UNIVERSIDADE FEDERAL DE LAVRAS

ARETHA FRANKLIN GUIMARÃES

PLANT-SOIL INTERACTIONS IN A SERPENTINE NEOTROPICAL FOREST

LAVRAS - MG
2021

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Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Botânica Aplicada, para obtenção do título de Doutora.

Profa. Dra. Marinês Ferreira Pires Lira
Orientadora
Prof. Dr. Eduardo van den Berg
Coorientador
Dra. Gabriela Siewerding Meirelles
Coorientadora
Prof. Dr. Nick Ostle
Coorientador

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

Guimarães, Aretha Franklin.
Plant-soil interactions in a serpentine neotropical forest / Aretha Franklin Guimarães. - 2021.
85 p. : il.

Orientador(a): Marinês Ferreira Pires Lira.
Coorientador(a): Eduardo van den Berg, Gabriela Siewerding Meirelles, Nick Ostle.
Tese (doutorado) - Universidade Federal de Lavras, 2021.
Bibliografia.

1. Interações solo-planta. 2. Traços funcionais. 3. Solos serpentinos. I. Lira, Marinês Ferreira Pires. II. Berg, Eduardo van den. III. Meirelles, Gabriela Siewerding. IV. Ostle, Nick. V. Título.

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PLANT-SOIL INTERACTIONS IN A SERPENTINE NEOTROPICAL FOREST

INTERAÇÕES SOLO-PLANTA EM UMA FLORESTA NEOTROPICAL COM SOLO SERPENTINO

Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Botânica Aplicada, para obtenção do título de Doutora.

APROVADA EM 09 de julho de 2021.
Dra. Flávia Regina Capelotto Costa - INPA
Dra. Marcela de Castro Nunes Santos Terra - UFLA
Dr. Guilherme Ferreira Demétrio - UFAL
Dr. Yuri Lopes Zinn - UFLA
Dr. Teotonio Soares de Carvalho – UFLA

Marinês Ferreira Pires Lira
Profa. Dra. Marinês Ferreira Pires Lira
Orientadora
Prof. Dr. Eduardo van den Berg
Coorientador
Dra. Gabriela Siewerding Meirelles
Coorientadora
Prof. Dr. Nick Ostle
Coorientador

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À minha mãe pelo apoio incondicional.
Ao meu marido, por me acompanhar nessa jornada.
À Aurora e Dante, para que tenham orgulho de sua mãe.
À Ubiran, Linda, Teresa e Celio, pelo acalento e incentivo à criança que fui.
Às minhas amigas do coração Simone, Gabriela, Carolina, Kaline, Polyanne, Aline, Marcela, Alana, Grécia e Natalia, pela troca de experiências, afeto e acalento.
Dedico

AGRADECIMENTOS

À Universidade Federal de Lavras e ao Departamento de Biologia.

Ao CNPq e CAPES, pela concessão da bolsa de Doutorado e Doutorado Sanduíche. O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Código de Financiamento 001.

Ao Programa de Pós-Graduação em Botânica Aplicada.

À Lancaster University por ter me recebido para o doutorado sanduíche.

Ao professor Nick Ostle pelos ensinamentos.

Ao meu orientador Eduardo van den Berg, por acreditar no meu projeto, me apoiando com ideias, sugestões e críticas. Pelos ensinamentos e pela paciência.

À professora Marinês pela orientação.

Aos meus professores, pelos ensinamentos transmitidos.

Ao meus colegas de doutorado da UFLA e LU, pelo apoio e pelas risadas.

À minha amiga Gabriela, que caminhou comigo até aqui.

À professora Emma Sayer pelos ensinamentos sobre escrita científica.

Obrigada aos meus amigos que partilharam essa jornada comigo: Poly, Marcela, Aline, Grécia, Carol, Michel, Simone,William, Kaline, Samuel, Ney, Maria Claudia e Janete.

À minha família pelo apoio incondicional.

MUITO OBRIGADA!

I am my mother's savage daughter
The one who runs barefoot cursing sharp stones
I am my mother's savage daughter
I will not cut my hair, I will not lower my voice

My mother's child is a savage
She looks for her omens in the colors of stones
In the faces of cats, in the fall of feathers
In the dancing of fire and the curve of old bones
(Sarah Hester Ross - Savage Daughter)

RESUMO

Os solos serpentinos possuem altos teores de metais pesados e ocorrem de forma espontânea em uma parte limitada do globo terrestre, criando vários tipos de restrições ambientais para os organismos que alí habitam. Quanto aos microorganismos presentes nesses solos, em alguns locais há alto grau de especialização, tendência a resistencia à metais pesados e alta diversidade da estrutura microbiana, e em outros locais exibindo padrão oposto a esse. Pouco da literatura existente diz respeito a solos serpentinos em áreas tropicais. Esses solos apresentam árvores com menor biomassa, menor altura e menor número de espécies, levando a uma flora e espécies hiperacumuladoras de metais pesados. Quanto à estrutura da comunidade microbiana serpentina, há pouca informação específica para os neotrópicos. Nesse sentido nosso trabalho visa preencher esta lacuna, com o objetivo de entender as interações existentes entre microorganismos do solo e plantas em uma área de solo serpentino nos neotrópicos. Nosso trabalho focou em cinco perguntas centrais: A) as interações entre microorganismos de solo-plant serão negativamente influenciadas pela presence de metais pesados no solo, assim como os traços funcionais da planta serão influenciados negativamente pela presence de metais pesados nas folhas; B) maior razão C:N em áreas de solo serpentino; C) o total de PLFA’s e fungos serão menores em áreas de solos serpentino; D) haverá um maior total de bactérias gram positivas e uma menor quantidade de bactérias gram negativas em áreas de solo serpentino e E) os traços funcionais exibirão uma tendência a nanismo em áreas de solo serpentino, enquanto valores de espessura foliar, área do xilema e floema apresentarão maiores valores, com tendência a xeromorfismo. Encontramos como padrão geral que bactérias gram positivas interagem com o ferro nas folhas; as razões C:N são similares entre as áreas avaliadas; não encontramos diferenças entre o total de PLFAs, total de fungos, total de bactérias gram positivas e negativas entre as áreas avaliada e finalemente encontramos uma tendência à nanismo e xeromorfismo nos traços funcionais de *Copaifera langsdorffii* Desf. em solos serpentinos.

Palavras-chave: Solos serpentinos. Flora ultramáfica. Metais pesados. Interação solo-planta.

ABSTRACT

Serpentine soils are those with high levels of high metals and occur spontaneously through limited areas of the world, imposing many environmental restrictions to the organisms inhabiting those soils. Regarding the soil microbes in serpentine soils, there's divergence between the authors, with some areas exhibiting adaptations and resistance to heavy metals, with high diversity of soil microbial structure, and other areas with the opposite pattern. Despite serpentine soils are being studied for decades in temperate and Mediterranean areas, we have few studies concerning the topic in tropical areas. As a central paradigm for the vegetation associated to serpentine soils, there's usually vegetation with lower biomass, lower tree's height and lower species number, leading to a depleted flora with many degrees of nutritional imbalance, high rates of endemic and/or heavy metal hyperacummulator species. However, new evidence seems to indicate that this pattern might be different for serpentine tropical flora, indicating that other mechanisms could be involved in the permanence of a higher species number in those areas despite the excess of heavy metal. Regarding the serpentine microbial structure, there's little specific information for the neotropical areas. In this sense, our study aims to fill this gap, aiming to understand the interactions between soil microbes and plants in a neotropical serpentine area. Our study focused in five main questions: A) the interactions between soil microbes-plants will be negatively affected by the presence of heavy metals in the soils, as well as the functional traits; B) higher C:N ratio in serpentine soil areas; C) total PLFAs and total fungi will be lower in serpentine areas; D) there will be higher amounts of total gram positive bacteria and lower gram negative bacteria in serpentine soils and E) functional traits will have a tendency to dwarfism in serpentine areas, while the functional traits leaf thickness, xylem and phloem area will exhibit a tendency to xeromorphism. As a general pattern, we found that gram positive bacteria interact with iron in the leaves; the C:N ratios are higher in serpentine 1 than serpentine 2, but the two areas are similar to the non-serpentine area; there was no difference between total PLFAs, total fugi, total gram positive bacteria and total gram negative bacteria in our study areas and finally, there's a tendency to dwarfism and xemorphism in the functional traits of *Copaifera langsdorffii* Desf. in serpentine soils.

Keywords: Serpentine soils. Ultramafic flora. Heavy metals. Plant-soil interactions.

SUMÁRIO

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Aretha Franklin Guimarães

FIRST PART

1 INTRODUÇÃO

Serpentine soils can be defined as soils derived from ultramafic rocks with 70% or more of iron-magnesium compounds (SALIHAJ; BANI; ECHEVARRIA, 2016; VIDAL-TORRADO et al., 2006), usually associated with low plant productivity. Serpentine soils are composed by parts of the Earth's mantle that poured out the crust (BROOKS et al., 1988), and the term has been used as a reference for any soil made from decomposed ultramafic material (OZE et al., 2004). Due to the high heavy metal concentration, those soils are usually inappropriate to agriculture, exhibiting low levels of nutrients such as potassium, low microbial activity and poor water retention capacity (HIPFGER et al. 2020). Also, the high amounts of heavy metals (mainly iron-magnesium compounds, as well as zinc, nickel and chrome) lead to the formation of soils with stressful conditions that only few organisms can thrive (ANACKER, 2014). These characteristics have long been studied and are known as the "serpentine syndrome", a group of features that are supposed to be a global pattern, where the ecosystems withholding serpentine soils exhibiting three major traits: 1) low plant productivity, 2) high levels of endemism and 3) vegetation with structure and composition different from the neighbouring areas (WHITAKKER, 1954). In such soils, some ecological processes (e.g. productivity functions like photosynthesis and nutrient uptake) responsible for maintaining the stability of an ecosystem are often impaired by the harsh conditions.

Although serpentine soils can be found in all continents, they occupy less than 1% of the Earth's surface (VITHANAGE et al., 2015), with most of them being found in the Circum-Pacific margin and Mediterranean Sea (HSEU et al., 2018). Those soils attract scientists attention because they are habitats that host "islands" of specialized and unique vegetation adapted to extreme edaphic conditions (CHIARUCCI; BAKER, 2007). Moreover, recent discoveries show that those soils are similar to the ones found in Martian regolith's, meaning that serpentine soils could be used in experiments as analogues to Martian soils (KANELLOPOULOS, 2020). Although they are worldwide distributed, to date we still lack data about vegetation on serpentine soils in the tropics (CANO et al., 2014), and the extend of the limitations imposed by those soils in the neotropics still needs careful consideration (VILELA; INDA; ZINN, 2019). The few studies carried in serpentine sites in tropical areas seem to point to a high number of species (CANO et al. 2014; SARMIENTO 2018;

GUIMARAES et al. 2019), counterpointing the current literature about those soils in colder climates. Therefore it is urgent to understand which mechanisms are involved in the adaptation of the tropical plant species that allow them to thrive in those environments against all the odds (GUIMARAES et al., 2019).

2 REFERENCIAL TEÓRICO

2.2 Soil microorganisms in serpentine soils

Microorganisms play an important hole in mediating ecosystem functioning processes: the microbiome is an important driver of plant success, influencing key traits to plants development such as growth, disease tolerance, water retention and abiotic stress tolerance (KÖBERL et al., 2013), and their diversity can also have a complementary effect on ecosystem productivity (LAFOREST-LAPOINTE et al., 2017). Microbes are also important in the modulation of the host plant immunity (ZAMIOUDIS; PIETERSE, 2012), nitrogen and carbon fixation (SCHLESINGER; ANDREWS, 2000) and its diversity is key in regulating plant-soil interactions (SEMCHENKO et al., 2018). More than simple decomposers, bacterial composition is an useful indicator of carbon availability in soils: gram-positive bacteria are commonly associated with complex soil organic matter derived carbon sources, while gram-negative bacteria are usually dependent on simple carbon plant-biomass derived sources (FANIN et al., 2019).

Microbes can avoid metal toxicity by chelating mechanisms, solubilizing those compounds and preventing them from entering the organism cell walls, providing efflux transporters to promote excretion from the cell to the environment, and if the heavy metals enter the cell, they can be contained by the formation of inclusion bodies with the harmful substance and by metabolizing some of these compounds (GALL; BOYD; RAJAKARUNA, 2015). Whenever soil microbial organisms become unable to cope with the heavy metals toxicity, they are harmed by it, leading to lower enzyme activity, reduction of soil microbial diversity, slowing decomposition and respiration rates (GALL; BOYD; RAJAKARUNA, 2015). The presence of heavy metals can also inhibit soil microorganism’s growth, resulting in soils with a lower microbial biomass (OIJAGBE et al. 2019).

In serpentine soils in a volcanic area in the Philippines, novel extremophilic archaeal communities were described, and they seem to be involved in methane oxidation by helping reducing sulphates (CURTIS et al., 2012). Microbes from serpentine sites can exhibit different genes related to heavy metals tolerance (PORTER et al., 2017), and also being important on diminishing metal toxicity and enhancing metal bioavailability to the roots of plants (BENIZRI; KIDD, 2018). Another study comparing serpentine and non-serpentine substrate evidenced that arbuscular mycorrhizal fungi contributes to plant growth promotion, root colonization and nutrient uptake in serpentine soils (DOUBKOVÁ; SUDA; SUDOVÁ, 2011). There's also an intriguing association of serpentine soils with specific microbial strands, with the predominance of actinomycetes and actinobacteria (COSTA et al., 2019; DEGROOD; CLAASSEN; SCOW, 2005), indicating an adaptation capacity of the microorganisms inhabiting serpentine sites. Although those characteristics can be commonly found in serpentine soils, there seems to be some divergence regarding the microbial diversity in those sites, where some authors indicate a high biodiversity of soil organisms, counterpointing what was usually found, i.e. that serpentine soils are depauperate and composed by a few specialised microbial species (BRANCO, 2010; VISIOLI et al., 2019).

