University of São Paulo
“Luiz de Queiroz” College of Agriculture

Effects of soil coverage upon the structuring and functioning of soil
microbiome

Luana Bresciani

Dissertation presented to obtain the degree of Master in
Science. Area: Soil and Plant Nutrition

Piracicaba
2021

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versão revisada de acordo com a resolução CoPGr 6018 de 2011

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Dedictory

I dedicate this work to my family (César, Luciana, Amanda, and Guilherme), and to all who believe in science, especially those who work for designing more sustainable and fair agriculture.

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Epigraph

“Everything is theoretically impossible until it is done.”

Robert A. Heinlein

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RESUMO

Efeitos da cobertura do solo na estruturação e no funcionamento do microbioma do solo

O aumento exponencial da população mundial aumentou a demanda por recursos energéticos e alimentares. Com isso, práticas alternativas são necessárias a fim de aumentar a produção agrícola e mitigar o uso de recursos naturais cada vez mais escassos. É necessário promover a qualidade do solo para uma produção mais sustentável, onde sua cobertura permanente, seja por resíduos ou culturas de cobertura, se torna fundamental. A cobertura do solo atua como uma barreira física à erosão, e aumenta a matéria orgânica do solo, melhorando a agregação de partículas de solo e a disponibilidade nutricional. No entanto, pouco se sabe sobre a influência da cobertura do solo na comunidade microbiana. Neste trabalho, estudamos o efeito da cobertura do solo nas comunidades microbianas nas plantações de café e cana-de-açúcar. O café foi avaliado comparando-se os sistemas convencional e consorciado, enquanto a cana-de-açúcar foi estudada em quatro níveis de palha mantida na superfície do solo [remoção total (TR), alta remoção (HR), baixa remoção (LR) e nenhuma remoção (NR)]. Avaliamos a comunidade bacteriana de 0 a 15 cm, dividida em quatro camadas no café, e de 0 a 20 cm, em cinco camadas na cultura da cana-de-açúcar. Neste medimos as atividades das enzimas β -glucosidase e fosfatase ácida, a abundância de genes funcionais de fixação biológica de nitrogênio e solubilização de fósforo (*nifH* e *phoD*, respectivamente) e a comunidade microbiana por sequenciamento de *16S rRNA*. Além disso, realizamos análise de co-ocorrência de rede e calculamos os parâmetros topológicos para avaliar as relações e as interações microbianas. Nossos resultados propõem que a cobertura do solo fornece um ambiente mais estável para o desenvolvimento, funcionamento e estruturação das comunidades microbianas. Estes indicam que as comunidades microbianas interagem com a cobertura do solo, e seus resultados podem aumentar os benefícios microbianos para o desempenho da cultura. As comunidades bacterianas observadas em ambos os sistemas sob cobertura de solo podem proteger a planta da invasão de patógenos e beneficiá-la pelo fornecimento nutricional e uso eficiente dos recursos do solo. No geral, este estudo contribuiu para entender melhor as comunidades microbianas em solos agrícolas, o que é essencial para o futuro design de melhores práticas para um sistema agrícola mais sustentável.

Palavras-chave: Café, Cana-de-açúcar, Ecologia microbiana

ABSTRACT

Effects of soil coverage upon the structuring and functioning of soil microbiome

The exponential increase of world population has raised the demand for energy and food resources. In order to achieve it, alternative practices are required to improve agricultural production using fewer natural resources. It is essential, among other factors, to achieve a better soil quality for sustainable production, which turns fundamental to keep soil permanently covered by crop residues or cover crops. Soil coverage acts as a physical barrier to soil losses and increases soil organic matter, enhancing the aggregate structure and nutritional availability. However, little is known about soil coverage's influence on the microbial community. Here, we studied the effect of soil coverage on bacterial communities in coffee and sugarcane plantations. Coffee was evaluated comparing conventional and intercropping systems, while sugarcane was studied in four levels of straw maintained in soil surface [total remove (TR), high remove (HR), low remove (LR), and no remove (NR)]. We assessed bacterial community from 0 – 15 cm, divided into four layers in coffee, and 0 – 20 cm, along with five layers in sugarcane crop. We evaluated β -glucosidase and acid-phosphatase activities, biological nitrogen fixation and phosphorus solubilization functional genes abundance (*nifH* and *phoD*, respectively), and bacterial community by *16S rRNA* sequencing. Further, we performed network co-occurrence analysis and topological parameters to evaluate bacterial assembly and interactions. Our results propose that soil coverage provides a more stable environment for bacterial communities' development, functioning, and structuring. Our findings indicate that bacterial communities interplay with soil coverage, and its outcomes may boost bacterial benefits for crop performance. The resulting communities observed in both systems under soil coverage can protect the plant from pathogen invasion and benefit the plant by nutritional supply and efficient use of soil resources. Overall, this study contributed to better understand bacterial communities in agricultural soils, which is essential to future design better practices for a more sustainable agricultural system.

