matter, but there was no correlation between dry matter and nodule number. This relation was closer, but not significant for the siratro plants as well.

Figure 6. Relation between nodule number per plant and shoot dry weight for both trap plants used in the experiments.

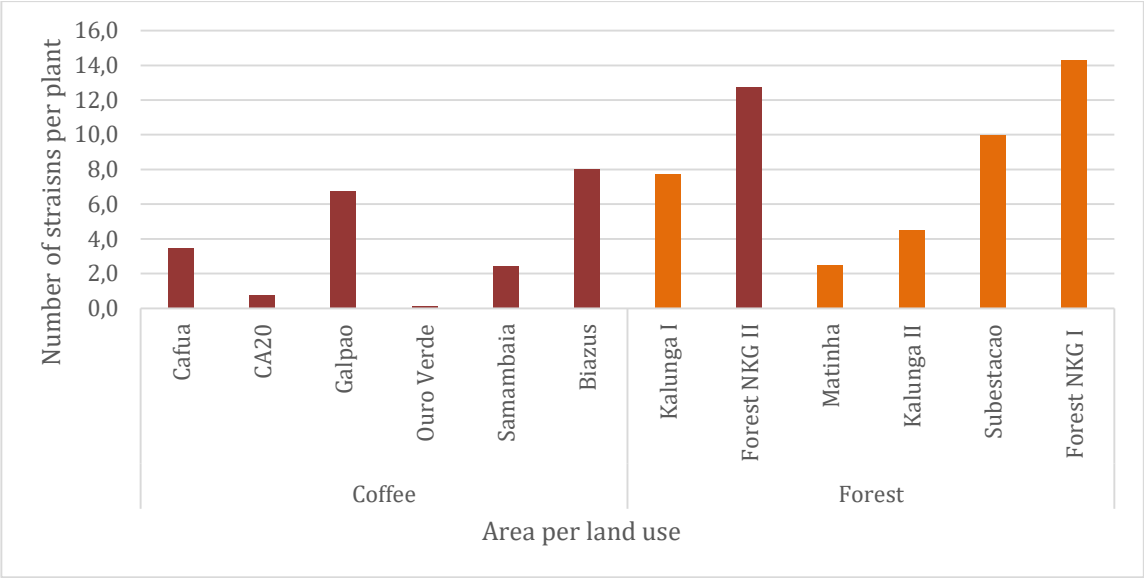


Fonte: Do autor, 2022.

Isolates

For the siratro experiment, there was a significant difference ($p<0.05$) between the number of strains per plant for the two different management areas with the forest presenting higher number of strains than the coffee (Figure 7).

Figure 7. Number of strains per plant for the two management areas considered in the siratro experiment, presenting a significant difference ($p<0.05$) between de the coffee and forest land use, multiple R squared 0.326 and adjusted R squared 0.2586.



Fonte: Do autor, 2022.

For the forest areas, isolated cultures were disposed into 95 groups, with the biggest one having 17 individuals. The characterization for the five biggest groups is shown in Table 8, among these, the majority were acidifying and fast growing, with other varying characteristics. In the case of the coffee areas, the numbers of groups were 72, with the biggest group having 9 individuals. Among the five biggest groups (Table 9), most of them were acidifying and fast growing, with other varying characteristics.

Table 8. Five biggest cultural groups out of 95 total, from the strains isolated in siratro plants from sampled soils from forest areas.

Group	Number	pH	Time of growth	Size	Shape	Color	Consistency	Indicator absorption
23	17	Acid	Fast	<1 mm	Point-like	Creme/Yellow	Dry	Yes/No
70	15	Acid	Fast	<1 - 2 mm	Circle	Yellow	Gummy/Aqueous	Yes
63	14	Acid	Intermediate/Fast	1-2 mm	Irregular	Creme/Yellow	Gummy	Yes/No
6	13	Acid	Fast	1-2 mm	Circle	Creme/White	Gummy	Yes
71	12	Neutral	Fast	<1 mm	Circle/Point-like	Creme	Aqueous/Gummy	No

Table 9. Five biggest cultural groups out of 72 total, from the strains isolated in siratro plants from sampled soils from coffee areas.

Group	Number	pH	Time of growth	Size	Shape	Color	Consistency	Indicator absorption
24	9	Acid	Fast	<1-2 mm	Circle	Creme/White	Gummy	No
33	6	Acid	Fast	2-4 mm	Circle/Irregular	Creme	Aqueous	Yes
49	5	Acid	Fast	<1-1 mm	Circle	Yellow	Dry	No
56	5	Acid	Fast	2-4 mm	Circle	Creme/Yellow	Dry	Yes
82	5	Neutral	Fast	<1-2 mm	Circle	Creme	Aqueous/Gummy	No

Genetic diversity of the strains via 16S rDNA sequencing

Of the 96 PCR products sent for sequencing, 55 sequences had more than 200 bp and were blasted for identification. Out of the 55 isolates identified, three were identified as *Rhizobium sp.* and three were identified as *Bradyrhizobium sp.* Among the rest of the identified isolates, the genera *Paenibacillus, Stenotrophomonas, Lysinibacillus, Bacillus, Firmicutes, Cohnella, Leifsonia* and *Sphingomonas* were found.

Gram coloration

From the 96 sequenced isolates, a total of 12 strains resulted in a Gram-negative coloration, coinciding with the *Rhizobium, Bradyrhizobium, Stenotrophomonas* and *Firmicutes* genera, along with three non-identified sequences (not identified due to poor quality of the sequenced). There were also 73 strains resulted in a Gram-positive coloration, coinciding with the *Lysinibacillus, Bacillus, Paenibacillus, Cohnella, Leifsonia* and *Firmicutes* genera, along with non-identified sequences. There were also five non-defined colorations, in which the process was done repeatedly but the differences were too subtle to be considered. All of these five non-defined did not have identification as well. These species coincide with others found in other areas of Minas Gerais, such as those found by CASTRO *et al*, 2017.

Carbon stock

The carbon stock data show that the area with the highest carbon sequestration was the Matinha and the area with the lowest carbon sequestration was CA20, as shown in Table 10.