Despite all the knowledge gathered in the past years, a few questions remain poorly understood (GRAHAM et al., 2016), and an important one of them is to understand how bellow and aboveground plant processes influences soil communities (EISENHAUER et al., 2015), especially in stressful environments such as serpentine soils. The relationships between microbial diversity and ecosystem functioning is still one of the 100 fundamental ecological questions yet to be addressed by scientists (SUTHERLAND et al., 2013), and we want to contribute to advances in the topic by answering how the network of soil-plant interactions allow some species to survive in a neotropical serpentine soils area in Brazil.

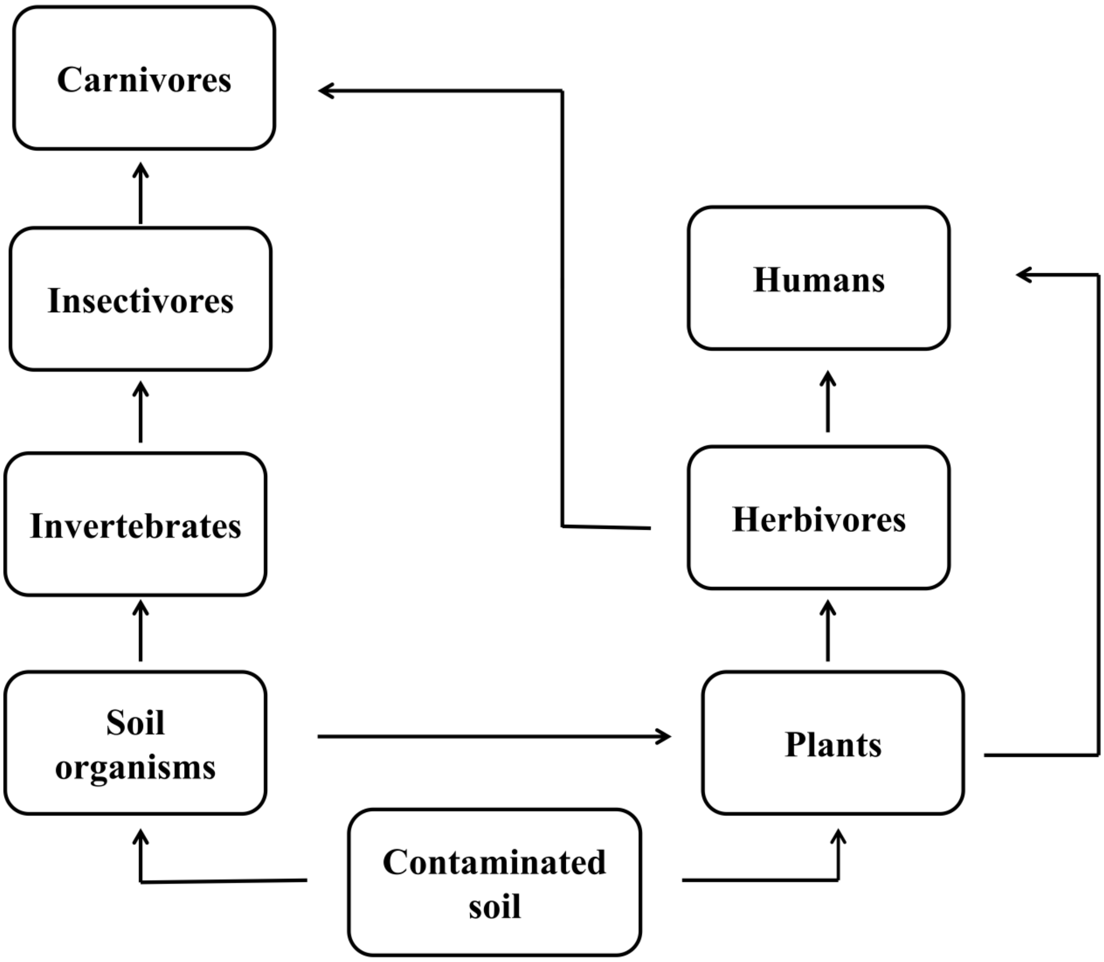
2.3 Plants and serpentine soils

Heavy metals input by the plants occurs by two distinct routes: directly from soil uptake or by acquiring the toxic substances that are released by soil microbes (GALL; BOYD; RAJAKARUNA, 2015). Some plants developed tolerance to accumulate extraordinarily high concentrations of heavy metals by translocation of the metals through the root-to-shoot systems and later detoxifying by sequestering those metals in the leaves without suffering the adverse effects caused by them. Such plants are called "hyperaccumulators" (RASCIO; NAVARI-IZZO, 2011), and they are commonly found in serpentine soils, being widely responsible for the high rates of endemism in those areas. In non-hyperaccumulator plants, heavy metals can induce DNA strand breaks and DNA base modifications, permutagenic damage, necrotic brown spots in leaves, chlorosis, cellular damage, and leaf death (NAGAJYOTI; LEE; SREEKANTH, 2010). If the presence of heavy metals in the soil is high, the osmotic potential in soil solution can be lower than in plant root cells, restricting the transport of water from soil to the plant and water delivery to the shoot system (RUCINSKA-SOBKOWIAK, 2016).

Another important consequence of the excess of heavy metals in plants is the production of reactive oxygen species (ROS, also known as free radicals), which triggers a series of malfunctions during the electron transport activities in the chloroplast membranes (RASCIO; NAVARI-IZZO, 2011), therefore affecting the photosynthesis and exposing the plant to oxidative stress. The presence of heavy metals also has a cascade effect in water transport: primary roots of plants exposed to contaminated soils tend to have a decreased elongation with inhibited growth, hormone synthesis and transport, leading to deposits of cellular debris and gums in the xylem, finally leading to a decrease in leaf size, leaf area, lamina thickness, intercellular spaces, density of stomata size and aperture, with a consequently decrease in stomata density (RUCINSKA-SOBKOWIAK, 2016). In serpentine soil sites, the most visible consequence of heavy metal toxicity usually is a dwarf and low biomass flora, with lower species diversity and many endemic plants (BROOKS et al., 1988), generally leading to the metal accumulation in the shoot system (DOUBKOVÁ; SUDA; SUDOVÁ, 2011).

Some plants developed coping mechanisms to deal with the presence of those heavy metals such as binding them with organic acids and storing them in vacuoles, for example (GALL; BOYD; RAJAKARUNA, 2015), and some serpentine plants are able to allocate the excess of heavy metals in the shoot tissues (DOUBKOVÁ; SUDA; SUDOVÁ, 2011). Some of the organisms that cannot metabolize heavy metals bioaccumulate them (i.e. accumulation of a substance from lower to higher trophic levels), and such bioaccumulation can be potentially harmful for humans as we have evidence that heavy metals can be found even in pollen and bees honey from organisms inhabiting serpentine areas (ATANASSOVA et al., 2016) (Figure 1). Once dealing with heavy metals toxicity is a pre-requisite for their existence, plants spontaneously inhabiting serpentine sites might be good proxies to be used for restoration projects and remediation of metal-contained area (BINI; MALECI; WAHSHA, 2016).

Figure 1 - Schematic view of organism's involved in bioaccumulation processes from heavy metal contaminated areas through the food web. Arrows represent the uptake by the organism described inside the box. This figure summarizes information in the review paper about bioaccumulation from Gall et al. (2015*b*). Plants and soil organisms' might uptake heavy metals directly from contaminated soils. Herbivores (e.g. cattle, sheep) then feed on contaminated herb, grass and other plants, and are consumed by humans. Invertebrates that feed from soil organisms are also potential bioaccumulators and can pass metal toxicity through the food web to insectivores and carnivores. Carnivores also can feed on herbivores from contaminated sites and became poisoned too.



(From the author, 2021)

2.4 Functional traits and soil microbes

Plant functional traits have been successfully used as a tool to test mechanisms and to evaluate ecosystem processes (DAWSON et al., 2019), as they are intrinsically linked to the overall plant productivity. They are a good proxy for plant growth, litter and habitat quality (SCHELLBERG; POTSH; RAUMBERG-GUMPENSTEIN, 2014), and at an individual level, they can be important determinants of soil carbon inputs and outputs (DEYN;

CORNELISSEN; BARDGETT, 2008). It is well established in the literature that functional traits have a dramatic impact in soil primary productivity: fast-growing plants with acquisitive traits (i.e. higher photosynthetic rates, lower lifespan, lower leaf dry mass content and carbon concentrations above and releases large amounts of recalcitrant carbon from their plant tissues, while slow-growing plants with conservative traits (i.e. higher leaf dry mass content, lifespan and concentrated carbon in plant tissues) provides slower but labile carbon fluxes (GLATZEL; VIENNA, 2008).

Plant traits associated to fast-growing, higher specific leaf area and low leaf dry mass content are linked to bacterial dominated communities (DE VRIES et al., 2012), and plants associated to slow-growing, higher leaf dry mass content and lower specific leaf area tend to select for recalcitrant carbon compounds, usually associated to higher fungal abundance (LAUBER et al., 2008; ORWIN et al., 2010). There’s growing evidence that microorganisms affect the functional traits by changing nutrient availability, plant development and stress tolerance (GOH et al., 2013). Since soil microbes respond promptly to heavy metal concentrations (STEFANOWICZ et al., 2020), the total microbial biomass decreases strongly in soils polluted by those metals (XU et al., 2019). Sites with high concentration of metals like serpentine soils are reported to have higher bacterial biomass (ORTIZ et al., 2020), as some bacterial strains tend to be more metal tolerant (ABOU-SHANAB; VAN BERKUM; ANGLE, 2007). The proportion of gram-positive to gram-negative bacteria is also an indicator of habitat quality, as gram-positive bacteria are thought to be associated with stress resistance and harsh environmental conditions when compared to gram-negative bacteria (FANIN et al., 2019).

Heavy metals cannot be degraded like other organic pollutant counterparts, with major consequences for the soils microbes (GALL; BOYD; RAJAKARUNA, 2015). Microbial growth can be retarded in the presence of heavy metals, reflecting in the overall accumulation of soil organic matter in contaminated soils, which is an indicative of a lack of organic matter decomposition in those sites (WENDEROTH; REBER, 1999). The most drastic consequence of the presence of heavy metals is the alteration of the microbial community structure with the suppression of some groups (e.g. fungi) in some cases (XU et al., 2019). However, the shifts in the soil microbial community can induce changes in the trees community, selecting for specific traits that allow these plants to survive (DE VRIES et al., 2012).

The majority of the studies about ecosystem functioning around the globe were developed in temperate areas, with a gap in the tropical area: only an infamous 1.6% of the studies were developed in those areas (CLARKE; YORK; RASHEED, 2017). Basic data about serpentine soils vegetation in the tropics and particularly in South America are lacking (CANO et al., 2014), and it is urgent to understand which mechanisms are involved in the adaptation of the tropical species that lead them to thrive in those environments (GUIMARAES et al., 2019). For all of what has been described for serpentine soils, and having in consideration that most of this theory was written based in model areas from temperate parts of the world, it is possible that we might be facing a paradigm shift regarding the theme in tropical areas. The high number of species found in tropical serpentine areas might be an indicative of novel soil-plant mechanistic adaptations towards heavy metal tolerance. Also, only a few studies about ecosystem functioning in serpentine soils addressed information regarding plant × serpentine soil mechanisms in the neotropics, especially information regarding microorganisms and their hole in plants tolerance to such environmental conditions.

In Brazil the situation might be even more urgent, as only a few previous studies have addressed information about the vegetation in areas of serpentine soils (ALMEDA; MATIRNS, 2015; BROOKS et al., 1988; GUIMARAES et al., 2019) – and it’s important to highlight that they are likely to support new endemic and/or hyperaccumulator species (i.e. plants with the ability to retain heavy metals in their tissues) (BROOKS et al. 1988). During the 80’s, the Brazilian government financed the first expedition in the country aiming to understand serpentine environments in Goiás State. The Brazilian Serpentine Expedition sampled approximately 1500 plants, belonging to 300 different taxa, and described that the most important belt of ultramafic bodies identified in Brazil dates from Precambrian age (1 to 2 billion years old), extending from the states of Goiás to 1000km northwards Pará, and branching southeast towards Minas Gerais (Brooks et al. 1988). In Minas Gerais State, this belt of ultramafic bodies culminates in the Quadrilátero Ferrífero (Iron quadrangle), with their southernmost extensions coming up to the Morro das Almas (Soul’s Hill) complex, an area with abundant occurrence of serpentinites (VILELA; INDA; ZINN, 2019).

Metal rich environments are among the most threatened and less studied in Minas Gerais State, Brazil (JACOBI; CARMO, 2008). The state is historically known for the exploitation of metals, and also the name refers to it – in English it could be translated as “general mines”, a reference to the historic period where Brazil was exploited by the Portuguese crown to provide raw material. The conversion of forested environments, especially metal-rich ones, in ore mining sites deserves careful attention, because the country’s current discourse is to encourage natural ecosystem conversion to cropland and pastures while denying its impact on biodiversity (FERRANTE; FEARNSIDE, 2019). In

Minas Gerais State, the exploitation of natural areas without proper care on its implications to human lives led to the environmental disasters that occurred with the breaking of the damns in Brumadinho and Mariana municipalities, two ore exploitation sites, where approximately 400 human lives were lost with major impacts for the biodiversity.