Keywords: Coffee, Sugarcane, Bacterial ecology

1. SOIL COVERAGE AS A BASIS FOR SUSTAINABLE AGRICULTURE

1.1 Origen and motivation of sustainable agriculture

The debate about improving agricultural sustainability and food production has been ruled date back to the 1950s and 1960s for supplying the rising world population (Pretty, 2008). At this period, agriculture was going through boost technological development, known as the Green Revolution (Evenson and Gollin, 2003; Khan, Zaidi, and Wani, 2007). Such change in crop management included the use of modern or high-yield crop varieties and intense external input (i.e. chemical fertilizers, pesticides, and antibiotics) (Kropff, Bouma, and Jones, 2001; Evenson and Gollin, 2003), that was rapidly adopted by farmers mainly in tropical and subtropical regions (Evenson and Gollin, 2003). Such innovations led to a substantial increase in the food supply since the 1960s with only a modest increase in cultivated areas. After changes, food production increased about 50% per capita until nowadays, while the global population has risen 2.5 times (Pretty et al., 2018). Thus, the Green Revolution is considered as one of the most successful human activities, that transformed food production and helped several countries to deal with their food supply deficiencies.

The Green Revolution introduced crop practices that changed the agricultural scenario worldwide. The access to modern crop varieties and external input, have made monoculture feasible and profitable (Jacques and Jacques, 2012; Pingali, 2012). Hence, this new agricultural system rapidly replaced agroecological and natural systems into industrial agriculture. The high-yield crop varieties are depended on high nutritional availability to reach their top performance (Evenson and Gollin, 2003), therefore requiring a large use of synthetic fertilizers, mainly composed of soluble salts or single elements (i.e. ammonia, nitrite, and triple superphosphate) (Zhang et al., 2018). Industrial agriculture also faces the increasing susceptibility of crops to diseases and pests, mainly as a result of monocropping (Horrigan, Lawrence, and Walker, 2002). In summary, these observations describe conventional agriculture as a hardly dependent system from external supplies, synthetic herbicides, pesticides, and N and P fertilizers, to ensure high crop yield.

At the same time, the Green Revolution has increased crop productivity and saved native area conversion to agriculture, there were evidenced several negative effects on environmental services (Horrigan, Lawrence, and Walker, 2002; Siebrecht, 2020). Conventional agriculture coupled with high and intensive use of harmful pesticides, overuse of soil fertilization, and heavy machinery has been jeopardized natural resources (Pellegrini and Fernández, 2018; Siebrecht, 2020). As a result, it became apparent air and water pollution, soil depletion and erosion, and biodiversity loss (Horrigan, Lawrence and Walker, 2002). Besides the entire conventional production chain is dependent on fossil fuel, resulting also in increasing greenhouse gas emissions (Horrigan, Lawrence and Walker, 2002; R. Harding and P. Peduzzi, 2012; Mahon et al., 2017). Due to these natural harmful and the excessive necessity of damaging inputs to yield success, conventional agriculture started been described as an unsustainable system (Dahlberg, 1991; Horrigan, Lawrence and Walker, 2002). Therefore, several environmental debates have aimed to develop alternative practices to enhance crop management sustainability while continue improving crop production (Mitchell et al., 2019; Siebrecht, 2020).