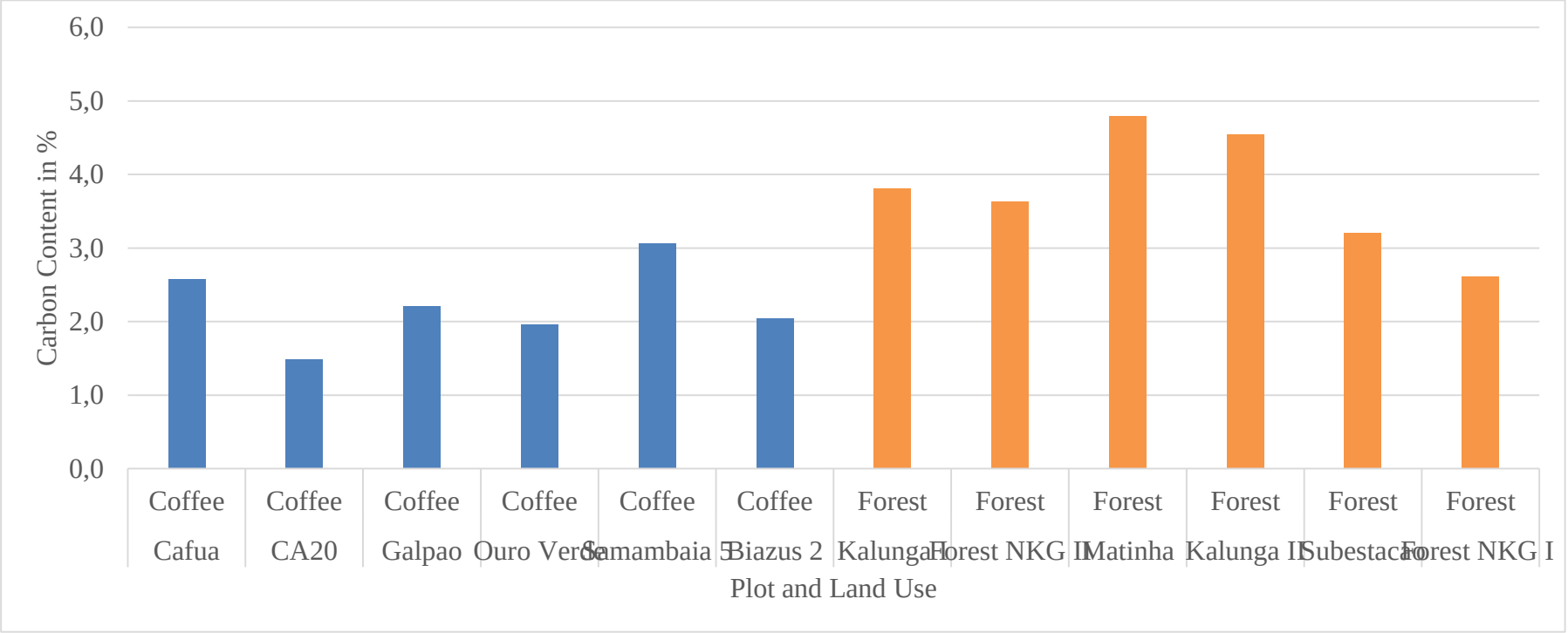
Table 10. Total Carbon for 0-10 cms, in percentage, for the sampled soils in Santo Antonio de Amparo and Lavras, Minas Gerais.

Area	Land Use	Total Carbon	
		%	
Cafua	Coffee	2,6	ab
Kalunga I	Forest	3,8	bc
CA20	Coffee	1,5	a
Forest NKG II	Forest	3,6	bc

Matinha	Forest	4,8	c
Galpao	Coffee	2,2	ab
Kalunga II	Forest	4,6	c
Ouro Verde	Coffee	2,0	ab
Subestacao	Forest	3,2	abc
Samambaia 5	Coffee	3,1	abc
Biazus 2	Coffee	2,1	ab
Forest NKG I	Forest	2,6	ab

There was a significant difference in carbon stocks between the coffee and the forest areas, in which the forest areas had a higher carbon content, due to a higher deposit of organic matter, as shown in Figure 8.

Figure 8. Total Carbon content in percentage for the areas studied. There was a significant difference in carbon content between the coffee and forest areas ($p>0.05$).



Fonte: Do autor, 2022.

7 CONCLUSIONS

There is a diverse rhizobia community in the soils under coffee cropping.

There is more rhizobia density and diversity in the Mata Atlântica biome than in the coffee monoculture.

There is higher organic carbon in the Mata Atlântica biome areas than in the coffee monoculture.

There is a relationship between rhizobia density and chemical attributes such as soil Ph and Cu content.

For this study, the trap plant Vigna unguiculata, cowpea, proved to be better at capturing bacterial diversity and density from these soils.

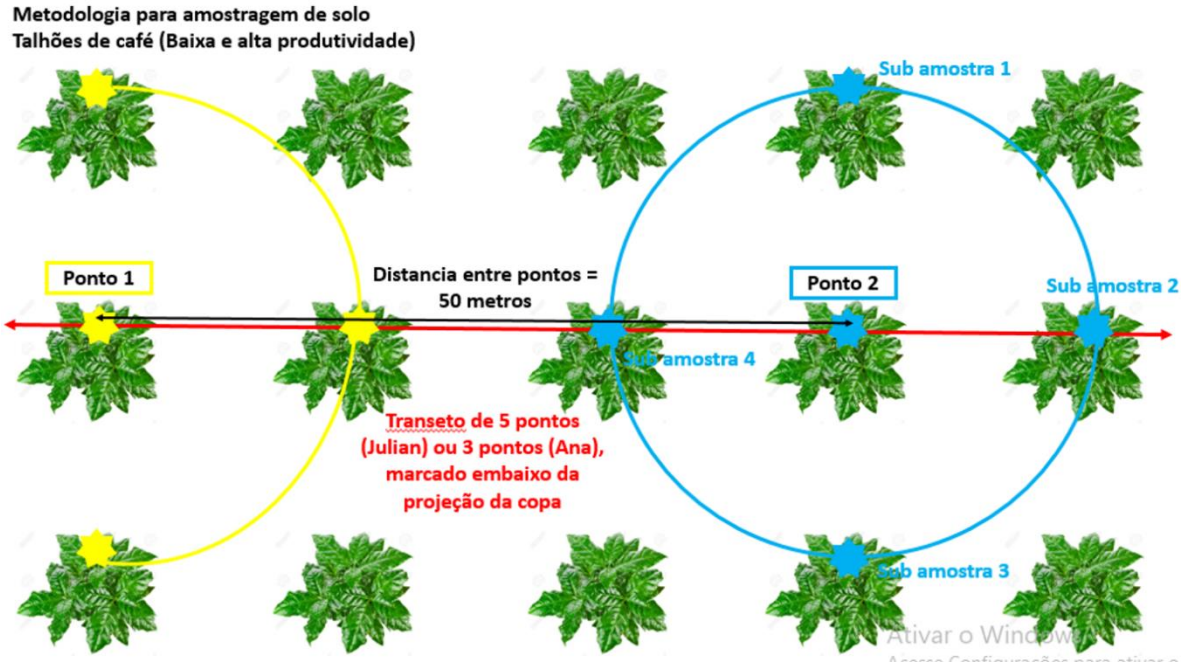
The coffee and forest areas in Santo Antonio de Amparo and Lavras are variable in terms of physical and chemical attributes.