During the Brazilian Serpentine Expedition, a series of studies were developed regarding serpentine soils and their associated flora (BROOKS et al., 1988; BROOKS; REEVES; BAKER, 1992; REEVES et al., 2007), however, those studies ceased in the late 90's. Since 2014 new papers regarding serpentine soils appeared (ARAUJO et al., 2014) about a serpentine soil area in Bom Sucesso, Minas Gerais State, which led to a series of investigations in the area (GUIMARAES et al. 2019, VILELA et al., 2020), although none of them addressed information regarding plant-soil interactions. Understanding the mechanistic relations in plant-soil interactions is crucial to address how they are able maintain the biodiversity functions, which are the physiological processes involved (FUJII et al., 2018) and for planning preventive and mitigation actions aiming the conservation of natural areas. The present study aims to fill this gap by gathering information about how the microbial community is structured in a naturally heavy-metal saturated environment and how does it compare to a non-serpentine area. The general objective of our study is to understand the network of interactions between below and aboveground organisms and their role in the permanence of a neotropical plant species in serpentine soils. For that, we selected *Copaifera langsdorffii* Desf., a native and common tree species in the region, as a model organism in order to access information about its functional traits. We then compared the species functional traits, soil microorganism structure and soil physicochemical properties in a serpentine and a non-serpentine area.

We believe that, since *Copaifera langsdorffii* plants were able to persist in serpentine environments, we believe that there might be some modifications in their soil microbial communities and/or in the plant functional traits that allow them to deal with the excess of heavy metals found in the soil. Serpentine plants and microorganisms are being successfully used in phytomining (HIPFINGER et al., 2020), phytoremediation (WÓJCIK et al., 2017) and phytostabilization projects (BOISSON et al., 2018), therefore understanding those environments is a key feature to help us improve the success of restoration and management projects in areas modified by human activities such as mining and industrial rejects.

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SECOND PART – MANUSCRIPT

Article: Uncoupled responses of plant functional traits and soil microbes to serpentine soils lead to *Bonsai effect* in the Neotropics.

(Preliminary version prepared for submission to the Journal Plant and Soil)

ABSTRACT

Purpose: We investigated the interactions between soil microbes and plants in a neotropical serpentine soil area. We hypothesized: A) the interactions between soil microbes-plants will be negatively affected by the presence of heavy metals in the soils; B) higher C:N ratio in serpentine soil areas; C) total PLFAs and total fungi will be lower in serpentine areas; D) there will be higher amounts of total gram positive bacteria and lower gram negative bacteria in serpentine soils and E) functional traits will have a tendency to dwarfism in serpentine areas, while the functional traits leaf thickness, xylem and phloem area will exhibit a tendency to xeromorphism.

Methods: We explored the relations between soil microbes (by using PLFAs), soil properties, vegetation attributes, leaf nutrients and leaf functional traits.

Results: We found that gram positive bacteria interact with iron in the leaves, while there was no other significant interaction between the remaining soil microbes and soil properties, functional traits and leaf nutrients; the C:N ratios are higher in serpentine 1 than serpentine 2, but the two areas are similar to the non-serpentine area; there was no difference between the soil microbes in our study areas and finally; there's a tendency to dwarfism and xemorphism in the functional traits of *C. Langsdorffii* in serpentine soils.

Conclusions: We found that even though we have differences between serpentine and non-serpentine sites, the only soil microbe that seems to be interacting with the heavy metals is the gram positive bacteria, possibly due to chelating mechanisms.

Keywords: Serpentine soils. Ultramafic flora. Heavy metals. Plant-soil interactions.

1 INTRODUCTION

Serpentine soils can be defined as parts of the Earth's mantle that poured out the crust forming ultramafic rocks (BROOKS et al., 1988) that, when decomposed, lead to soils with at least 70% of iron-magnesium compounds (SALIHAJ; BANI; ECHEVARRIA, 2016) with high concentrations of heavy metals such as zinc, nickel and chrome. Such peculiar features can be found in less than 1% of the world (VITHANAGE et al., 2015), and the majority of them are distributed in the Circum-Pacific area and Mediterranean Sea (HSEU et al., 2018). Those soils are known for its low water retention capacity, imbalance of essential nutrients such as Ca and P, and nutrient deficiency, making them hostile to many plant species (ANACKER, 2014). Combined, those environmental characteristics constitute a strong selective filter, leading to a global pattern of features that have been defined as "serpentine syndrome" such as 1) low plant productivity, 2) high levels of endemism and 3) vegetation with structure and composition different from the neighbouring area (i.e. lower plant diversity and biomass) (WHITAKKER, 1954). Also, serpentine outcrops holds approximately 75% of the hypperaccumulating species in the world (BAKER et al., 2000), constituting "islands" of specialized and unique vegetation adapted to extreme edaphic conditions (CHIARUCCI; BAKER, 2007). Recently, serpentine soils are gaining attention due to recently found similarities with Martian regolith's soil (KANELLOPOULOS, 2020), suggesting that they could be used as soil analogues to gather information about Mars (CANNON et al., 2019).

The high concentration of heavy metals in serpentine soils has dramatic consequences for soil biota like inhibited microbial growth (OIJAGBE et al., 2019), lower enzyme activity, reduction of soil microbial diversity, slower decomposition and respiration rates (GALL; BOYD; RAJAKARUNA, 2015). Dealing with metal stress has a great energy cost, therefore the total amount of soil microbial biomass decreases when soil organisms need to live in serpentine soils (GILLER; WITTER; MCGRATH, 2009). In sites with higher heavy metal concentrations, microorganisms sensitivity tends to be different: bacteria are usually more resistant to the presence of heavy metals (STEFANOWICZ et al., 2020) due to their ability to promote changes in their cell walls structure (i.e. enlargement of the cell walls), by producing extracellular polysaccharides and by binding or precipitating metals inside or outside their cells (PACWA-PŁOCINICZAK et al., 2018). Fungi, for its part, deal with heavy metals by using mechanisms like biosorption to cell wall and pigments, sequestration, intercellular compartmentalization and crystallization (TORRES-CRUZ et al., 2018). Some fungi are able to release organic acids in soil to solubilize heavy metals (GALL; BOYD; RAJAKARUNA, 2015), and many are capable to translocate Fe (iron) from mineral to organic horizons, therefore promoting its degradation (LLADÓ; LÓPEZ-MONDÉJAR; BALDRIAN, 2018). Specific studies with microorganisms from serpentine sites indicate that bacterial populations tend dominate over fungi (PAL et al., 2005; SENEVIRATNE et al., 2015), but authors diverge about it (STEFANOWICZ et al., 2020; XU et al., 2019). Evidence tend to indicate that some strains of bacteria (e.g. *Bacillus* and *Pseudomonas* sp.) have specific genetic mechanisms to deal with excess of heavy metals (JACOB et al., 2018), being more likely to survive in metal contaminated sites (GALL; BOYD; RAJAKARUNA, 2015; XU et al., 2019), while fungi populations tend to decrease (XU et al., 2019). The overall patter for microorganisms in the presence of heavy metals varies greatly (CHU, 2018), with both fungi (MAYNARD et al., 2019) and bacteria biomass tending to decrease (ABDU; ABDULLAHI; ABDULKADIR, 2017; STEFANOWICZ et al., 2020).

In plants, metal uptake has three well described patterns: 1) metals are excluded/restricted from entering the plant 2) there’s a restriction of translocation (through the action of heavy metal transporters like P-type ATPase – HMAs (BAIG et al., 2020)) from the roots to the shoot system and 3) accumulation of the metals in plant tissues (KAMAL et al., 2004). Once inside the plant tissues, heavy metals can weaken the plant defence system and lead to oxidative stress by producing reactive oxygen species (ROS) inside the cells, which act as electron carriers damaging DNA, proteins, lipids and cell membranes (DELANGIZ et al., 2020). Heavy metals can unchain cascade effects such as decreased elongation in roots, inhibited plant growth, inhibition and down regulation of hormone synthesis and transport, deposits of gums and debris in xylem in order to try to avoid the metals to enter the cells, decreases in leaf size, decreases in lamina thickness, reductions in intercellular spaces, decrease in the density of stomata size and aperture with a consequently decrease in stomata density (RUCINSKA-SOBKOWIAK, 2016). The overall consequence of the excess of heavy metals in serpentine soils is usually a dwarf low biomass flora (BROOKS et al., 1988). Also, plants inhabiting serpentine soils exhibit a myriad of traits such as reduced vein density (KAWAI et al., 2019), disrupted patterns of xylem and phloem cells (SONG et al., 2013) with reduction in their size (ZAHOOR et al., 2018), reduction of leaf size (ALAOUI-SOSSÉ et al., 2004), inhibition of cell expansion (GALL et al., 2015) culminating in reduction of plant growth (JIANG et al., 2019), thicker cuticle in the leaves (MOHAMMADI JAHROMI et al., 2019) and smaller specific leaf area (KAYAMA et al., 2009). Heavy metals can also bioaccumulate when they are not metabolized by plants, and can be found even in pollen and bee’s honey (ATANASSOVA et al., 2016), being potentially harmful for humans. Studying those kinds of ecosystems can be a good proxy for restoration and remediation of metal-contaminated areas, since resisting to heavy metal toxicity is a pre-requisite for organisms inhabiting serpentine sites (BINI; MALECI; WAHSHA, 2016).

Soil microbes interact with plant functional traits by two distinct mechanisms: A) they enhance resource acquisition by accessing nutrients that wouldn't be available to plants and B) by increasing resource acquisition, making the process more efficient, which typically increases plant tolerance to stress (FRIESEN et al., 2011). Further, soil microbes-plant interactions have a great impact in soil primary productivity, where traits associated to fast-growing plants, higher specific leaf area and low leaf dry mass content (i.e. acquisitive traits (POORTER; BONGERS, 2006)) release labile carbon compounds from their tissues (BARDGETT; FREEMAN; OSTLE, 2008), and are commonly linked to bacterial dominated communities (DE VRIES et al., 2012). Plants with slow-growing, higher leaf dry mass content and lower specific leaf area tend to produce recalcitrant carbon compounds, usually associated to higher fungal abundance (Lauber et al. 2008, Orwin et al. 2010). The rule of thumb for soil microbes-plant interactions is assessed in terms of biomass: plants tend to display better fitness proxies (i.e. higher biomass) in the presence of microorganisms (GOH et al., 2013). Soil microbes also have a fundamental contribution to plants life, as they mineralize soil organic matter, making them available for plant growth, as well as binding metals to reduce their toxicity (Camenzid et al. 2018). However, despite a great effort from scientists on the past decades to "illuminate the black box" of soil-plant interactions, many of them remain to be addressed by researchers (BARDGETT; VAN DER PUTTEN, 2014; BENNETT et al., 2019; PHILLIPS et al., 2020). Also, systematic estimations regarding the relations between plant-associated microbes and how they affect plant functional traits still need to be addressed (ESCALAS et al., 2019; FRIESEN et al., 2011). Additionally, we have little data that specifically concerns soil microbial community structure and their relations with plant functional traits in serpentine neotropical areas, which is particularly flagrant in Brazil, a country that holds one of the most biodiverse floras of the globe (GOMES-DA-SILVA; FORZZA, 2020).

In our study we sought to fill a part of this gap by quantifying C and N content, soil microbial community structure (i.e. total fungi, total bacteria, gram positive and gram negative), soil properties, and leaf nutrients and functional traits for the species *Copaifera langsdorffii* Desf. in serpentine soils. We compared those parameters in serpentine and non-serpentine areas to access the effects of heavy metals in the soil-plant interactions. In this sense, we address the following question: how does the relationships between soil properties, soil microbes, leaf nutrients and plant functional traits allows *C. langsdorffii* trees to thrive in the adverse conditions imposed by the presence of serpentine soils (Figure 1). Therefore, we hypothesize that:

A) Soil microbes-plant interactions will be negatively affected by the presence of heavy metals, i.e. soil properties will negatively affect the soil microbial structure, plant functional traits and plant nutrients; soil microbial structure will positively affect the soil properties; and leaf heavy metals will negatively affect plant functional traits.

B) Higher C:N ratios in serpentine areas as a result of a more xeromorphic vegetation (i.e. higher inputs of recalcitrant carbon in soils, derived from thicker leaves and high lignin content plant material).

C) Soil total PLFA's and total fungi will be lower in serpentine areas, since the presence of heavy metals in the soils negatively affect those organisms (STEFANOWICZ et al., 2020).

D) There'll be a higher total of gram positive bacteria and lower total gram negative bacteria species in serpentine areas, once gram positive bacteria are supposed to be more tolerant to heavy metal stress than gram negative ones (DE VRIES; SHADE, 2013).

E) Plant functional traits will exhibit a tendency to dwarfism (such as lower height, circumference at breast height, specific leaf area, cuticle thickness, stomata density and chlorophyll content (RUCINSKA-SOBKOWIAK, 2016) in serpentine soil areas, while leaf thickness, xylem area and phloem area will have higher values in serpentine areas.