Sustainable agriculture discussion has been widely boosted since Earth Summit, the first global United Nations Conference on Environment and Development in 1992 (Siebrecht, 2020). Earth Summit Conference concerned that future agriculture systems would face heavy demands for food, fiber, and energy while increasing natural resource constraints (Mitchell et al., 2019). The Conference Report, Agenda 21, described emerging

environmental problems associated with Green Revolution implemented techniques, such as intensive synthetic fertilization and pesticide inputs (United Nations Conference on Environment & Development, 1992). It was important for promoting many governmental food safety regulations including restricting the indiscriminate use of agrochemicals (Tilman et al., 2002). At Agenda 21 chapter fourteen, FAO, endorsed by United Nations members, articulated the basis of sustainable agriculture to mitigate environmental health issues. The chapter elucidated 12 elements for improving crop sustainability, such as integrated pest management, land conservation, crop diversification, and sustainable plant nutrition (United Nations Conference on Environment & Development, 1992). Although FAO was not able to precisely prescribe which activity should be adopted by each farm, it was important to enhance field sustainable practices and guide international research studies.

1.2 Sustainable intensification and conservation agriculture

Global demand for food production is expected to increase about 70% in the next three decades with even more constrained and scarce natural sources (FAO, 2009; Tilman et al., 2011). Thus, the term ‘sustainable intensification’ has become popular as a proposal to supply 9.5 to 11 billion people's demand for agricultural products without degrading or exhausting the natural environment (Cassman, 2017). Sustainable intensification involves social and economic aspects of the agricultural system and aims to improve crop yield by precise management, improving soil structure and quality (Cassman, 2017; Mitchell et al., 2019). However, these changes require high expenditures in innovation and often technological tools for accessing driving variables, identifying issues, and best recommend the practices for a particular situation in the field (Cassman, 2017). Due to this, while increasing benefits for the environment, sustainable practices must show results in higher yields.

Several numbers of alternatives have been proposed to address sustainable intensification and increasing profits (Hobbs, Sayre, and Gupta, 2008). FAO of the United Nations has described the adoption of conservation agriculture (CA) as one of the most efficient sustainable approaches to achieve global production demanded in the future (Mitchell et al., 2019). Conservation agriculture is based on three principles, minimal mechanical soil disturbance, permanent soil organic cover, and diversification of cropping species (FAO, 2017) and it is widely applicable in the majority of world agricultural areas (Kassam et al., 2009; Theodor Friedrich, 2012). Based on the cover or straw crop residues released on soil, CA is a progressive approach to sustain manifold ecological functions and further improving biodiversity (Mitchell et al., 2019). Besides, CA is a carbon (C) based system that may mitigate climate change while enhancing soil C stock (Hobbs, Sayre, and Gupta, 2008).

Currently, CA has been worldily practiced on more than 130 million ha, and its scope daily increase, mainly in North, South America, Australia, and New Zealand (Theodor Friedrich, 2012; Shrestha, Timsina and Kandel, 2020). South America is responsible for the biggest area under CA, where was primarily adopted in face of producing in areas with large soil erosion rates (Junior RC, de Araujo AG, 2012; Shrestha, Timsina and Kandel, 2020). As an illustration CA boost expanded in Brazil with soybean, maize, and other crops in Cerrado, at the beginning of the 1980s (Mitchell et al., 2019). The replacement aimed to reduce soil degradation and massive soil erosion resulted from the intensive use of disc plows to prepare the soil for planting (Mitchell et al., 2019). The diminution of soil erosion besides preventing soil loss and promoting nutrient use efficiency, decreased water pollution, silting of rivers and water reservoirs (Horriggan, Lawrence, and Walker, 2002; Mitchell et al., 2019). CA has extended for more than 25 million ha in Brazil, enhancing active and unproductive areas, considered the third-largest in extension in the world, next to the USA and Argentina (Friedrich, Derpsch and Kassam, 2017).

CA represents a sustainable and profitable practice to attend the 21st-century call-in production system. However, its adoption requires a change and commitment of all stakeholders involved and interested. Sustainable policies can provide incentives to farmers to transform their productions and continue improving over time. In summary, CA benefits can be highlighted in six main reasons: (1) better crop economy by less use of machinery, fuel, and time; (2) flexible operations for planting, fertilization, and weed control; (3) higher and more stable yield (long term effect); (4) soil protection against erosion; (5) better nutritional and (6) water use efficiency (Friedrich, Derpsch and Kassam, 2017). Off-farm CA provides several environmental services in C-sequestration, water quality, and enhance biodiversity. Overall, CA offers multidisciplinary benefits as sustainable intensification practice, not only requiring fewer natural resources for high yield but also reducing climate change effect, water pollution, and enhancing above and belowground biodiversity for a healthier environment.