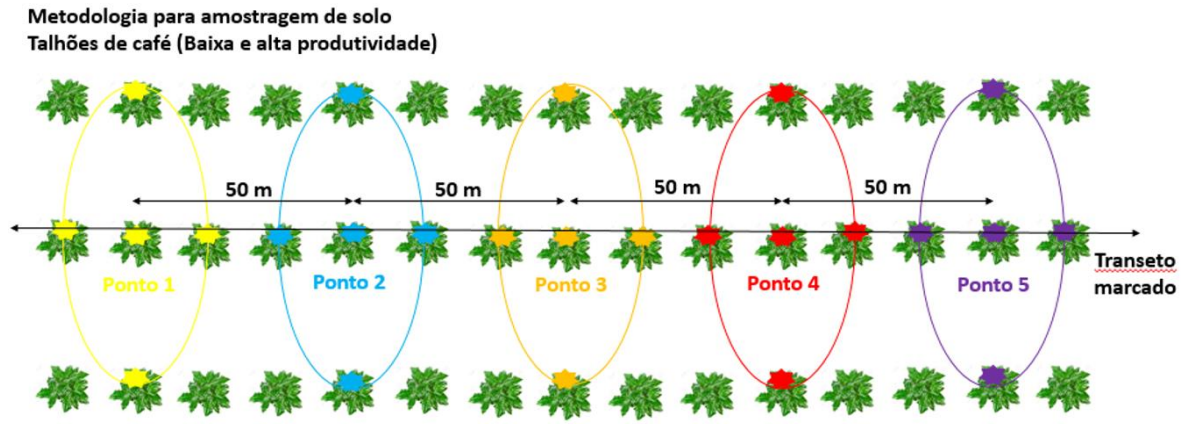
Soil Ph and copper content were related to nodulation in the areas under this study.

The bacteria in these areas proved to e highly resilient, since the physical and chemical characteristics and the carbon content were very variable, and still, they were able to nodulate.

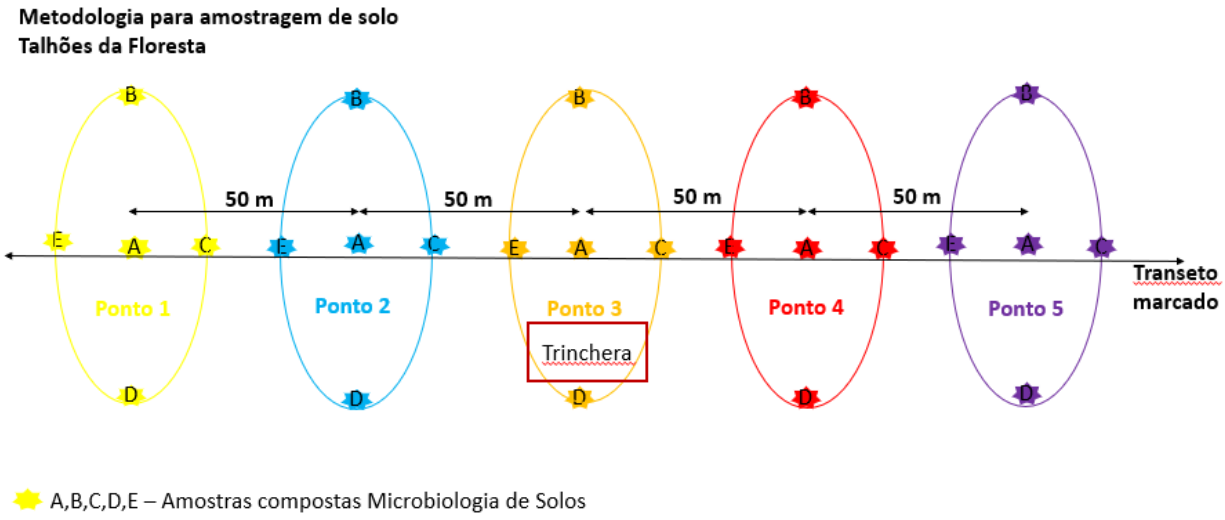
8 ANNEX

Annex 1 – Sampling in the coffee areas





Annex 2 – Sampling in the Mata Atlantica



9 REFERENCES

AHMAD, M.T.; ASGHAR, H.N.; SALEEM, M.; KHAN, M.Y.; ZAHIR, Z.A. Synergistic effect of rhizobia and biochar on growth and physiology of maize. **Soil Fertility and Crop Nutrition**, v. 107, n. 6, p. 2327-2334, 2015. Disponivel em: https://doi.org/10.2134/agronj15.0212

ALBAREDA, M.; RODRIGUEZ-NAVARRO, D.N.; TEMPRANO, F.J. Soybean inoculation: Dose, N fertilizer supplementation and rhizobia persistence in soil. **Field Crops Research**, v. 113, p. 352-356, 2009. Disponivel em: 10.1016/j.fcr.2009.05.013.

ALVAREZ, V.V.H.; NOVAIS, R.F; BARROS, N.F. CANTARUTTI, R.B.; LOPES, A.S. Interpretacao dos resultados das analises de solos. In: RIBEIRO, A.C.; GUIMARAES, P.T.G.; ALVAREZ, V.V.H., editores. Recomendacoes para o uso de corretivos e fertilizantes em Minas Gerais: 5ª aproximação. Viçosa, MG: Comissao de Fertilidade do Solo do Estado de Minas Gerais; 1999. P. 25-32.