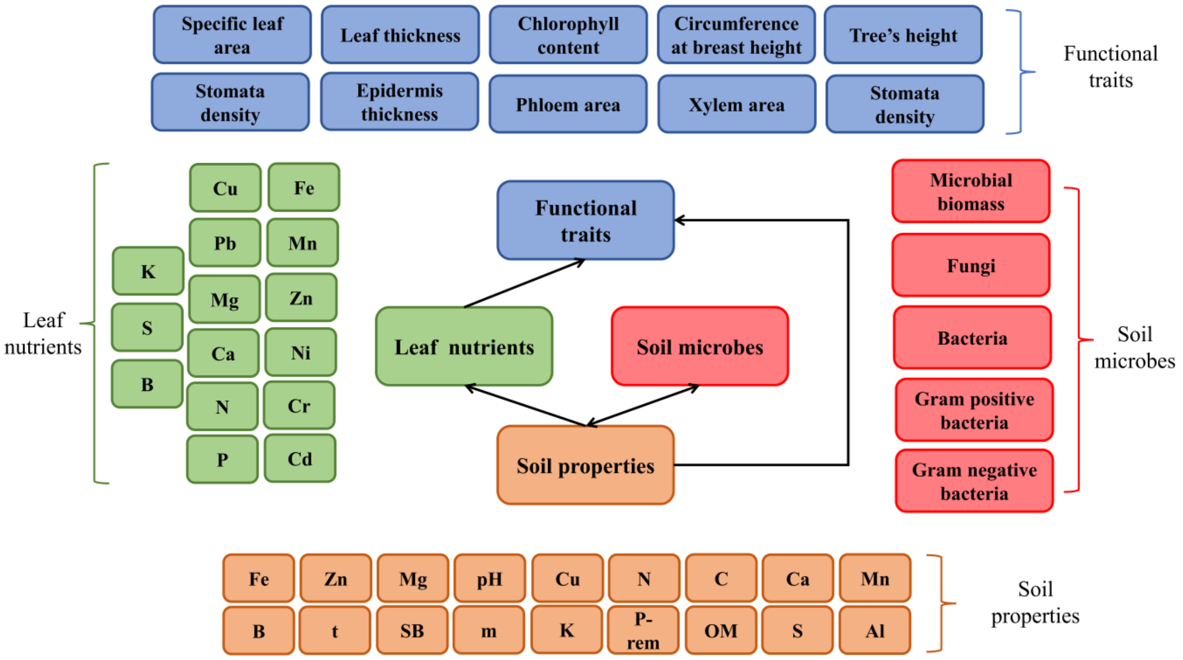

Figure 1 - Conceptual model proposed to access the relationships between soil microbes (red boxes), soil properties (orange boxes), plant functional traits (blue boxes) and leaf nutrients (green boxes) from the model plant *Copaifera langsdorffii* Desf. in serpentine and non-serpentine areas in Minas Gerais State, Brazil. The black arrows indicate the direction of the relationship and double arrows indicate that one variable influences the other in both ways. (From the author, 2021).

2 MATERIAL AND METHODS

2.2 Study areas.

Field work was carried out in three different areas (Figure 2), Morro das Almas (*Souls Hill*, serpentine 1), Serpentine 2 and Parque Ecológico Quedas do Rio Bonito (non-serpentine), located in Minas Gerais State, Brazil. Serpentine 1 and Serpentine 2 are located at Bom Sucesso municipality, under the coordinates 21º01'58"S and 44º45'28"W; 21°06'07.4"S and 44°46'02.4"W, respectively. Both areas are situated in an altitude of 952m above the sea level, and the region is known for being an ecotone between the Atlantic Forest and Cerrado (Brazilian savannah), therefore it is usual to find there tree species belonging to both domains. Serpentine 1 and Serpentine 2 geologic and floristic patterns were under a series of previous investigations (ARAUJO et al., 2014; GUIMARAES et al., 2019; VILELA et al., 2020a; VILELA; INDA; ZINN, 2019) due to the fact that their soils hold a naturally high amount of heavy metal compounds (i.e. Fe_2O_3 on 72,33%), which characterizes serpentine soil (ARAUJO et al., 2014; VILELA et al., 2020b). Although both serpentine 1 and serpentine 2 are located in the same municipality and under the same kind of soil (i.e. serpentine soil), the vegetation structure in those areas is different. Serpentine 1 area (Figure 3) has a rocky shallow soil with a thin layer of litter, as it is located in the convex part of a steep hill with lower water retention capacity, many areas of exposed soil, lots of *Bromeliaceae* sp. and *Bambusa* sp. and sparse vegetation with an open canopy. Serpentine 2 has a deeper soil than serpentine 1, and it is located in a concave part of the hill, which improves the soil water retention capacity, it is covered by a thicker layer of litter, little to no exposed soil, few exposed rocks, lots of vine and a closed canopy. It is important to highlight that, although topography is an important filter that influences not only species distribution but also heavy metal distribution (Rezapour et al. 2014), the stress-dominance hypothesis postulates that stressful environmental conditions are the major driver of community composition, with strong influences in functional traits (Ács et al. 2019). Comparisons between mountain ranges are possible if soil formation factors (parent material, time, vegetation and slope) are kept constant (ZINN et al., 2018). Also, the highest parts of the hills in serpentine 1 and serpentine 2 differ only by 6m. In this sense, since there were some particularities regarding the sampling areas, we sought to decrease bias due to possible topographic influences in plants functional responses by sampling in a gradient from the bottom of the valley to the top of the hill whenever it was possible.

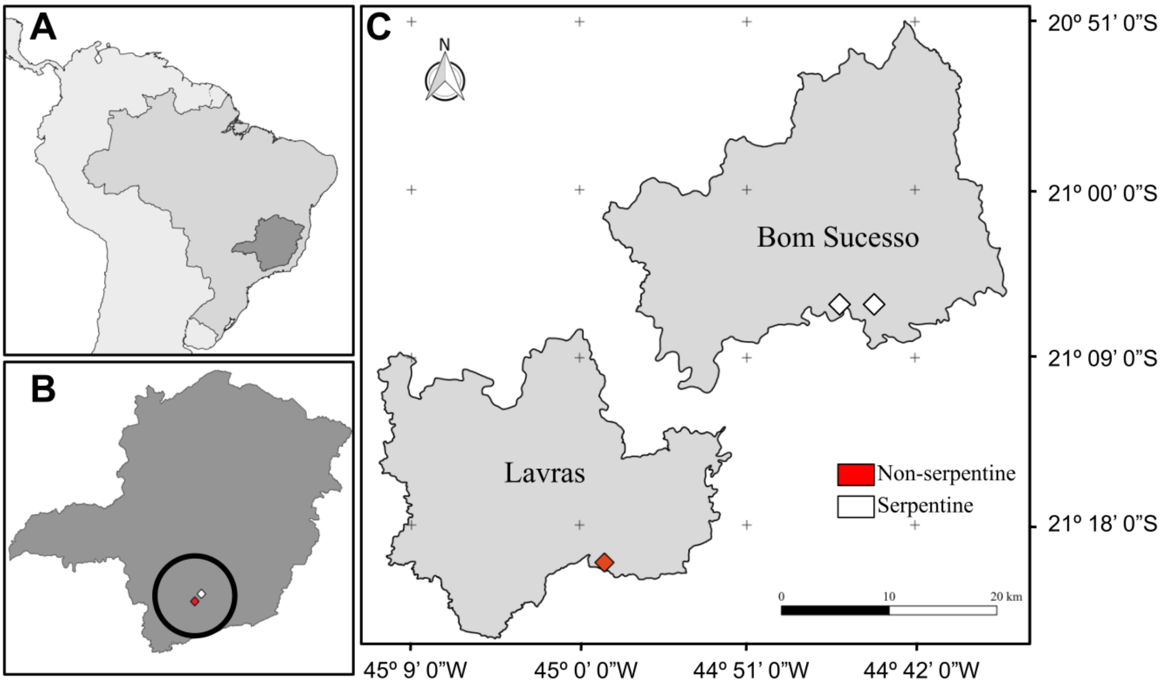

Figure 2 – Map from the study areas. A) South America, Brazil and Minas Gerais State; B) Minas Gerais State and the three sampling areas: Bom Sucesso municipality (in white, Serpentine 1 and serpentine 2) and Parque Ecológico Quedas do Rio Bonito park in Lavras municipality (in red, the non-serpentine area); C) Serpentine 1 and Serpentine 2 areas in the municipality of Bom Successo and the non-serpentine area in Parque Ecológico Quedas do Rio Bonito, in the municipality of Lavras, Minas Gerais State, Brazil. (From the author, 2021).

The non-serpentine sample area is located in the Parque Ecológico Quedas do Rio Bonito, Lavras municipality, 21º19’S and 44º59’W, in an altitude ranging from 950m to 1200m (OLIVEIRA-FILHO; FLUMINHAN-FILHO, 1999). The area belongs to the Atlantic Domain (Oliveira-Filho and Fontes 2000) and the soils in the sampled area can be described as Litolic Neosol (DALANESI; OLIVEIRA-FILHO; FONTES, 2004). The non-serpentine area soil is deeper than serpentine 1 and serpentine 2, with a thick layer of litter, no exposed soil, little to no exposed rocks and closed canopy.

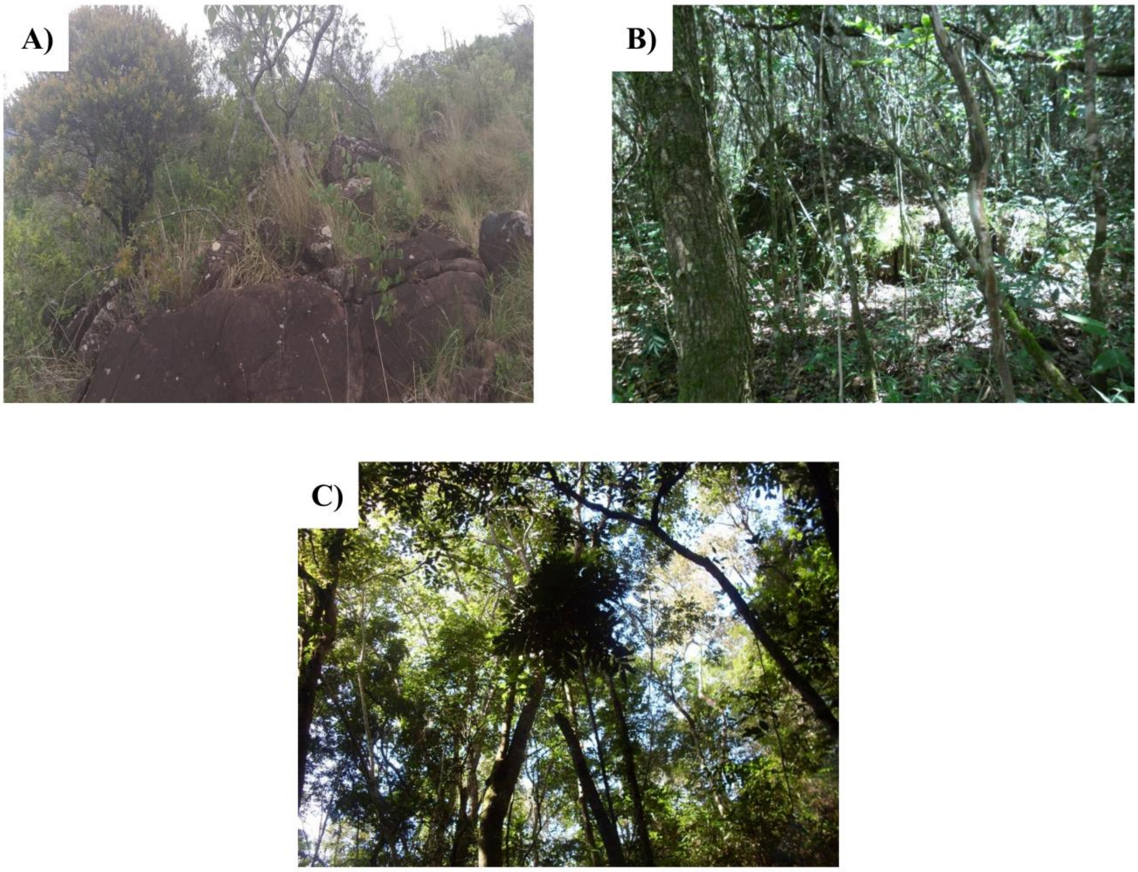

Figure 3 – A) Serpentine 1 with sparse vegetation on shallow, rocky soils; B) Serpentine 2 with closed forest vegetation on deeper soils than Serpentine 1, few rocks; C) Non-serpentine forest vegetation area at Parque Ecológico Quedas do Rio Bonito on deeper soils. The three areas are located at Minas Gerais State, Brazil. Photo A) was taken by Eduardo de Paiva Paula and photos B) and C) were taken by the author. (From the author, 2021).

The three areas are subjected to the same climate, and can be characterized by two distinct seasons: a rainy one that goes from October to March, and a dry one, that goes from April to September, holding a mean annual precipitation of 1776 mm and mean temperature of 19ºC. The three areas are classified as Semideciduous Forest (Oliveira-Filho and Fontes 2000, Guimaraes et al. 2019), and they lose roughly 30% of their leaves during the dry season. All field work was carried in November 2019, during the wet season.

2.3 Data sampling.

Copaifera langsdorffii Desf. is a native tree from the Fabaceae family, widely spread in regions of Brazil (BFG, 2018). *C. langsdorffii* is also known as Copaiba or Pau-d'óleo, usually presents elliptic leaflets with 3-5 pairs of leaves, and a rotund legume (COSTA, 2009) and can reach up to 35m (COSTA et al., 2012). Its oil, extracted from the trunk, has been used by native Brazilian indigenous populations for centuries due to its pharmacological features such as antiseptic, anti-inflammatory and antimicrobial action (CAVALCANTE;

CAVALCANTE; BIESKI, 2017), and its widely used due to its healing properties on wounds (LIMA et al., 2011; TRINDADE; DA SILVA; SETZER, 2018). We chose this species because it is a well distributed and easily found in the forests of Minas Gerais State, being abundant in the non-serpentine soils of the region, but also in the studied serpentine soil. From each of the 30 *C. langsdorffii* trees sampled (10 trees each sampling site) we collected information about functional traits and leaf nutrients.