1.3 Conservation agriculture, ecosystem services, and soil health

Soil health is an essential concept to ensure soil ability to continue providing ecosystem services (Karlen DL., 2012; Amundson et al., 2015; CDFA. California Department of Food and Agriculture, 2018). However, intensive use of external input and continuous tillage in conventional agriculture have hindered the maintenance of soil health (Foley et al., 2011; Amundson et al., 2015). This comes with soil C loss, soil structure depletion, and unstable soil functionality including nutritional dynamics (Cassman, 1999; Karlen DL., 2012). On the other hand, the three principles of conservation agriculture (i.e., minimum soil disturbance, the permanent soil covering, and diversified cropping species) have focused on enhancing and protecting soil from degradation, loss, and resource exhaustion (Mitchell et al., 2019). CA approaches consider soil as a living system in which C is widely required. Consequently, the no-tillage, stability, and C-diversified input provided by CA practices culminate in impacting on physical, chemical, and biological properties of soil.

1.3.1 Soil carbon sequestration and climate change mitigation

Soils are the largest organic C source storing roughly three times more C compared to the atmosphere (Sanderman, Hengl, and Fiske, 2017). Natural ecosystem conversion to land use easily reduces soil C stock by up to 50% over 50 years in temperate climates and up to 70% over 10 to 25 years in tropical climates (Lal, 2016). This comprehends about 8% of global C emitted to the atmosphere per year (Le Quéré et al., 2015; Potma Gonçalves et al., 2018). Soil disturbance by continuous tillage, widely practiced in conventional agriculture, has been described as the principal cause of soil organic C (SOC) loss (Lal, 2004). With multiple benefits, the decrease of SOC negatively affects soil quality and climate change (Sun et al., 2020). However, soil C sequestration and consequent storage are a result of land use and field practices. Under good management, agricultural fields have the potential to maintain and sequester C, significantly reducing CO₂ and CH₄ in the atmosphere (Corsi et al., 2012; Palm et al., 2014; Minasny et al., 2017).

Even though CA practices were not initially developed to enhance soil C stock, it is now considered as an alternative to sink C from the atmosphere (Palm et al., 2014). Briefly, soil C is a balance of C input and C decomposition, fully affected by several abiotic and biological factors (Giller et al., 2009; Govaerts et al., 2009; Powlson et al., 2011). The three principles of CA show benefits to increase soil C stock (Palm et al., 2014). Crop

rotation, species diversification, and soil permanent coverage increase C input in the soil system. Diversifying crops provide different root patterns and root depth, increasing further above and belowground biomass (West and Post, 2002; Palm et al., 2014). On the other hand, minimum soil disturbance, the main practice that affects soil C content, is negatively correlated to mineralization rate (Reicosky, 1997; Kabiri, Raiesi, and Ghazavi, 2016; Valkama et al., 2020). Soil disturbance (i.e., tillage or soil inversion) destroys soil structure exposing C to microbial oxidation and further release to the atmosphere (Reicosky, 1997; Teixeira et al., 2013; Reicosky, Sauer and Hatfield, 2015; Kabiri, Raiesi and Ghazavi, 2016). Together, it is expected that crop diversification, residue retention, and minimum disturbance provide high C input in soil with less decomposition, and finally a positive C budget.

However, the three principles of CA are commonly studied separately, although it is important to recognize they interact. CA provides several modifications of soil properties and functions that together deliver multiple ecosystem services (Palm et al., 2014). Soil C content is primarily determined by climate, soil type, and nutritional and water availability (Palm et al., 2014). According to Li et al., 2018 metadata analysis, CA practices have the potential to affect soil microclimate while protecting soil surface with crop residues. CA practices also improve water retention, and nutritional availability, and efficient use (Mitchell et al., 2019). These complex and integrated processes have made it difficult to determine clear patterns of C stock, mainly with the current independent studies. However, it is known that CA practices contribute to soil quality and food security, such as maintaining or improving soil C storage as crucial to help climate change adaption as well as their mitigation.