ARORA, N.K.; KANG, S.C.; MAHESHWARI, D.K. Isolation of siderohore-producing strains of Rhizobium melitoli and their biocontrol potential against Macrophomina phaseolina that causes charcoal rot of groundnut. **Current Science**, v. 81, n. 6, p. 673-677, sept. 2001. Disponivel em: https://www.jstor.org/stable/24106362

ASHWIN, R.; BAGYARAJ, D.V.; RAJU, B.M. Dual inoculation with rhizobia and arbuscular mycorrhizal fungus improves water stress tolerance and productivity in soybean. **Plant Stress**, v. 4, 2022. Disponivel em: https://doi.org/10.1016/j.stress.2022.100084

BEN-LAOUANE, R.; AIT-EL-MOKHTAR, M.; ANLI, M.; BOUTASKNIT, A.; RAHOU, Y.A.; RAKLAMI, A.; OUFDOU, K.; WAHBI, S.; MEDDICH, A. Green compost combined with mycorrhizae and rhizobia; A strategy for improving alfalfa growth and yield under field conditions. **Gesunde Pflanzen**, v. 73, p. 193-207, 2021. Disponivel em: DOI:10.1007/s10343-020-00537-z

BOONKERD, N; WEAVER, R.W. Survival of cowpea rhizobia in soil as affected by soil temperature and moisture. **Applied and Environmental Microbiology**, v. 43, n. 3, p. 585-589, mar. 1982. Disponivel em: 10.1128/aem.43.3.585-589.1982

BROOS, K.; BEYENS, H.; SMOLDERS, E. Survival of rhizobia in soil is sensitive to elevated zinc in absence of the host plant. **Soil Biology and Biochemistry**, v. 37, p. 573-579, 2005. Disponivel em: doi:10.1016/j.soilbio.2004.08.018

CAO, Y.; TIE, D.; ZHAO, J.L.; WANG, X.B.; YI, J.J.; CHAI, Y.F.; WNG, K.F.; WANG, E.T.; YUE, M. Diversity and distribution of Sophora davidii rhizobia in habitats with difference irradiances and soil traits in Loess Plateau area of China. **Systematic and Applied Microbiology**, v. 44, 2021. Disponivel em: https://doi.org/10.1016/j.syapm.2021.126224

CARMO, F.L. do; SANTOS, H.F. dos; MARTINS, E.F.; ELSAS, J.D. van; ROSADO, A.S.; PEIXOTO, R.S. Bacterial structure and characterization of plant growth promoting oil degrading bacteria from the rhizopheres of mangrove plants. **The Journal of**

Microbiology, v.49, n. 4, p. 535-543, 2011. Disponivel em: 10.1007/s12275-011-0528-0

CASTRO, J.L. de; SOUZA, M.G.; RUFFINI, M.; GUIMARAES, A.A.; RODRIGUES, T.L.; MOREIRA, F.M.S. Diversity and efficiency of rhizobia communities from iron mining areas using cowpea as a trap plant. **Revista Brasileira de Ciencia do Solo**, 2017. Disponivel em: https://doi.org/10.1590/18069657rbcs20160525

CERRI, C.C.; MOREIRA, C.S.; ALVEES, P.A.; TOLEDO, F.H.R.B.; CASTIGIONI, B.A.; RODRIGUES, G.A.A.; CERRI, D.G.P.; CERRI, C.E.P.; TEIXEIRA, A.A.; CANDIANO, C.A.C.; REIS, M.R.; D'ALESSANDRO, S.C.; TURELLO, L. Estoques de carbono e nitrogenio no solodevido a mudança do uso da terra em áreas de cultivo de café em Minas Gerais. **Coffee Science**, Lavras, v.12, n.1, p. 30-41, jan./mar. 2017. Disponivel em: 10.25186/cs.v12i1.1194

CHANDRA, N.; KUMAR, S. Antibiotics producing soil microorganisms. In: Antibiotics and antibiotics resistance genes in soils, Soil Biology 51, 2017. Disponivel em: DOI 10.1007/978-3-319-66260-2_1

CHATEL, D.L.; PARKER, C.A. Survival of field-grown rhizobia over the dry summer period in western Australia. **Soil Biology and Biochemistry**, v. 5, p. 415-423, 1973. Disponivel em: https://doi.org/10.1016/0038-0717(73)90068-0

Companhia Nacional de Abastecimento. Acompanhamento da safra barileira: café, v.1, n.1, 2021. Disponivel em: http://www.conab.gov.br

COSTA, E. M. da; LIMA, W. de; OLIVEIRA-LONGATTI, S.M.; Souza, F.M. de. Phosphate solubilising bactéria enhance Oryza sativa growth and nutrient accumulation in an oxisol fertilized with rock phosphate. **Ecological Engineering**, v. 83, 2015, p. 380-385. Disponivel em: http://dx.doi.org/10.1016/j.ecoleng.2015.06.045

DEL VALLE, I. del; WEBSTER, T.M.; CHENG, H.Y.; THIES, J.E.; KESSLER, A.; MILLER, M.K., BALL, Z.T., MACKENZIE, K.R.; MASIELLO, C.A.; SILBERG, J.J.; LEHMANN, J. Soil organic matter attenuates the efficacy of flavonoid-based plant-microbe communication. **Science Advances**, v. 6, n. 5, 2020. Disponivel em: doi: 10.1126/sciadv.aax8254.

DUCHENE, O.; VIAN, J.F.; CELETTE, F. Intercropping with legume for agrecological cropping systems: Complementarity and facilitation processes and the importance of microorganisms, a review. **Agriculture, Ecosystems and Environment**, v. 240, p. 148-161, 2017. Disponivel em: http://dx.doi.org/10.1016/j.agee.2017.02.019

EMPRESA DE PESQUISA AGROPECUARIA DE MINAS GERAIS. Diagnostico da cafeicultura mineira – régios tradicionais: Sul/Sudoeste de Minas, Zona da Mata, Triangulo Mineiro/Alto Paranaiba. Serie Documentos no. 46, ISSN 0102-2164, Belo Horizonte, 2010.