2.4 Soil sampling.

For each of the 30 *C. langsdorffii* trees sampled, we collected soil samples utilizing a cross design, with the first sample being collected in the centre of the cross (right above the tree), followed by four other soil samples taken 1.5 m from the centre at each cross arm, totalizing five samples that were then mixed together in a single sample. Each soil sample was collected at a depth of 0-10 cm after removing the litter layer. We also estimated visually the percentage the relative soil cover by graminoids, herbs, exposed soil and shrubs in the 1.5m radius of each *C. langsdorffii*.

Soil samples were stored in black plastic bags and taken to the Soil Analyses Laboratory at Universidade Federal de Lavras for the analyses of pH, organic matter (OM content), K, Ca, Mg, P reminiscent, Al, SB, t, V, m, Zn, Fe, Mn, Cu, B and S. Soil pH was evaluated through suspension in water (1: 2.5), and available contents of potassium (K+), phosphorus (P), sodium Na2+, boron (B), Zn (zinc), manganese (Mn2+), iron (Fe2+) and copper (Cu2+) extracted by the Mehlich-1 solution (MEHLICH, 1953); exchangeable calcium (Ca2+), magnesium (Mg2+) and aluminium (Al3+) extracted by 1 mol L -1 KCl (MCLEAN et al., 1958); potential acidity (H+ Al) by SMP extractor (SHOEMAKER; MCLEAN; PRATT, 1961); sum of bases (SB), cation exchange capacity at pH 7.0 (CEC), extracted by Ca 0.5 mol L-1 acetate; and available sulphur (S) extracted by monocalcium phosphate in extracting acetic acid (WALKLEY; BLACK, 1934). Texture (sand, silt and clay contents) were estimated using the Bouyoucos method (BOUYOUCOS, 1951). Base saturation (V%) and Aluminium saturation (m%) were also calculated.

The remainder of the samples were kept in a freezer (-20ºC) and then freeze dried to be transported to the Plant-Soil Laboratory at Lancaster Environment Centre, Lancaster University, for carbon, nitrogen and microbial analyses. We utilized an Elemental Analyzer – Elementar Vario EL III run in C:N mode to measure Carbon (C) and Nitrogen (N). The microbial community structure was investigated using phospholipid fatty acid analysis (PLFA) (Bardgett et al. 1996), using 1.5g of freeze-dried soil and an Agilent 6890 Gas Chromatograph (detector FID and column 60m Agilent RTx-1 capillary column - 60m x

0.32mm ID, 0.25um film thickness). The PLFAs analysis allow us to make quantitative inferences about the microbial community in a given area (WILLERS; JANSEN VAN RENSBURG; CLAASSENS, 2015). We utilized the following biomarkers for fungi: 18:2 ω6,9 and 18: 1ω9 (Deyn et al. 2011); gram positive bacteria: 15:0i, 15:0a, 16:0i, 17:0i, 17:0a (considering ester-linked branched-chain fatty acids) and for gram negative bacteria: 16:1ω7, 7,cy-17:0, 18:1ω7,7,8cy-19:0 (considering cyclopropyl saturated and monosaturated fatty acids) (Rinnan and Bååth 2009). We calculated total PLFA - hereafter used as a proxy for soil microbial biomass and soil microbial structure (FROSTEGARD; BAATH, 1996) - as the sum of all the PLFAs cited previously and the identified biomarkers: 14:0, 15:0, 16:1, 16:1ω5, 16:0,17:1ω8, 7Me-17:0, br17:0, br18:0, 18:1ω5, 18:0, 19:1. We then calculated Fungi:Bacteria and Gram positive: Gram negative bacteria ratios.

2.5 Functional traits and leaf nutrient sampling.

To collect the plant functional traits we sampled 5 leaves from 10 different *C. langsdorffii* trees in each of the three sampling areas, totalizing 150 samples – 50 leaves per sampling area. We measured the following functional traits: leaf area (LA – cm^2), leaf dry-matter content (LDMC – g/g), leaf thickness (LT - mm), chlorophyll content (CLO – SPAD unit), circumference at breast height (CBH – cm) and trees height (m). We used LA and LDMC ratio to calculate the specific leaf area (SLA) values. All the samples were processed in 24h. We used plastic black bags to avoid desiccation and transported the samples to laboratory facilities next to our study area, where they were processed and analysed at Vegetal Anatomy Laboratory, at Universidade Federal de Lavras.

We calculated the leaf area based on images, using ImageJ software. Fresh leaves were weighted in a 0.0001g precision scale (Shimadzu AX200) and after that digitalized using a digital scanner. Latter, the fresh leaves were dried at 70ºC temperature in a greenhouse to determine leaf dry mass content. Chlorophyll content was measured in the field using a field SPAD (Chlorophyll Meter SPAD 502) to avoid its degradation during the transport. Circumference at breast height was measured using a measuring tape, while trees height was estimated using a graduated telescopic stick. Leaf thickness was measured using a field micrometer (Electronic Digital Outside Micrometer) in the field. All protocols used in this study followed the handbook proposed by Pérez-Harguindeguy et al. (2013). To analyze leaf nutrient contents, we grinded the dried leaves in a Willey mill and sent the samples to the Soil Analyses Laboratory at Universidade Federal de Lavras, for leaf nutrients (N, P, K, Ca, Mg, S, Mn, Zn, Cd, Pb, Cr, Ni, B, Cu and Fe) assessment. The nitrogen content was obtained by using sulphuric acid digestion method with posterior distillation and titration. The remaining leaf nutrients were obtained by using the digestion method with nitric acid and perchloric acid (MALAVOLTA, 1997).

To access information about leaf anatomy we sampled leaves from the same 30 *C. langsdorffii* trees that we utilized to measure the functional traits. The leaves were collected in the field and immediately placed in a plastic jar filled with FAA solution (Formaldehyde Alcohol Acetic Acid, 10%:50%:5% + 35% water) to preserve the tissues and then transported to laboratory facilities. We utilized the mean values obtained from three leaves (9 leaflets) to measure the following anatomy parameters: adaxial epidermis, cuticle thickness, stomata density, xylem area and phloem area. The paradermic sections were obtained by dissociation (BERSIER; BOCQUET, 1960) and stained with 1% safranine (KRAUS; ARDUIN, 1997). The leaves were subjected to a dehydration process in an alcohol series (70%, 80%, 90% and 100%) (JOHANSEN, 1940) in order to perform a transversal section. The material was placed in a pre-infiltration solution (ethyl alcohol at 100% and base resin, 1:1) for approximately 24h, following the manufacturer instructions (Leica Historesin Embedding Kit). The fragments were placed in another base resin (100%) for an additional 24h at 4ºC using the Incorporation Historesin kit. The transversal sections were obtained using a semi-automated rotary microtome Yidi YD-335 (Jinhua Yidi Medical Appliance CO., LTD, Zhejiang, China) and stained with toluidine blue 1% (w v−1). The sections were photographed using an optic microscope coupled with a digital camera. All the q anatomical measures were taken utilizing software ImageTool (UTHSCSA ImageTool Version 3.0).

2.6 Data analyses.

To investigate the influence of soil and leaf nutrients in soil microbes and plant functional traits, all non-parametric variables were log adjusted (Zuur et al. 2010) and then standardized in a way that all of them had a variance of 1 and a mean of 0, in order to make comparisons in the same scale. This was implemented using the scale function from the base package in R (TEAM, 2015). We then utilized a principal components analysis (PCA) to reduce the number of soil and leaf nutrients set. We included all variables that appeared in the axis 1, 2 and 3 from this PCA to build a structural equation model (SEM). To avoid multicollinearity, we tested for correlations (Pearson’s correlation) between the variables and build the models in a way that correlated variables would be in different models (WESTON; GORE, 2006), to access the relationships between soil microorganisms, soil properties, functional traits and leaf nutrients. The SEM analysis allowed us to test the relationship between a set of variables and to calculate all the relations between the independent (explanatory) variables and their capacity to explain a determinate phenomenon (response variable) (Grace 2006, Veen et al. 2010, Eisenhauer et al. 2015). Inside the SEM, we also utilized a set of hypothetical generalized linear models (GLMs) with Gaussian family distribution, using only the interactions based in the biological theory to build SEM1. Soil microbes and soil properties were utilized as predictor variables. Leaf functional traits and leaf nutrients were utilized as response, as we verified the theoretical interactions as illustrated in figure 4. The GLM models were later compiled using the function "psem" in the package "piecewiseSEM" in R studio version 4.0.3 (Team 2020). After testing a few hypothetical models, the non-significant variables were excluded and we utilized the Shipley's test of directional separation to test the assumption that all variables are conditionally independent. The conditional independency implies that there aren't missing relations between the variables that are not connected (Shipley 2000). The relations that were not significant, but identified as missing relations, were maintained. We utilized Fisher's C test to test the adjustment of the model, where the models with good adjustment presented a p > 0.05 from the R^2 values from the response variables (Nakagawa and Schielzeth 2013).

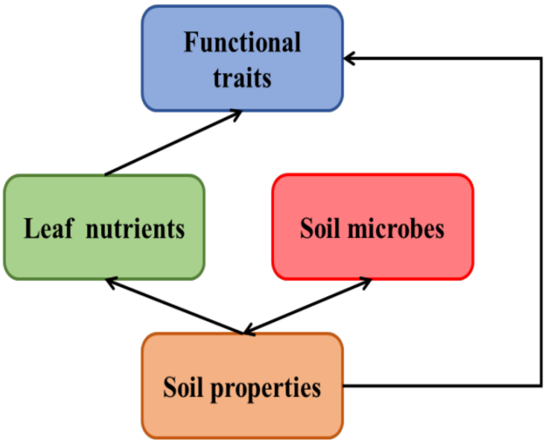

Figure 4: Theoretical model from the connections between soil microbes, soil properties, leaf nutrients and functional traits. The arrows indicate the direction of the interactions. All variables were collected from *Copaifera langsdorffii* Desf. trees in three different soils (serpentine 1, serpentine 2 and a non-serpentine area) in Minas Gerais state, Brazil. The black arrows indicate the direction of the relationship and double arrows indicate that one variable influences the other in both ways. (From the author, 2021)

To investigate how soil carbon and nitrogen, soil microbes and functional traits (hypothesis B, C, D and E) varied between the serpentine soils and the non-serpentine one, we utilized ANOVA tests followed by Tukey for normal distribution data, and Kurskall-Wallis followed by Dunn test with Bonferroni correction for non-parametric data utilizing the "car" package (WEISBERG, 2019) in R studio environment (TEAM, 2015).

3 RESULTS

3.1 Soil-plant interactions.

The parameters selected as the ones with the best explanatory contribution to the separation of the three areas in terms of soil and leaf nutrients using PCA were Mg leaf, Zn leaf, Cd leaf, Ni leaf, B leaf, Cu leaf, Fe leaf, N leaf, Mn leaf, pH soil, K soil, Mg soil, Al soil, Fe soil, N soil and C soil (Figure 5). The PCA showed the separation of the groups regarding differences in soil and leaf nutrients, with a cumulative proportion of explanation of 59.62% (eingenvalues of PC1 9.7861, PC2 of 4.1655 and PC3 of 2.1366) using the three first axis of the analysis.

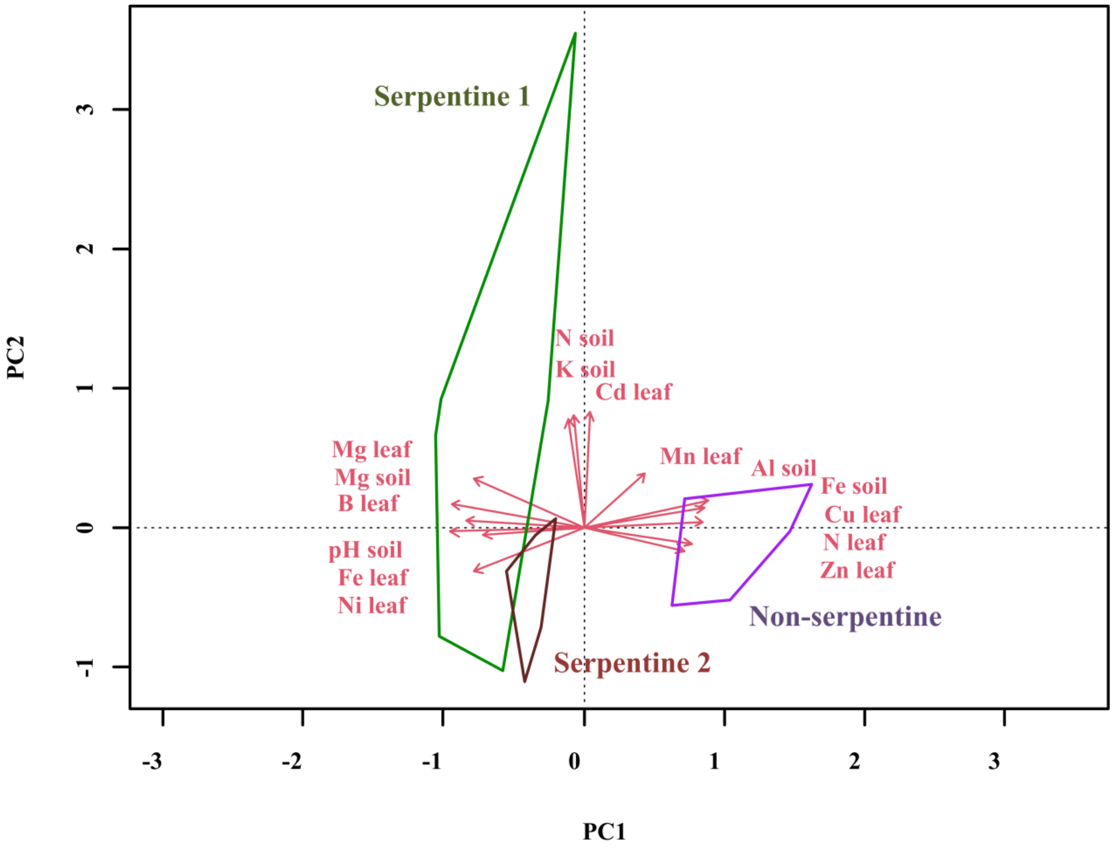

Figure 5 –Principal components analysis (PCA) showing the similarity of the three areas (serpentine 1, serpentine 2 and non-serpentine) in Minas Gerais State, Brazil, based on their soil and leaf nutrients. Polygon represents the sites where *Copaifera langsdorffii* Desf. trees were collected and the straight red lines indicate the influence of each soil or leaf nutrient. Green polygon refers to serpentine 1 area, the brown polygon represent serpentine 2 area and the purple polygon represent the non-serpentine area. (From the author, 2021)