1.4 Conservation agriculture, soil quality, and water and nutrients provision

The soil quality concept is generally referenced to soil capacity to sustain plant and animal productivity, maintain environmental quality, and provide plant and animal health (Doran and Parkin, 1994). This definition shows the complexity of the soil system based on environmental quality (Bünemann et al., 2018). Soil quality addressed to the agricultural environment includes soil properties and functions that interfere in supporting plant growth and crop productivity, protecting watersheds by efficient water use, infiltration and evaporation, and preventing water and air pollution (i.e. chemical fertilizers, pesticides, and organic waste) (Karlen et al., 1997; Bünemann et al., 2018). Thereby, soil quality indicators in the field are related to physical, chemical, and biological properties of soil fairly directly relevant to optimize soil conditions to plant performance (Bünemann et al., 2018). Examples of these go from soil macroporosity, aggregation, water movement to biological activity.

Although soil quality is partially determined by fixed properties (i.e. soil texture and mineralogy), it can be modified by soil organic matter (SOM) content and biological activity, both related to the management system (Palm et al., 2007; Verhulst et al., 2010). CA practices from residue maintenance, crop diversification, and minimum soil disturbance have been described as contributing to good soil structural distribution, water balance, and stability (Verhulst et al., 2010; Palm et al., 2014). The increase of organic matter input by crop retention in the soil surface, or cover crop, further influences soil nutritional availability, cation exchange capacity, and further pH (Verhulst et al., 2010). Thereby, it is followed summarized below the effect of CA practices in soil water and nutrient availability through the changes in soil physical and chemical properties.

1.4.1 Soil physical changes under conservation agriculture: soil structure, water use efficiency, and soil erosion

Soil structure controls several soil functions, moderating water retention and infiltration, gas exchange, C sequestration, and erosion susceptibility (Rabot et al., 2018). Such a concept refers to the size, shapes, and arrangements of soil particles and pores (Lal, 1991; Bronick and Lal, 2005). It defines soil capacity of retaining or transmitting organic and inorganic substances, besides supporting vigorous root growth and plant development (Lal, 1991; Bronick and Lal, 2005). Soil structure is often described using soil aggregation stability degree as an indicator, considering soil's ability to continue intact after exposed to disturbances (Verhulst et al., 2010). Soil aggregation is the result of soil particles, flocculation, and cementation rearrangement (Duiker and Lal, 1999). Soil aggregation development is mediated by SOC and biota that perform an essential role as a binding agent and decomposition controller (Bronick and Lal, 2005). Thereby, SOC decomposition rate and remaining time in soil, directly influence soil aggregation and effectiveness.

Favorable soil structure and high aggregation stability are very related to land use and crop practices (Bronick and Lal, 2005). In this sense, continuous tillage is the principal factor affecting soil structure in agricultural systems (Lal, 2004). Intense tillage destroys soil structure by breaking aggregates and releasing SOC for the atmosphere (Reicosky, Sauer, and Hatfield, 2015). This aggressive and continuous practice reduce soil porosity and directly impact water efficiency use (Kladivko, 2001; Doral Kemper, Schneider, and Sinclair, 2011; Mitchell et al., 2019). On the other hand, the three principles of the CA act as reducing soil disturbs while increasing SOC input. SOC is the key factor for enhancing soil structure (Verhulst et al., 2010). Its continuous retention covering soil promotes aggregates rearrangement for improving and maintaining a more stable environment. The increase of SOC in the near-surface also improves soil hydraulic properties (Blanco-Canqui et al., 2011). CA practices are expected to enhance the rates of soil water infiltration, runoff, availability, erosion, and others soil functions.

Understanding that C and water cycles are interdependent is important to design crop practices to enhance water use efficiency by plants. Briefly, water is a component of the photosynthetic equation, making it essential for C fixation and biochemical energy production (Mitchell et al., 2019). In turn, C management can optimize water use efficiency for plants absorbing nutrients, translocating photosynthates, and providing evaporation cooling (Mitchell et al., 2019). CA practices contribute to water use efficiency, first, increasing water infiltration in soil. Soil pores' quantity, distribution, and arrangement determine soil's capacity of water retention, infiltration rate, and permeability (Mitchell et al., 2019). CA management from minimum tillage and large input of organic C enhance soil structure and stability providing macro pores for water infiltration as well as for root development (Kladivko, 2001; Doral Kemper, Schneider, and Sinclair, 2011). CA practice also provides a substantial reduction of soil water loss by evaporation. Crop residues avoid water evaporation keeping the soil surface cooler once it is protected from the sun (Turmel et al., 2015; Mitchell et al., 2019).