FIGUEREIDO, N.O.; PRATES JUNIOR, P.; CARDOSO, I.M.; FERNANDES, R.B.A. Importancia dos microrganismos no crescimento de milho. Cadernos de Agroecologia, ISSN 2236-7934, Anais do XI Congresso Brasileiro de Agroecologia, São Cristovao,

Sergipe, v. 15, n. 2, 2020. Recuperado de: https://cadernos.aba-agroecologia.org.br/cadernos/article/view/5163

FRAGA, M.E.; BRAZ, D.M.; ROCHA, J.F.; PEREIRA, M.G.; FIGUEREIDO, D.V. Interacao microrganismo, solo e flora como condutores da diversidade na Mata Atlantica. **Acta Botanica Brasilica,** v. 26, n. 4, dez. 2012. Disponivel em: https://doi.org/10.1590/S0102-33062012000400015

FUKAMI, J.; OSA, C. de la; OLLERO, F.J.; MEGIAS, M.; HUNGRIA, M. Co-inoculation of maize with Azospiriullum brasilense and Rhizobium tropici as a strategy to mitigate salinity stress. **Functional Plant Biology**, v. 45, n. 3, p. 328-339, 2017. Disponivel em: doi: 10.1071/FP17167.

GACHOMO, E.W.; KEFELA, T.; HOUNGNANDAN, P.; BABA-MOUSSA, L.; KOTCHONI, S.O. Bradyrhizobium japonicum IRAT FA3 increases biomass, yield and drought tolerance in plants**. Journal of Biology and Nature**, v. 1, n.1., p. 12-23. Disponivel em: https://doi.org/10.1094/MPMI-05-21-0118-R

GONCALVES, T. A floresta estacional decidual no Brasil: distribuicao geografica e influencia dos aspectos pedogeomorfologicos na vegetacao . **Revista Monografias Ambientais REMOA/UFSM**, v.14, n.1, p. 144-153, jan/avr. 2015. Disponivel em: https://doi.org/10.5902/2236130815213

GUDINO-GOMEZJURADO, M.E.; LEITE, R.A.; CARVALHO, T.S.; PFENNING, L.H.; MOREIRA, F.M.S. Phosphate-solubilizing fungi co-inoculated with Bradyrhizobium promote cowpea growth under varying N and P fertilization conditions. **Scientia Agricola**, v. 79, n. 5, 2022. Disponivel em: http://doi.org/10.1590/1678-992X-2021-0061

HIRSH, A.M. How rhizobia survive in the absence of a legume host, a stressful world indeed. In: Symbioses and Stress: Joint Ventures in Biology, ISBN: 978-90-481-9448-3, 2010. Disponivel em: DOI 10.1007/978-90-481-9449-0.

HOWIESON, J.G. Rhizobial persistence and its role in the development of sustainable agricultural systems in Mediterranean environments. **Soil Biology and Biochemistry**, vol. 27, no. 4, p. 603-610, 1995. Disponivel em: https://doi.org/10.1016/0038-0717(95)98638-5

JACK, C.N.; WOZNIAK, K.J.; POTTER, S.S.; FRIESEN, M.L. Rhizobia protect their legume hosts against soil-borne microbial antagonists in a host-genotype-dependent manner. **Rhizosphere**, v. 9, p. 47-55, 2019. Disponivel em: https://doi.org/10.1016/j.rhisph.2018.11.005.

JARAMILLO, P.M.D.; GUIMARAES, A.A.; FLORENTINO, L.A.; SILVA, K.B.; NOBREGA, R.S.A.; MOREIRA, F.M.S. Symbiotic nitrogen-fixing bacterial populations trapped from soils under agroforestry systems in the Western Amazon. **Scientia Agricola**, v. 70, n. 6, p. 397-404, nov./dec. 2013. Disponivel em: https://doi.org/10.1590/S0103-90162013000600004

JURBUNG, S.D.; SHEK, K. L.; MCGUIRE, K. Soil microbial composition varies in response to coffee agroecosystem management. FEMS **Microbiology Ecology**, v. 96, n. 9, p. 1-11, 2020. Disponivel em: https://doi.org/10.1093/femsec/fiaa164

KAVIYA, N.; UPADHAYAY, V.K.; SINGH, J.; KHAN, A.; PANWAR, M.; SINGH, A.V. Role of Microorganisms in soil genesis and functions. In: Mycorrhizosphere and Pedogenesis, Springer Nature, 2019. Disponivel em: https://doi.org/10.1007/978-981-13-6480-8_2

KEBEDE, E. Competency of rhizobial inoculants in sustainable agricultural production and biocontrol of diseases. **Frontiers in Sustainable Food Systems**, v. 5, 2021. Disponivel em: doi: 10.3389/fsufs.2021.728014

KEMPEL, A.; BRANDL, R.; SCHADLER, M. Symbiotic soil microorganisms as players in aboveground plant-herbivore interactions – the role of rhizobia. **Oikos**, v. 118, p. 634-640, 2009. Disponivel em: doi: 10.1111/j.1600-0706.2008.17418.x,

KENNEDY, A.C.; SMITH, K.L. Soil microbial diversity and the sustainability of agricultural soils. **Plant and Soil**, v. 170, p. 75-86, 1995. Disponivel em: http://www.jstor.org/stable/42947361

KIMITI, J.M.; ODEE, D.W. Integrated soil fertility management enhances populations and effectiveness of indigenous cowpea rhizobia in semi-arid eastern Kenya. **Applied Soil Ecology**, v. 45, p. 304-309, 2010. Disponivel em: doi:10.1016/j.apsoil.2010.05.008

KRISHNAN, S. Sustainable Coffee Production. **Oxford Research Encyclopedia, Environmental Science,** Oxford, p. 1-29, 2017. Disponivel em: https://doi.org/10.1093/acrefore/9780199389414.013.224

KUMAR, A.; MEENA, V.S.; ROY, P.; VANDANA, KUMARI, R. Role of rhizobia for sustainable agriculture: Lab to land. In: Plant growth promoting rhizobacteria for Agricultural Sustainability, Springer Nature, 2019. Disponivel em: https://doi.org/10.1007/978-981-13-7553-8_7