In SEM analysis, a P>0.05 is desirable and means that the theoretical model was supported by our data (LEFCHECK, 2019), and our final model using the structural equation model (figure 6) presented a good adjustment (Fisher's C = 207.056; P-value = 0.248; Df= 194). The variables that did not appear in figure 6 were the ones which the structural equation model did not find any effect (positive or negative). The overall structural equation model showed that the only soil microbe variable that had a relationship with the other variables was the total gram positive bacteria, which influenced positively the Fe leaf. Fe soil, N soil and C soil were the only soil variables with a direct effect in the plant functional traits without passing by the leaf nutrients compartment. The soil properties had a direct influence in the leaf nutrients, and leaf nutrients seem to mediate the soil effects on functional traits. Most of the functional traits (leaf thickness, tree’s height, cuticle thickness, phloem area and specific leaf area, - SLA) were affected mainly by the leaf nutrients Fe leaf, Mn leaf, B leaf, Zn leaf and Mg leaf. Leaf thickness was negatively affected by Fe leaf, while trees height was positively affected by N soil and B leaf, and negatively affected by C soil, Fe leaf and Mn leaf. Cuticle thickness was negatively affected by Fe soil. Specific leaf area was negatively affected by Mg leaf and Phloem area was positively affected by Zn leaf.

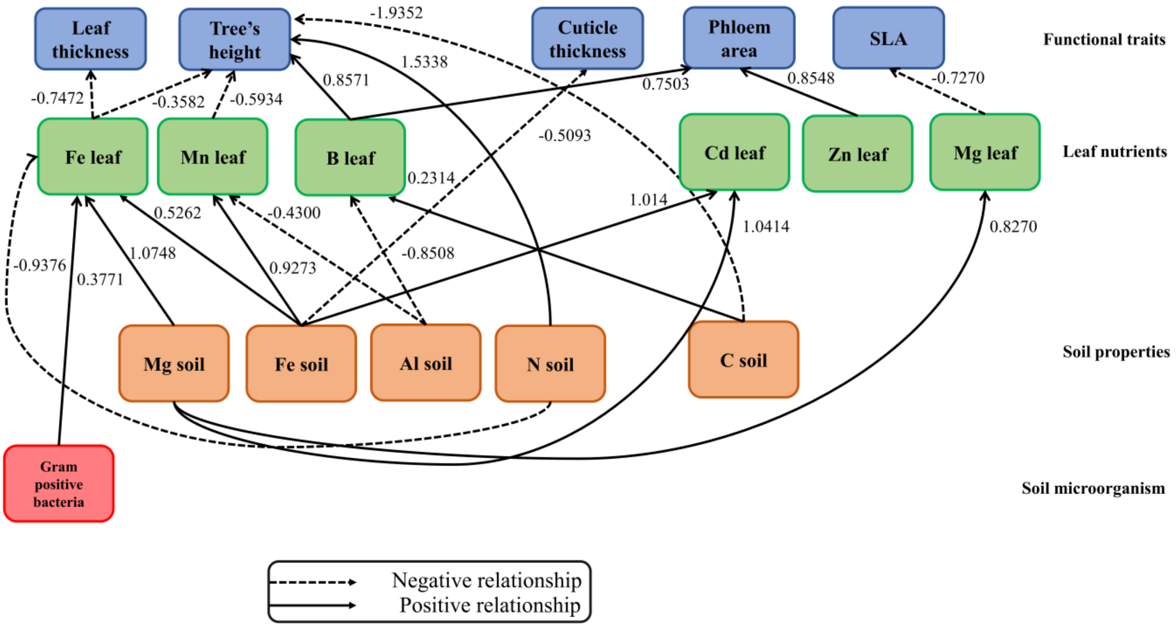

Figure 6 - Structural equation model (SEM) showing the connections between soil properties (orange), leaf nutrients (green), soil microbes (red) and functional traits (blue) with the respective path correlation coefficient values from two serpentine areas and a non-serpentine area in Minas Gerais State, Brazil. (From the author, 2021)

3.2 Soil carbon:nitrogen ratios and vegetation.

We found a significant difference between soil carbon/nitrogen ratios in serpentine 1 and serpentine 2, while the non-serpentine area occupied an intermediary position and was statistically similar to both serpentine sites (One-way ANOVA: p = 0.03458) (Figure 7, Table 1).

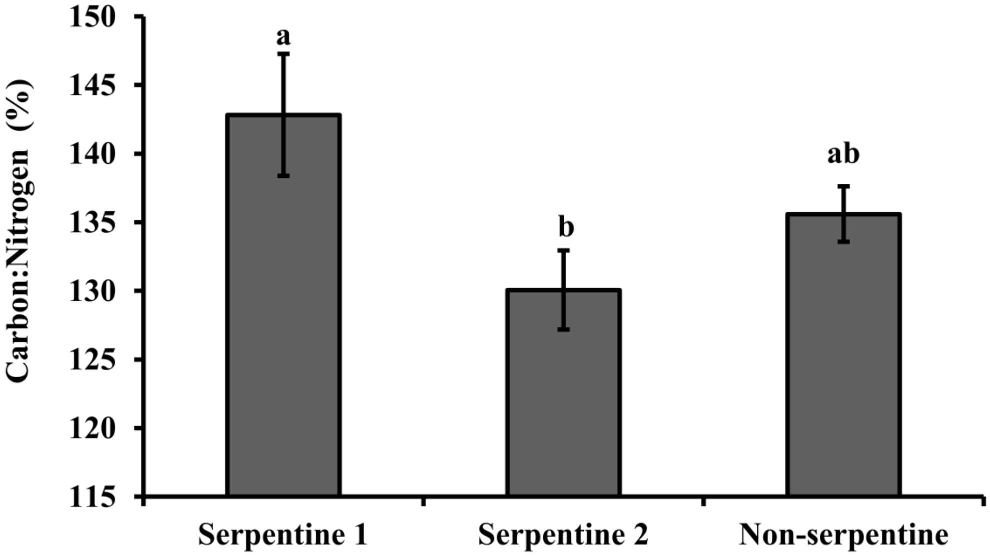

Figure 7 – Comparison between soil carbon/nitrogen ratios for two serpentine sites and a non-serpentine site area from Minas Gerais State, Brazil using one-way ANOVA: p = 0.03458) followed by Tukey test. Same letters above the error bars means no difference between the carbon/nitrogen ratios in the areas analysed, while different letters mean a significant difference between the areas. (From the author, 2021)

We found a significant difference in the percentage of graminoid cover between serpentine 1 and serpentine 2, and intermediate values for the non-serpentine area (Kruskal-Wallis: p = 0.02696) (Table 1). There was no difference between serpentine 1 and serpentine 2 regarding herbs cover, while in the non-serpentine area there was higher cover of herbs Kruskal-Wallis: p = 0.002528). There was no difference in the percentage of exposed soil between the three areas analysed (ANOVA: p = 0.1643). The bushes cover was similar between serpentine 1 and serpentine 2, but both of them were lower than the non-serpentine area (Kruskal-Wallis: p = 4.44E-06).

3.3 Total PLFAs and fungi.

There was no difference in the total PLFAs and total fungal between the three areas analysed (Kruskal-Wallis: p = 0.1054; Kruskal-Wallis: p = 0.2288; ANOVA: p = 0.3101 and ANOVA: p = 0.1997, respectively).

3.4 Total gram positive and gram negative bacteria.

There was no difference between the three areas for the total gram positive bacteria (ANOVA p = 0.1867) and total gram negative bacteria (ANOVA p = 0.231) (Table 1).

3.5 Functional traits.

There was no difference in the chlorophyll content (one-way ANOVA: p = 0.06984), leaf adaxial epidermis (one-way ANOVA: p = 0.0974), stomata density (one-way ANOVA: p

= 0.252), xylem area (Kruskal-Wallis: p = 0.1054) and phloem area (Kruskal-Wallis: p = 0.2288) between the compared areas.

There were differences between the three areas for the following functional traits: leaf thickness, circumference at breast height, tree’s height, specific leaf area and cuticle thickness (Figure 8). For leaf thickness, serpentine 1 had higher values, while there was no difference between serpentine 2 and non-serpentine (Kruskal-Wallis: p = 0.01028). Non-serpentine area had higher values of circumference at breast height, while there was no difference between serpentine 1 and serpentine 2 (Kruskal-Wallis: p = 0.007571). Trees were taller in the non-serpentine area, followed by intermediate height in serpentine 2 and lower height in serpentine 1 (Kruskal-Wallis: p = 0.00001). For the specific leaf area, serpentine 1 had lower values than non-serpentine, while serpentine 2 exhibited similar intermediate values between serpentine 1 and non-serpentine (Kruskal-Wallis: p = 0.01624). Cuticle thickness was higher in serpentine 1 area, while there was no difference between serpentine 2 and non-serpentine (Kruskal-Wallis: p = 0.0004566) (Figure 9).

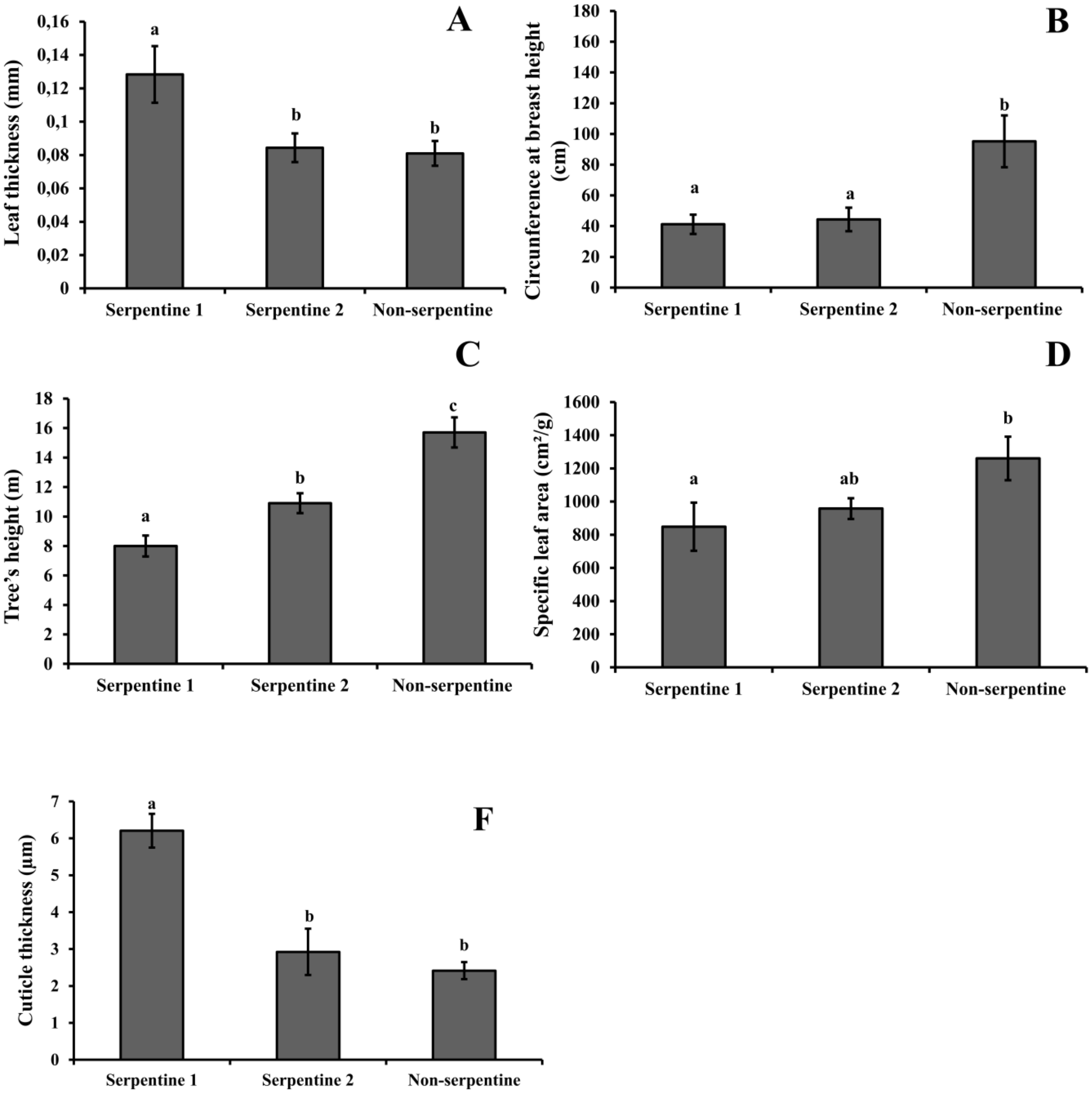

Figure 8 - Comparision between functional traits for the three areas (serpentine 1, serpentine 2 and a non-serpentine area) in Minas Gerais State, Brazil. Same letters above the error bars means no difference between the functional traits in the areas analysed, while different letters mean a significant difference between the areas. A: leaf thickness (Kruskal-Wallis: $p = 0.01028$); B: circumference at breast height (Kruskal-Wallis: $p = 0.007571$), C: Tree's height (Kruskal-Wallis: $p = 0.00001$); D: specific leaf area (Kruskal-Wallis: $p = 0.01624$) and F: cuticle thickness (Kruskal-Wallis: $p = 0.0004566$). (From the author, 2021)

Table 1 – Summary of the mean values and standard deviation (SD) of all soil microbes, soil properties and functional traits in the three studied areas (Serpentine 1, Serpentine 2 and Non-serpentine). Statistically similar groups at the P≤0.05 level are indicated with same lower-case letters, as tested through ANOVA with Tukey's pairwise comparisons and Kruskal-Wallis with Dunn Test pairwise comparisons.