Topsoil residues and the improvement of soil structure act as reducing water runoff, and consequently, soil erosion (Turmel et al., 2015). As described before, CA practices were mostly introduced to feasible crop production in areas with massive erosion rates (Junior RC, de Araujo AG, 2012; Shrestha, Timsina, and Kandel, 2020). Minimum disturbance and permanent crop residue protect soil from structure stresses and decrease its susceptibility to erosion by raindrop impact and wind by 90% or more (Steiner, 1989; Mitchell et al., 2019). Less erosion reduces agricultural chemical offsite movement, increasing their efficiency (Mitchell et al., 2019). CA practices have been decreased the best topsoil wash into rivers and deposited in the sea. Reduced sedimentation has

multiple benefits for the environment and human beings. It includes better fish habitats and higher dams' longevity, besides mitigating soil degradation.

CA practices may result in more plant-available water and more sustainable crop production (Verhulst et al., 2010; Mitchell et al., 2019). The improvement in soil structure by enhancing SOC promotes the bio-pore network in the soil, which increases water infiltration and storage (Hudson, 1994; Rawls et al., 2003; Murphy, 2014; Basche, 2017; Mitchell et al., 2019). Retention of crop residues and constant protection of soil from the sun also promotes an increase in bio-pore network reducing water evaporation and runoff (Hudson, 1994; Rawls et al., 2003; Basche, 2017). All of these “transform soil into sponges” and lead water use efficiency for plants (Basche, 2017). In resume, CA practices enhance soil physical properties for crop production through three main mechanisms: (1) adding SOM to the soil surface and enhancing soil aggregation and stability; (2) decreasing soil disturbance and structure break; (3) protecting soil from raindrop impact, sun and wind. Consequently, CA practices play important role in soil protection, water quality, and water use efficiency, thus, provide optimized and sustainable agriculture production.

1.5 Soil chemical changes under conservation agriculture: soil organic matter, nutrient availability, soil pH, and cation exchange capacity

SOM is the principal modulator of soil chemical propriety, essential for soil functions and plant growth supply (Liu et al., 2006; Murphy, 2014). Together with the improvement in soil structure, SOM acts enhancing SOC stock and nutritional reservoir (Liu et al., 2006; Verhulst et al., 2010). SOC is considered a key indicator of soil quality because of its impact on soil aggregation and nutrient efficient use (Turmel et al., 2015). SOC and nutrient availability are dependent on SOM content and chemistry, which can be divided into labile and humus pools (Turmel et al., 2015). Labile SOM (i.e., dissolved organic C, particulate organic C, easily oxidizable C, and microbial biomass) is easily decomposed by microorganisms, while the humus pool is highly resistant to decomposition allowing soil C storage (Blair, Lefroy, and Lisle, 1995; Ghani, Dexter, and Perrott, 2003; R J Haynes, 2005; Turmel et al., 2015). The decomposition process, mainly from labile fraction, is responsible for SOM's influence in soil aggregation and nutrient supplying to soil living organisms (R. J. Haynes, 2005; Murphy, 2014). Soil labile organic matter plays important role in soil functions, although it is really sensitive to soil disturbances and crop management (R. J. Haynes, 2005).

CA practices of continuous input of SOM in soil system and minimum soil stresses imply increasing or maintaining C retention in the soil profile (Liebig, Archer, and Tanaka, 2014; Turmel et al., 2015). These may contribute to soil cation exchange capacity (CEC), buffering against changes of soil pH, and nutrient availability (Murphy, 2014). CEC is a soil fertility indicator, described as the soil capacity to hold cation for exchange with soil solution (Turmel et al., 2015). CEC is regulated by soil clay or minerals and humus in soil organic matter, which can be dependent on soil pH (Murphy, 2014). The residue maintenance in soil under CA management results in increasing SOM and a range of compounds such as humic and fluvic acids, and aminoacids and phenolic compounds (Stevenson, 1994; Murphy, 2014). When soil pH reaches values around 7, these compounds form negatively charged sites, which are available for cation exchange, increasing soil cation hold capacity (Murphy, 2014). However, this increase in soil CEC may be limited in the soil surface, and it is unclear if this change occurs under the first five centimeters of soil (Duiker and Beegle, 2006; B. Govaerts et al., 2007; Turmel et al., 2015). Crop continuous retention in the soil can affect pH considering residue content of nitrogen and concentration of organic anions (Tang

and Yu, 1999; Butterly et al., 2011). Processes of mineralization of nitrogen and decomposition of organic anions consume or release H^+ anions to soil solution, and the balance of these reactions determines the final soil pH (Xu and Coventry, 2003).