LEITE, A.A.; CARDOSO, A.A.S.; LEITE, R.A., OLIVEIRA-LONGATTI, S.M.; LUSTOSA FILHO, J.F.; MOREIRA, F.M.S., MELO, L.C.A. Selected bacterial strains enhance phosphotus availability from biochar-based rock phosphate fertilizer. **Annals of Microbiology**, v. 70, n. 6, 2020. Disponivel em: https://doi.org/10.1186/s13213-020-01550-3

LIMA, A.S., NOBREGA, R.S.A.; BARBERI, A.; SILVA, K. de; MOREIRA, F.M.S. Nitrogen-fixing bactéria communities occuring in soils under different uses in the Western Amazon Region as indicated by nodulation of siratro (Macroptilium atropurpureum). **Plant Soil,** v. 319, p. 127-145. Disponivel em: DOI 10.1007/s11104-008-9855-2

LINDSTROM, K.; MURWIRA, M.; WILLEMS, A.; ALTIER, N. The biodiversity of beneficial microbe-host mutualism: the case of rhizobia. **Research in Microbiology**, v. 161, p. 453-463, 2010. Disponivel em: doi:10.1016/j.resmic.2010.05.005

MACHADO, D.L.; PEREIRA, M.G.; CORREIA, M.E.F.; DINIZ, A.R.; MENEZES, C.E.G. Fauna edáfica dinâmica sucessional da Mata Atlantica em floresta estacional semidecidual na bacia do Rio Paraiba do Sul – RJ. **Ciencia Florestal**, Santa Maria, v. 25, n. 1, p. 91-106, jan/,ar. 2015. Disponivel em: https://doi.org/10.5902/1980509817466

MARTINS, S.A.; SHURT, D.A.; SEABRA, S.S.; MARTINS, S.J.; RAMALHO, M.A.P.; MOREIRA, F.M.S.; SILVA, J.C.P. da; SILVA, J.A.G. da; MEDEIROS, F.H.V. de. Common bean (Phaseolus vulgaris L.) growth promotion and biocontrol by rhizobacteria under Rhizoctonia solani suppressive and conductive soils. **Applied Soil Ecology**, v. 127, p.129-135. Disponivel em: https://doi.org/10.1016/j.apsoil.2018.03.007

MASEFIELD, G.B. The effect of organic matter in soil on legume nodulation. **Experimental Agriculture**, p. 113-119, 1965. Disponivel em: https://doi.org/10.1017/S0014479700018676

MEDEIROS, R.V.V.; RODRIGUES, P.M.A. A economia caffeira no Brasil e a importância das inovações para essa cadeia. **A Economia em Revista**, v. 25, n. 1, jun. 2017. Disponivel em: https://doi.org/10.4025/aere.v25i1.35511

MELLONI, R.; MOREIRA, F.M.S.; SIMAO, R.; NOBREGA, A.; SIQUEIRA, J.O. Eficiencia e diversidade fenotípica de bactérias diazotroficas que nodulam caupi (Vigna unguiculata L. Walp) e feijoeiro (Phaseolus vulgaris L.) em áreas de mineração de bauxita em reabilitação. **Revista Brasileira de Ciencias do Solo**, v. 30, p. 235-246, 2006. Disponivel em: https://doi.org/10.1590/S0100-06832006000200005

MELO, A.P.; OLIVARES, F.L.; MEDICI, L.O.; TORRES-NETO, A.; DOBBS, L.B.; CANELLAS, L.P. Mixed rhizobia and Herbaspirillum seropedicae inoculations with acid-like substances improve water-stress recovery in common beans. **Chemical and Biological Technologies in Agriculture**, v. 4, n. 6, 2017. Disponivel em: https://doi.org/10.1186/s40538-017-0090-z

MICHEL, D.C.; GUIMARAES, A.A.; COSTA, E.M. da; CARVALHO, T.S. de; BALSANELLI, E.; WILLEMS, A.; SOUZA, E.M. de; MOREIRA, F.M.S. Bradyrhizobium uaiense sp.nov., a new highly efficient cowpea symbiont. **Archives of Microbiology**, 2020. Disponivel em: https ://doi.org/10.1007/s0020 3-020-01827 -w

MOREIRA, F.M.S.; GILLIS, M.; POT, B.; KERSTERS, K.; FRANCO, A.A. Characterization of rhizobia isolated from different divergence groups of tropical Leguminosae by comparative polyacrylamide gel electrophoresis of their total proteins. **Systematic and Applied Microbiology**, v. 16, n. 1, p. 135-146, avr. 1993. Disponivel em: https://doi.org/10.1016/S0723-2020(11)80258-4

MOURA, E.G.; CARVALHO, C.S.; BUCHER, C.P.C.; SOUZA, J.L.B.; AUIGAR, A.C.; FERRAZ JUNIOR, A.S.L.; BUCHER, C.A.; COELHO, K.P. Diversity of rhizobia and importance of their interactions with legume trees for feasibility and sustainability of the tropical agrosystems. **Diversity**, v. 12, n. 206, 2020. Disponivel em: doi:10.3390/d12050206

NOORI, F.; ETESAMI, H.; ZARINI, H.N.; KHOSHKHOLG-SIMA, N.A.; SALEKDEH, G.H.; ALISHAHI, F. Mining alfalfa (Medicago sativa L.) nodules for

salinity tolerant non-rhizobial bacteria to improve growth of alfalfa under salinity stress. **Ecotoxicology and Environmental Safety**, v. 162, p. 129-138, 2018. Disponivel em: doi: 10.1016/j.ecoenv.2018.06.092.