	Measurements	**Serpentine 1**	**Serpentine 2**	**Non-serpentine**	**SD**
Soil microbes	Total bacteria (nmol. g^{-1} dry soil)	19.02 **a**	15.12 **a**	13.44 **a**	7.09
	Total fungi (nmol. g^{-1} dry soil)	1.65 **a**	2.25 **a**	1.39 **a**	1.92
	Total gram negative (nmol. g^{-1} dry soil)	10.88 **a**	8.34 **a**	8.13 **a**	3.96
	Total gram positive (nmol. g^{-1} dry soil)	7.86 **a**	6.27 **a**	5.15 **a**	3.30
	Total PLFAs (nmol. g^{-1} dry soil)	34.31 **a**	27.91 **a**	25.43 **a**	13.20
Soil properties	pH (soil)	5.49 **a**	5.39 **a**	4.25 **b**	0.60
	K (soil) (mg/dm^3)	84.61 **a**	45.29 **b**	62.25 **a**	25.15
	Ca (soil) (cmol/dm^3)	0.71 **a**	0.15 **b**	0.14 **b**	0.33
	Mg (soil) (cmolc/dm^3)	3.90 **a**	1.70 **b**	0.11 **c**	1.74
	H+Al (soil) (cmolc/dm^3)	5.14 **a**	4.33 **a**	12.52 **b**	4.26
	SB (cmolc/dm^3)	4.84 **a**	1.97 **b**	0.42 **c**	2.04
	CEC (cmolc/dm^3)	4.87 **a**	2.08 **b**	2.85 **c**	1.49
	V (%)	48.15 **a**	31.43 **b**	3.52 **c**	19.89
	m (%)	0.66 **a**	5.50 **b**	84.56 **c**	39.26
	Organic matter (soil) (dag/kg)	5.04 **a**	2.86 **b**	3.34 **b**	1.34
	Rem-P (mg/L)	22.88 **a**	22.18 **a**	19.27 **a**	3.87
	Zn (soil) (mg/dm^3)	1.10 **a**	0.97 **a**	0.77 **b**	0.37
	Fe (soil) (mg/dm^3)	33.78 **a**	51.02 **a**	88.38 **b**	27.82
	Mn (soil) (mg/dm^3)	75.07 **a**	46.11 **b**	10.18 **c**	29.98
	Cu (soil) (mg/dm^3)	0.09 **a**	1.16 **b**	0.58 **c**	0.49
	B (soil) (mg/dm^3)	0.04 **a**	0.02 **b**	0.06 **a**	0.05
	S (soil) (mg/dm^3)	3.01 **a**	2.74 **a**	2.86 **a**	1.21
	C:N (soil) (%)	142.82 **a**	130.05 **b**	135.59 **ab**	11.32
	Carbon (soil) (%)	4.13 **a**	2.46 **b**	3.04 **a**	1.04
	Nitrogen (soil) (%)	0.29 **a**	0.19 **b**	0.22 **ab**	0.06

Vegetation attributes	Graminoids (%)	4.20 **a**	22 **b**	24.7 **ab**	24.08
	Herbs (%)	3 **a**	5 **a**	24.5 **b**	14.74
	Exposed soil (%)	1.5 **a**	2 **a**	0.000001 **a**	2.52
	Bushes (%)	0.80 **a**	0.000001 **a**	18.5 **b**	10.61
Functional Traits	Chlorophyll content (spad unit)	4.2 **a**	22 **b**	24.7 **ab**	5.78
	Leaf thickness (mm)	0.12 **a**	0.08 **b**	0.08 **b**	0.04
	Specific leaf area (cm²/g)	848.93 **a**	957.94 **ab**	1260.40 **b**	403.47
	Circunference at breast height (cm)	3 **a**	5 **a**	24.5 **b**	42.65
	Tree's height (m)	3 **a**	10.9 **b**	15.7 **c**	4.08
	Adaxial epidermis thickness (µm)	0.12 **a**	0.08 **b**	0.08 **b**	1.26
	Cuticle thickness (µm)	6.20 **a**	2.92 **b**	2.41 **b**	2.22
	Stomata density (nº of stomata/mm²)	42.2 **a**	37.06	37.06 **a**	7.70
	Xilem area (µm)	29136.13 **a**	31208.69 **a**	21993.94 **a**	9539.12
	Phloem area (µm)	35.07 **a**	40.24667 **a**	40.1 **a**	10535.84
Leaf nutrients	N (g/kg)	19.73 **a**	21.61 **a**	24.22 **b**	2.90
	P (g/kg)	1.38 **a**	1.51 **a**	1.79 **b**	0.29
	K (g/kg)	8.24 **a**	7.08 **a**	10.61 **a**	3.61
	Ca (g/kg)	7.15 **a**	6.15 **a**	5.22 **a**	2.30
	Mg (g/kg)	5.18 **a**	3.9 **a**	2.35 **b**	1.67
	S (g/kg)	1.33 **a**	1.38 **ab**	1.5 **b**	0.15
	Mn (mg/Kg)	529.97 **a**	807.38 **a**	808 **a**	279.30
	Zn (mg/Kg)	24.51 **a**	28.35 **a**	40.11 **b**	10.39
	Cd (mg/Kg)	0.38 **a**	0.14 **a**	0.1 **a**	0.49
	Pb (mg/Kg)	0.96 **a**	0.82 **a**	0.69 **a**	0.36
	Cr (mg/Kg)	4.12 **a**	4.7 **a**	3.29 **a**	2.10
	Ni (mg/Kg)	7.75 **a**	8.93 **a**	3.29 **b**	3.33
	B (mg/Kg)	17.8 **a**	18.02 **a**	3.72 **b**	8.77
	Cu (mg/Kg)	6.51 **a**	8.16 **b**	10.76 **c**	2.45
	Fe (mg/Kg)	226.81 **a**	253.41 **a**	112.38 **b**	94.76

(From the author, 2021).

4 DISCUSSION

Our study allowed us to investigate soil microbes-plant interactions under natural field conditions for the first time in the Brazilian serpentine soils by comparing soil microbes-plant interactions in serpentine and non-serpentine soils. We hypothesized that (A) soil microbes-plant interactions will be negatively affected by the presence of heavy metals, and we found that our results partially support this hypothesis, since there was a direct relationship between soil gram positive bacteria and iron in the leaves, while there was no direct relationship between the other soil microbes and the other variables. Also, the functional traits leaf thickness, tree's height, cuticle thickness, phloem area and specific leaf area were affected directly by Fe soil, Fe leaf, Mn leaf, B leaf, Zn leaf and Mg leaf. Our second hypothesis (B), that C:N ratios would be lower in serpentine areas as a result of the presence of heavy metals, wasn't supported by our data. Our third hypothesis (C), that soil total PLFAs and total fungi biomass would be higher in non-serpentine areas and fourth hypothesis (D) that serpentine areas would have a higher total gram positive bacteria and lower gram negative bacteria were not supported by our findings, since there was no difference between the three areas for the parameters analysed. Our results partially support hypothesis (E) – i.e. plant functional traits would exhibit a tendency to dwarfism and xeromorphism in serpentine soil areas - since there were differences between serpentine and non-serpentine soils for five (leaf thickness, circumference at breast height, height, specific leaf area and cuticle thickness) out of the ten functional traits.

4.1 General patterns.

Our results in SEM partially support our first hypothesis, and they indicate that there wasn't any effect of the soil properties in the soil microbes. The only soil microbe group that had an effect in a leaf nutrient (iron) was the gram positive bacteria. The remaining soil microbes did not mediate the effects of the heavy metals on *C. langsdorffii* functional traits. Regarding the functional traits, we found that they are directly linked to the metal levels in the leaves, and the last ones are affected by the metals in the soils. Mg (magnesium) and Fe (iron), the main components in serpentine soils, are the soil properties that appear in the SEM as affecting most of the leaf nutrients, and as a consequence, the heavy metals in the leaves too. Cuticle thickness was the only functional trait that was directly linked to a soil heavy metal – i.e. Fe soil – being negatively affected by it. N (nitrogen) and C (carbon) in the soil appears in the SEM as the ones affecting directly tree's height. Moreover, some components of the soil (Mg, Fe), besides increasing their respective levels in the leaves, also affect other heavy metal levels in the leaves (Mg soil → Fe leaf, Cd leaf), (Fe soil → Mn leaf, Cd leaf). Lastly, the Al soil affected negatively the Mn leaf and B leaf.

The total gram positive bacteria affected positively the iron (Fe) in the leaves. This interaction could indicate that gram positive bacteria is playing a crucial role in chelating the iron, consequently being absorbed by the *C. langsdorffii* trees and accumulated in the leaves (FRANCIS; HOLSTERS; VEREECKE, 2010). Microorganisms mediate plant functional traits by two distinct routes: 1) they provide new biochemical capabilities (e.g. modifying, increasing and/or avoiding nutrient uptake) and 2) by promoting changes in known plant pathways (e.g. by producing plant hormones to increase/decrease nutrient uptake) (FRIESEN et al., 2011). One of those biochemical capabilities is the interaction with siderophores - low molecular weight molecules with strong iron-specific binding capacity that leads to the chelation of heavy metals (GIONGO et al., 2008) - which, coupled with the gram positive bacteria thick layer of peptidoglycan membrane, makes them more tolerant to the stressful conditions (FANIN et al., 2019) such as the presence of serpentine soils. An additional mechanism of iron regulation is the presence of Fur regulator, a transcription factor found in bacteria that control the expression of specific proteins that balance the efflux and acquisition of this metal throw the cellular membranes, controlling its homeostasis (SANTOS; BATISTA; DA SILVA NETO, 2020). In this sense, bacteria interacts with iron by removing the excess of this metal in soils and thus by solubilizing those compounds, reducing Fe^{3+} to Fe^{2+}, forming stable complexes that decrease their toxicity and increase their bioavailability to plants (Rajkumar et al. 2010), and the positive relationship that we found between gram positive bacteria and iron could be an indicative of that.

Moreover, it has been largely discussed that native microbial populations from long-term exposed soils are likely to be well-adapted to the high concentration of soil heavy metals (GILLER; WITTER; MCGRATH, 2009) and that soil microbes-plant interactions are capable of alleviating the excess of heavy metals in the soils, changing the plant responses in order to maintain their homeostasis (ETESAMI, 2018), which might explain why the soil microbes (except the gram positive bacteria) didn’t show a strong relationship with the other variables. In this sense, our results in SEM indicate that gram positive bacteria are the ones mediating the Fe effects in the leaves, possibly by alleviating the toxic effects caused by the presence of iron.

We also found evidence for two distinct patterns when analysing the effects of soil and leaf nutrients in the functional traits using the SEM analysis. Firstly, we found that Fe leaf and Mn leaf are negatively affecting leaf thickness and tree’s height, while Fe soil is negatively affecting cuticle thickness. Fe and Mn are known for forming iron oxalates, a free radical that is capable to compromise plant cell integrity (BRIAT; LOBRÉAUX, 1997). When in excess in the leaves, Fe also induces the release of abscisic acid (ABA), and coupled with the presence of manganese, can cause modifications in the photosynthesis that lead to the formation of reactive oxygen species (ROS). ROS increases the oxidation potential in the cells, leading to a cascade of reactions that provide damage to large molecules such as proteins, lipids and nucleic acids, with the overall restriction of plant growth (ADAMSKI et al., 2011). Therefore the presence of Fe and Mg in the leaves is capable of harming the photosynthetic activity of plants, finally leading to smaller plants.

Secondly, there's a positive effect of some leaf nutrients (B leaf and Zn leaf) in the functional traits tree's height and phloem area, which seems to be antagonistic to the negative effects promoted by Fe (leaf and soil) and Mn soil. Boron (B) in the leaf might be compensating the adverse effects caused by Fe and Mn, as boron has been pointed to prevent damage and inhibition of growth in plants (GALL et al., 2015). Zinc (Zn) leaf also appears in SEM as affecting positively phloem area, and this heavy metal is a co-factor for enzymes, regulation of growth hormones and is capable of activating antioxidant enzyme activities (AWAN; SHOAIB, 2019). The positive effect of boron and zinc in the functional traits mentioned indicates that those leaf nutrients might be acting as a counterpoint to the negative oxidant effects caused by the presence of the other heavy metals (mainly Fe and Mn).