SOM is central to nutrient cycling and is the principal store of nutrients for plants in most soils. Its content associated with microorganism activity ensures that organic material is mineralized, and nutrient released in an available form for plants (Murphy, 2014). CA management providing higher content of SOM in soil surface contributes to enhancing nutrient source, its use efficiency, and prevent rapid leaching through soil surface (Verhulst et al., 2010; Mitchell et al., 2019). Nutritional supply from SOM is related to its composition, decomposition rate, and soil storage capacity (Murphy, 2014; Turmel et al., 2015). Usually, SOM composition is described using C: N: P: S ratio content (Murphy, 2014; Turmel et al., 2015). To illustrate, legume residues with a low C: N ratio can stimulate soil N mineralization, whereas cereal residues with a high C: N ratio can immobilize soil N while the decomposition process (Aulakh et al., 1991; Govaerts et al., 2006). Several studies have described an increase of topsoil P content with residue retention (Verhulst et al., 2010). It can be attributed to humic and low weight molecules that can minimize Al-oxide site adsorption of P (Haynes and Mokolobate, 2001). However, these effects are dependent on residues composition and lability that together with soil structure, define soil fertility and nutritional supply for plant growth.

1.6 Conservation agriculture, system environment, and soil biology

Soil organisms respond directly to environmental conditions and different crop managements that imply beneficial or deleterious changes in biological properties (Gupta, Roper, and Thompson, 2020). Soil biodiversity is generally divided into two groups: microbiota and soil fauna (i.e., macro-, meso fauna) classified based on their size (Kladivko, 2001; Turmel et al., 2015). Macrofauna is organisms larger than 2 mm (i.e., earthworms, termites, and large arthropods) and, apparently, especially sensitive to soil disturbances (Gallandt, 2005; Verhulst et al., 2010). On the other hand, mesofauna is an average between 0.2 – 2 mm size (i.e., microarthropods and small Oligochaeta). Microbiota is smaller organisms (i.g. bacteria, fungi) that can live in micropores and water-filled space (Verhulst et al., 2010). CA changes in tillage, crop diversity, and soil coverage can offer several benefits, leading to changes in soil fauna composition and their metabolic capacities (Beauregard et al., 2010; Wu et al., 2016; Liu et al., 2020).

SOC is the key factor affecting soil biological properties which in turn, plays influencing SOC dynamics and their impact on the soil system (Busari et al., 2015). Soil fauna also responds to soil structure and chemistry, which are affected by SOM and SOC content. CA practices have facilitated soil aggregates development and maintenance, where organic matter is protected from losing (Six, Elliott and Paustian, 2000). Bacteria produce organo-mineral products during SOM decomposition which are associated with soil particles and enhance micro aggregates stability (Tisdall, 1994). Further, the mycelium of arbuscular mycorrhiza fungi directly impacts soil aggregation, entrapping soil particles together by the hyphal network (Tisdall, 1994; Peng, Guo and Liu, 2013; Zheng, Morris and Rillig, 2014). Fungi mycelium also exudate glomalin, a hydrophobic protein that may act as an adhesive agent, clustering soil particles and contributing to soil aggregates hydrophobicity (Chenu, 1989; Wright and Upadhyaya, 1996; Rashid et al., 2016). On the other hand, Larger organisms, such as earthworms and termites, play forming bio pores diversifying pore size distribution and soil aggregation (White and Kirkegaard, 2010a, 2010b; Bottinelli et al., 2015; Rillig et al., 2015). Thus, SOM supply for microorganisms indirectly improves their beneficial role in soil aggregation.