Nyoki & Ndakidemi, 2014

NYOKI, D.; NDAKIDEMI, P.A.. Effects of phosphorus and Bradyrhizobium japonicum on growth and chlorophyll content of cowpea (Vigna unguiculata L. Walp). **American Journal of Experimental Agriculture,** v. 4, n. 10, p. 1120-1136, 2014. Disponivel em: DOI: 10.9734/AJEA/2014/6736

OLAYINKA, A.; ADETUNJI, A; ADEBAYO, A. Effect of organic amendments on nodulation and nitrogen fixation by cowpea. **Journal of Plant Nutrition**, v. 21, n. 11, p. 2455-2464, 1998. Disponivel em: 10.1080/01904169809365577

OMAR, S.A.; ABD-ALLA, M.H. Biocontrol of fungal root rot diseases of crop plants by the use of rhizobia and bradyrhizobia. **Folia Microbiologica**, v. 43, p. 431-437, 1998. Disponivel em: https://doi.org/10.1007/BF02818587

OSORIO FILHO, B.D.; BINZ, A.; LIMA, R.F.; GIONGO, A.; SA, E.L.S. Promocao de crecimento de arroz por rizobios em diferentes níveis de adubação nitrogenada. **Ciencia Rural**, Santa Maria, v. 46, n. 3, p. 478-485, mar. 2016. Disponivel em: https://doi.org/10.1590/0103-8478cr20141066

PEDROSA, M.V.B.; ALVES, L.P.; PASCHOA, R.P.; AMARAL, A.A. do. Importancia ecológica dos microrganismos do solo. **Enciclopedia biosfera,** Goiania, v. 11, n. 22, p. 100-114, 2015. Recuperado de: https://conhecer.org.br/ojs/index.php/biosfera/article/view/1366

PENA-CABRIALES, J.J.; ALEXANDER, M. Survival of Rhizobium in soils undergoing drying. **Soil Science Society of America Journal**, v. 48, p. 962-966, 1979. Disponivel em: https://doi.org/10.2136/sssaj1979.03615995004300050030x

PEREIRA, A.B. Mata Atlantica: Uma abordagem geografica. **Nucleus**, v. 6, n. 1, p. 27-53, 2009. Disponivel em: DOI:10.3738/1982.2278.152

PIRES, R.C.; REIS JUNIOR, F.B. dos; ZILLI, J.E.; FISHCER, D.; HOFMANN, A.; JAMES, E.K.; SIMON, M.F. Soil characteristics determine the rhizobia in association with different species of Mimosa in central Brazil. **Plant Soil**, 2017. Disponivel em: https://doi.org/10.1007/s11104-017-3521-5

PITOMBO, L.M.; REGANHAN-CONEGLIAN, C. Potential of Inga sp. (Inga uruguensis Hook. And Arn) in the phytoremediation of oily compounds. **Soil and Sediment Contamination**, v. 22, n. 7, p. 829-838, 2013. Disponivel em: https://doi.org/10.1080/15320383.2013.768206

PODLESAKOVA, K.; FARDOUX, J.; PATREL, D.; BONALDI, K.; NOVAK, O.; STRNAD, M.; GIRAUD, E.; SPICHAL, L.; NOUWEN, N. Rhizobial synthesized cytokinins contribute to but are not essential for the symbiotic interaction between photosynthetic bradyrhizobia and Aeschynomene legumes. **American**

Phytopathological Society Publications, v. 26, n. 10, p. 1232-1238, 2013. Disponivel em: doi/10.1094/MPMI-03-13-0076-R

POOLE, P.; RAMACHANDRAN, V.; TERPOLILLI, J. Rhizobia: from saprophytes to endosymbionts. **Nature Reviews, Microbiology**, v. 16, p. 291-303, 2018. Disponivel em: doi:10.1038/nrmicro.2017.171

PUEPPKE, S.G. Nodule distribution on legume roots: Specificity and response to the presence of soil. **Soil Biology and Biochemistry**, v. 18, n. 6, p.601-606, 1986. Disponivel em: https://doi.org/10.1016/0038-0717(86)90082-9

ROCHA, W.S.; SANTOS, M.M. dos; COLONIA, B.S.O.; CHAGAS JR., A.F. The ability of rhizobacteria to solubilize phosphate and synthesize of indoleacetic acid in cowpea. **Revista Verde de Agroecologia e Desenvolvimento Sustentavel**, v. 13, n. 1, p. 116-121, 2018. Disponivel em: http://dx.doi.org/10.18378/rvads.v13i1.5275

RODRIGUES, T.L.; COSTA, E.M.; RIBEIRO, P.R.A.; CARVALHO, F. de; RUFFINI, M.; SILVA, A.O.; TEIXEIRA, A.F.S.; PEREIRA, T.A.; SALES, F.R.; MOREIRA, F.M.S. Diversity and biotechnologial potential of rhizobia isolated from lima bean nodules collected at a semi-arid region. **Soil Science Society of America Journal,** v.85, n. 5, p. 1663-1678, 2021. Disponivel em: https://doi.org/10.1002/saj2.20272

SA, O.A.A.L. de; RIBEIRO, P.R.A.; RUFFINI, M.; CRUNIVEL, I.A.F.; CASAGRANDE, D.R.; MOREIRA, F.M.S. Microsymbionts of forage peanut under different soil and climate conditions belong to a specific group of Bradyrhyzobium strains. **Applied Soil Ecology**, v. 143, p. 201-212, 2019. Disponivel em: https://doi.org/10.1016/j.apsoil.2019.07.018

SAGHAFI, D.; GHORBANPOUR, M.; LAJAYER, B.A. Efficiency of Rhizobium strains as plant growth promoting rhizobacteria on morpho-physiological properties of Brassica napus L. under salinity stress. **Journal of Soil Science and Plant Nutrition**, v.18, n. 1, 2018. Disponivel em: http://dx.doi.org/10.4067/S0718-95162018005000903

SIDDIQUI, I.A.; EHTESHAMUL-HAQUE, S.; ZAKI, M.J.; GHAFFAR, A. Greenhouse evaluation of rhizobia as biocontrol agent of root-infecting fungi in okra. **Acta Agrobotanica**, v. 53, n. 1, 2000. Disponivel em: DOI: https://doi.org/10.5586/aa.2000.002

SILVA, A.O.; GUIMARAES, A.A.; COSTA, A.M. da, RODRIGUES, T.L.; CARVALHO, T.S.; SALES, F.R.; MOREIRA, F.M.S. Plant growth promoting rhizobacterial communities from an area under the influence of iron minin and from adjacent phytophysiognomies have high genetic diversity. **Land degradation and development**, v. 31, n. 16, p. 2237-2254. Disponivel em: https://doi.org/10.1002/ldr.3593