Lastly, as a result from the SEM we have N (nitrogen) soil influencing positively the tree's height and C (carbon) soil influencing negatively. Tree's height is directly linked to light and energy acquisition, which means that when competing for light resources in a forest, taller trees are the ones holding an advantage. In this sense, Nitrogen acquisition and usage is an important component for plant growth and development, with a strong influence in plant structure and architecture (LUO et al. 2020). Increases in nitrogen are usually related to increases in wood production (VALENTINE; MÄKELÄ, 2012), as nitrogen regulates DNA synthesis, cell division and cell growth, which assures that the plant will reach the expected height (LUO; ZHANG; XU, 2020). Moreover, *C. langsdorffii* trees are capable of forming N-fixing nodules in their roots, increasing the acquisition of nitrogen from the soil (BARBERI et al., 1998), which could help explain the direct link found between tree's height and nitrogen soil in our SEM analysis. Carbon appears in the SEM as negatively affecting the tree's height, which is an unexpected result, as trees usually do not acquire carbon from the soil. We speculate that this finding might be a reflex of the xeromorphic trees with higher C:N ratios found in serpentine 1, and will be discussed in the further sections.

4.2 Soil carbon:nitrogen, functional traits and vegetation.

We hypothesized that B) carbon:nitrogen ratios would be lower in serpentine soils, which was not supported by our findings. Serpentine 1 had the highest percentage of carbon:nitrogen ratios when compared to serpentine 2. Both serpentine 1 and 2 had similar values to the non-serpentine area, which indicates that, although holding high amounts of heavy metals, both serpentine soils organic matter cycling are still happening in at similar ratios. The non-serpentine area also had higher amounts of graminoids and bushes cover, which is an indicative of higher plant productivity (i.e. plant biomass that will be latter transformed in organic matter) in the non-serpentine area. We speculate that the similarities in the C:N ratios between the non-serpentine and serpentine areas could be merely a consequence of the higher inputs of organic matter deposition in the non-serpentine area. Also, in serpentine 1, the vegetation had lower height and circumference at breast height, with higher leaf thickness and cuticle thickness, indicating a tendency to xeromorphism, typically found in serpentine soils (ANACKER, 2014). It is important to highlight that serpentine 1 forest is located on a hill top, with larger areas of exposed soil, large rocks, and lower grass, non-grass and bushes cover (i.e. shallow litter cover) (Table 1), while serpentine 2, despite still being serpentine soil, is located in the bottom of a concave valley, which could favour water retention when compared to serpentine 1. Although we didn’t measure water retention in our study, we speculate that this tendency to xeromorphism could indicate a higher accumulation of lignin (i.e. recalcitrant carbon) (LAMMEL et al., 2015) in *C. langsdorffii* tissues, which reflects higher C:N ratios in serpentine 1. Serpentine 2 and non-serpentine areas, on the other hand, presents higher percentage of grasses, non-grasses and bushes, which typically produces small chain labile C roots exudates (e.g. glucose) (PAUSCH; KUZYAKOV, 2017), therefore reflecting in a lower C:N ratio in the soil.

The patterns for the C:N ratios found in our study are contrary to the scarce literature available regarding soil carbon:nitrogen ratios in serpentine soils. In Turkey, serpentine soils had lower C:N ratios as a consequence of lower microbial activity induced by the presence of heavy metals in those soils (SAGLIKER et al. 2018). Although the amounts of heavy metals in our study are undoubtedly high in serpentine soils, it is possible that the higher C:N ratios in our study are likely a consequence of higher speed of organic matter cycling in tropical regions (GMACH et al., 2020), together with topography, somehow balances the negative effects of high heavy metal content in serpentine soils, counterpointing what was found by SAGLIKER et al. (2018).

4.3 Soil microbial community structure.

We hypothesized (C) that the total PLFAs and total fungal would be higher in non-serpentine areas, and (D) that there would be a higher total gram positive bacteria (more resistant to stress) and lower gram negative bacteria. Our data don't support these hypotheses. Despite the direct effect of the gram positive bacteria in soil Fe using SEM, we found that there was no difference in soil microbes between serpentine and non-serpentine areas using Anova/ Kruskal-Wallis tests (table 1- same total PLFAs, total fungal, total bacteria, total gram positive bacteria and total gram negative bacteria). The lack of differences between those parameters indicates that the three sites (serpentine 1, 2 and non-serpentine) have similar bacterial community structure, which was unexpected. Authors seem to diverge regarding serpentine soil microbes: while in a study conducted in Portugal, they found a weak relationship between serpentine soils and a distinct soil microbial community (FITZSIMONS; MILLER, 2010), Degrood et al. (2005) found lower values of PLFas when comparing serpentine to non-serpentine areas in California, and Xu et al. (2019) found a decrease in the total PLFAs when accessing the effects of heavy metals on soil microbes in Australia. The lack of difference between PLFAs in serpentine and non-serpentine sites in our study might be an indicator of selection for heavy metal tolerance among microbial populations in serpentine sites (ABOU-SHANAB; VAN BERKUM; ANGLE, 2007) and that the pattern in tropical serpentine soils might different from non-tropical soils. The findings of Ortiz et al. (2020), which investigated serpentine soils in Costa Rica, indicated microbial heavy-metal tolerance: when comparing the effects of serpentine soils in *Tabebuia* trees, they found serpentine tolerant bacteria adapted to heavy-metal rich soil conditions, which corroborates to our heavy metal tolerance suggestion. Our study have not carried any molecular analysis to evaluate microbial community composition, therefore, even though we found no difference in the PLFA analysis between systems, these soils might still show differences in genus, species composition and differences in the abundance between certain microbial groups. We speculate that the bacterial community in our study site might present molecular mechanisms of adaptation, similar to those found by Abou-Shanab et al. (2007), or possibly metalloregulatory proteins (i.e. proteins that regulates the presence of metals in the cells) (CHANDRANGSU; RENSING; HELMANN, 2017) that facilitate them to persist in such hard environmental conditions, but such possibilities can only be investigated accessing the microbial composition.

The lack of differences in microbial community structure between the two serpentine sites and the non-serpentine site could indicate an adaptation from the soil microbes towards heavy metal tolerance, which deserves closer attention. Considering that our findings are contrary to the existing literature regarding microbial community structure in serpentine soils, which found lower amounts of soil microbes (measured using phospholipid fatty acids technique) (DEGROOD; CLAASSEN; SCOW, 2005; XU et al., 2019), we suggest that more studies investigating soil microbes and heavy metal toxicity in serpentine soils should be conducted in natural field conditions - focusing particularly in determining the microbial functional diversity, investigation of specific metal resistant genotypes and DNA analysis - in order to better understand the mechanisms involved in serpentine soil microbes toleration to heavy metals in tropical areas.

4.4 “*Bonsai effect*” and functional traits.

Regarding the differences in the functional traits between the areas analysed, we found three distinct patterns: serpentine 1 has a clear tendency to xeromorphism, reflected in higher leaf thickness and cuticle thickness, and lower tree’s height and specific leaf area. Interestingly, serpentine 2 functional traits are generally occupying an intermediate position (when comparing the areas using ANOVA/Kruskall-Wallis) between serpentine 1 and the non-serpentine area, with functional traits that are sometimes similar to serpentine 1, similar to the non-serpentine and sometimes different from both of them. The non-serpentine area is on the other end of the extremes, with the presence of taller trees. Since serpentine 2 is located in the bottom of a valley, the topography of this site might be contributing to the accumulation of litter and a better water retention than serpentine 1. Some of the adverse effects caused by the presence of serpentine soils seems to be somehow, alleviated in serpentine 2. Although those differences between serpentine 1 and serpentine 2 topography might be influencing the functional traits analyzed, they are not enough to completely compensate the effects caused by the presence of serpentine soils, as some of those traits are still similar to the serpentine 1 area (e.g. circumference at breast height and specific leaf area), showing a clear tendency to xeromorphism and dwarfism, which is completely different from the functional traits in the non-serpentine area.

The presences of Fe and Mn, the main micronutrients in excess in serpentine soils, can damage the photosynthetic activity of plants through the formation of iron oxalates, a free radical that is capable to compromise plant cell integrity (Briat and Lobréaux 1997, Santos et al. 2017). When in excess, iron induces the release of abscisic acid (ABA) that, coupled with the presence of manganese, form reactive oxygen species (ROS), which are capable to provide damage to large molecules such as proteins, lipids and nucleic acids, leading to a restriction of growth (Adamski et al. 2011). The presence of serpentine soils in our study caused a "*bonsai effect*", leading to miniaturized *C. langsdorffii* trees with different degrees of growth restrictions in their functional traits (reduced circumference at breast height, trees height and specific leaf area), showing a tendency to dwarfism according to the results of the ANOVA/Kruskall-Wallis tests.

When comparing serpentine 1 to non-serpentine, and serpentine 2 to non-serpentine, trees had respectively 50% and 30% smaller heights in serpentine areas, ~ 55% lower circumference at breast height in serpentine areas and leaves had ~28% smaller specific leaf area in serpentine soils. Similar morphological differences were also found by Ortiz et al. 2020, which are linked to geological differences, when comparing the effects of different soil types (including serpentine soils) in *Tabebuia heterophylla* (DC.) Britton in a study conducted in Costa Rica. Reduction of growth was also found in Japan for larch seedlings in serpentine sites when compared to non-serpentine ones (KAYAMA et al., 2009). Reduced growth and xeromorphism were the only patterns found in our study that are similar to those described in serpentine areas outside the tropics.

There's still lack experimental evidence designed to access the interactions between soil properties in tropical soils, (Camenzid et al. 2018), particularly the combined effects of heavy metals and microbial community and their consequences to serpentine neotropical flora. In this sense, we suggest that other aspects of the soil microbiome should be investigated (e.g. litter nutrient and cycling as potentially alleviating the adverse effects caused by the excess of heavy metals (Stefanowicz et al. 2020) in serpentine soils in order to access specific processes regarding heavy metal uptake, aiming to determine if they are mediated by a subset of specific resistant organisms and if those processes reflect the response of the entire serpentine soil microbial community. We also recommend that such studies should be carried in other serpentine tropical areas to evaluate if this pattern is constant in the tropics. This information could be provided by using the combined results of microbial RNA, such as 16s analysis and enzyme activity to access if there are particular species of soil microbes and/or enzymes that are responsible for heavy metal uptake.

5 CONCLUSION

Our study allowed us to understand, for the first time in the neotropics, how soil microbes and *C. langsdorffii* plants interact in natural field conditions in serpentine and non-serpentine soils. We demonstrated that even though we have differences in parent material between serpentine and non-serpentine sites, the only soil microbe that seems to be interacting with the heavy metals is the gram positive bacteria, possibly due to chelating mechanisms and/or by the double lipid bilayer that confers adaptability to higher heavy metal concentrations in the soils. The other soil microbes (total gram negative and total fungi) were not directly affected by the presence of the serpentine soils in terms of microbial biomass and did not mediate any relationship with the plant traits. Even though we could find evidence for the adverse effects of soil Fe and Mn in *C. langsdorffii* functional traits, with a clear tendency to xemorphism and dwarfism, those adverse effects aren't enough to prevent those species to inhabit serpentine soils. We suggest that other species from tropical serpentine soils should be closely examined to determine if there's a pattern regarding soil microbes-plant interactions that allow tropical trees to thrive in serpentine soils.

Our study helped us advance the surveys made by Guimaraes et al. (2019) regarding the serpentine flora of *Morro das Almas*, which recommended that a closer investigation regarding the plant species should be conducted in order to understand the soil-plant adaptations that led to a high species diversity in that area, a pattern that is contrary to what has been described in the literature for non-tropical serpentine soils. It is also important to highlight that currently in Brazil there's no specific legislation protecting serpentine areas, and that they are actually target areas for mining and ore exploration, which increases the need for conservation and protection of those areas. Our study also has a potential for phytostabilization and phytoremediation projects, since plants from serpentine soils are being successfully used in projects aiming the recovery of degraded areas (BINI; MALECI; WAHSHA, 2016; BOISSON et al., 2018; MIZUNO et al., 2018).

Although we didn't access the contribution of specific microbial groups (e.g. actinomycetes, arbuscular mycorrhizal fungi, and others (WILLERS; JANSEN VAN RENSBURG; CLAASSENS, 2015)), nor did we access how those groups of microbes are distributed in serpentine and non-serpentine areas, as well as the contribution of those groups for the soil microbial structure, we believe that this is an important information that could be incorporated in future studies. This kind of information could be accessed by using specific microbial biomarkers in the PLFA technique, and such information can help researchers to access the relative importance of specific microbial groups, along with providing environmental context (FRIESEN et al., 2011).

Understanding the mechanisms involved in microbes permanence in naturally stressed environments are important in developing strategies for the mitigation and remediation of polluted sites (TURNER et al., 2020). The same approach we utilized in our study could be used in different context to verify if our results can be found in other serpentine soils in the neotropics. Future studies in serpentine soils should investigate whether: A) it is a pattern for neotropical serpentine soil microbes to show similar microbial composition when compared to non-serpentine areas and B) if other mechanisms such as metal uptake by the roots, histidine chelation of the heavy metals (KRÄMER et al., 1996), enzyme activity and differential microbial species might be influencing the permanence of such plants in the Soul’s Hill. A more pervasive way to access those mechanisms would be conducting additional experiments measuring plant functional traits under the inoculation of different strains of microorganism from serpentine soils and access their impacts in plants, to determine which of those impacts are provided by specific serpentine microbes (FRIESEN et al., 2011). Future studies should explore the combined results of microbial DNA (to access if there are particular species of microbes responsible for heavy metal uptake/adaptability), root anatomy and enzyme activity to advance in the knowledge of the mechanisms involved in the permanence of tree species in neotropical serpentine soils.

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