SILVA, M.C.S. da; VELOSO, T.G.R.; ENTRINGER, T.L.; BULLERGAHN, V.B.; ANASTACIA, L.M.; PEREIRA, L.L.; KASUYA, M.C.M. Diversity of nitrogen-fixing bactéria in coffe crops (Coffea arábica L.), **Revista Ifes Ciencia**, v. 6, n. 3, p. 12-21, 2020. Disponivel em: 10.36524/ric.v6i3.852

SINGH, J.S.; PANDEY, V.C.; SINGH, D.P. Efficient soil microorganisms: A new dimension for sustainable agriculture and environmental development. **Agriculture, Ecosystems and Environment,** v. 140, p. 339-353, 2011. Disponivel em: doi:10.1016/j.agee.2011.01.017

SOUSA, L.P. de, GUERREIRO-FILHO, O.; MONDEGO, J.M.C. The rhizosphere microbomes of five species of coffee trees. **Microbiology Spectrum**, v. 10, n. 2, mar/avr. 2022. Disponivel em: 10.1128/spectrum.00444-22

STEINAUER, K.; CHATZINOTAS, A.; EISEHAUER, N. Rood exudate cocktails: the link between plant diversity and soil microrganisms? **Ecology and Evolution**, v. 6, p. 7387-7396, 2016. Disponivel em: DOI: 10.1002/ece3.2454

STONE, B.W.; LI, J.; KOCH, B.J.; BLAZEWICZ, S.J.; DIJKSTRA, P.; HAER, M.; HOFMACHEL, K.S.; LIU, X.J.A.; MAU, R.L.; MORRISSEY, E.M.; PETT-RIDGE, J.; SCHWARTZ, E.; HUNGATE, B.A. Nutrients cause consolidation of soil carbon flux to small proportion of bacterial community. **Nature Communication**s, v. 12, n. 3381, 2021. Disponivel em: https://doi.org/10.1038/s41467-021-23676-x

SU, Y.; HE, Z.; YANG, Y.; JIA, S.; YU, M.; CHEN, X.; SHEN, A. Linking soil microbial community dynamics to straw-carbon distribution in soil organic carbon. **Scientific Reports**, v. 10, n. 5526, 2020. Disponivel em: https://doi.org/10.1038/s41598-020-62198-2

THAMER, S.; SCHADLER, M.; BONTE, D.; BALHORN, D.J. Dual benefit from a belowground symbiosis: nitrogen fixing rhizobia promote growth and defense against a specialist herbivore in a cyanogenic plant. **Plant Soil**, v. 341, p. 209-219, 2011. Disponivel em: DOI 10.1007/s11104-010-0635-4

THIES, J.E.; BOHLOOL, B.B.; SINGLETON, P.W. Subgroups of the cowpea miscellany: Symbiotic specificity within Bradyrhizobium spp. for Vigna unguiculata, Phaseolus lunatus, Arachis hypogaea and Macroptilium atropurpureum. **Applied and Environmental Microbiology**, v.57, n.5, p. 1540-1545, 1991. Disponivel em: doi: 10.1128/aem.57.5.1540-1545.1991

WAHAB, A.M.A.; ABD-ALLA, M.H.; EL-ENANY, A.E. Stimulation of nodulation, nitrogen fixation and plant growth of faba bean by cobalt and copper additions. In: Fertilizers and environment, v. 66, ISBN: 978-94-010-7210-6, p. 127-130, 1996.

WERNECK, M. S.; REZENDE, S. G.; BRINA, A. E.; FRANCESCHINELLI, E.V. Composicao floristica do componente arboreo e afinidade fitogeografica de uma floresta semidecidua em Nova Lima, MG. **Brazilian Journal of Botany**, v. 33, n. 4, p. 547-561, dez. 2010. Disponivel em: https://doi.org/10.1590/S0100-84042010000400004

YADAV, A.N.; VERMA, P.; KOUR, D.; RANA, K.L.; KUMAR, V.; SINGH, B.; CHAUAHAN, C.S.; SUGITHA, T.C.K.; SAXENA, A.K.; DHALIWAL, H.S. Plant microbiomes and its beneficial multifunctional plant growth promoting attributres. **International Journal of Environmental Sciences and Natural Resources**, v. 3, n. 1, jun. 2017. Disponivel em: DOI : 10.19080/IJESNR.2017.03.555601

ZABALOY, M.C.; GOMEZ, M.A. Diversity of rhizobia isolated from an agricultural soil in Argentina based on carbon utilization and effects of herbicides on growth. **Biology and Fertiliy of Soils**, v. 42, p. 83-88, 2005. Disponivel em: 10.1007/s00374-005-0012-2

ZEIJL, A. van; CAMP, R.H.M.O. den; DEINUM, E.E.; CHARNIKHOVA, T.; FRANSSEN, H.; CAMP, H.J.M.O. den; BOUWMEESTER, H.; KOHLEN, W.; BISSELING, T.; GEURTS, R. Rhizobium lipo-chitooliosaccharide signaling triggers accumulation of cytokinins in Medicago truncatula roots. **Molecular Plant**, v. 8, n. 8. P. 1213-1226, 2015. Disponivel em: 10.1016/j.molp.2015.03.010

ZENGENI, R.; MPEPEREKI, S.; GILLER, K.E. Manure and soil properties affect survival and persistence of soyabean nodulating rhizobia in smallholder soils of Zimbabwe. **Applied Soil Ecology**, v. 32, p. 232-242, 2006. Disponivel em: doi:10.1016/j.apsoil.2005.06.001

Zilli *et al.*, 2013

ZILLI, J.E.; PEREIRA, G.M.D.; FRANÇA JUNIOR, I.; SILVA, K. de; HUNGRUA, M.; ROUWS, J.R.C. Dinamica de rizobios em solo do cerrado de Roraima durante o período de estiagem. **Acta Amazonia**, v. 43, n. 2, p. 153-160, 2013. Disponivel em: https://doi.org/10.1590/S0044-59672013000200004

ZILLI, J.E.; VALICHESKI, R.R.; RUMJANEK, N.G.; SIMOES, J.L.; FREIRE FILHO, F.R.; NEVES, M.C.P. Eficiencia simbiótica de estirpes de Bradyrhizobium isoladas de solo do Cerrado em caupi. **Pesquisa Agropecuária Brasileira**, Brasilia, v. 41, n. 5, p. 811-818, mai. 2006. Disponivel em: https://doi.org/10.1590/S0100-204X2006000500013