Constant soil coverage protects the soil surface from water loss, erosion, and extreme temperature (Ward et al., 2013). Cooler soil temperature, good soil structure, and high input of SOM soil enhance the nutritional supply and provides a stable environment for soil living organisms. Soil ecosystem engineers, particularly earthworms and termites, are usually found in higher abundance and diversity under CA practices and play important role in nutritional cycling (Verhulst et al., 2010; Nieminen et al., 2011; Palm et al., 2014). They break down the litter and introduce organic materials into the soil enhancing soil microbes' contact and, thus, stimulating the mineralization process (Lavelle, 1997; Bossuyt, Six and Hendrix, 2006). SOM mineralization releases nutrients to soil solution while enhancing soil fertility for flora and fauna supply. CA practices of increasing environment stability are essential to provide biodiversity and deliver environmental services and nutrient cycling. Crop residues covering soil result in a slower decomposition rate and high nutrient efficiency in agricultural systems (Palm et al., 2014).

1.6.1 Microbial biomass, abundance, community composition

Soil microbial biomass (SMB), composed mainly of bacteria and fungi, plays a key role in soil aggregation process and nutrient cycling, enhancing soil quality and health (Mäder et al., 2002; Palm et al., 2014; Zuber and Villamil, 2016; Zhang et al., 2017). SMB represents part of the labile pool of SOM, not only as a nutrient recycler agent but also as a sink and source of nutrients for plants (Mäder et al., 2002; Kallenbach and Grandy, 2011; Zhang et al., 2017). SMB defines the ability of soil to store and cycle nutrients (e.g. C, N, P, and S) and organic matter (Dick, 1992; Carter et al., 1999; Verhulst et al., 2010; Oldfield, Wood and Bradford, 2018). Its structure and activity affect SOM dynamics and active fractions (Acosta-Martínez et al., 2003; Liu et al., 2014). SOC inputted from plant biomass is generally considered the principal factor regulating SMB, acting as an energy source in soil systems (Franzluebbers et al., 1999; Verhulst et al., 2010). Thus, SMB quickly responds to environmental factors, such as temperature, and moisture change, as well as crop management practices (Debosz, Rasmussen and Pedersen, 1999; B Govaerts et al., 2007). This supports its use as a soil quality indicator, detecting early changes in soil C stability (Kallenbach and Grandy, 2011).

Soil constant coverage and residue retention practices are described as important factors stimulating SMB and activity (Turmel et al., 2015). Practices of no-tillage and soil permanent coverage act reducing soil temperature and moisture fluctuation and increasing soil SOC sequestration (Balota et al., 2003; Helgason, Walley and Germida, 2010a, 2010b; Sapkota et al., 2012; Liu et al., 2016). CA practices provide a nutrient-rich and stable environment for microbial growth and proliferation (Carter, 1992; Salinas-García et al., 2002; Brussaard, 2013; Palm et al., 2014). However, such practices change microbial community structure and functionality, which can select specific groups based on their colonization ability or competencies (Gallandt, 2005; Verhulst et al., 2010; Singh, Pandey and Singh, 2011; Yang, Siddique, and Liu, 2020). Liu et al., 2020 suggested that no-till and crop residues maintenance favor soil-specific groups recruitment increasing soil substrates availability and bacterial diversity. Considering the copiotroph-oligotroph concept, several studies describe the increase of Proteobacteria phylum, a fast-growing copiotroph, correlated to the increase of nutrient availability in no-till and crop retention systems (Fierer, Bradford and Jackson, 2007; Jenkins et al., 2010; Liu et al., 2020). On the other hand, Acidobacteria, an oligotroph phylum that slowly grows in a nutrient-rich environment, have been shown to decrease under CA (Jenkins et al., 2010; Kielak et al., 2016; Liu et al., 2020). These results illustrate how environmental conditions can change the microbial composition.

Considering agricultural systems, some specific microbial groups are particularly interested to enhance soil system and plant performance. Fungi, especially arbuscular mycorrhizal fungi, play an important function in nutrient

acquisition and drought resistance for plants (Oehl et al., 2005; Turmel et al., 2015). Under the CA system, these groups are highly stimulated by minimum soil disturbances (Verhulst et al., 2010). Continuous tillage destroys the fungi mycorrhizal hyphae network and may transport them to the soil surface, reducing or deleting their beneficial effects for current and next crops (Verhulst et al., 2010). On the other hand, considering soil-born diseases, CA can facilitate different pest species that use residual crops as substrates for growth (Verhulst et al., 2010). Such diseases include necrotrophic leaf and stem, and inflorescence-attacking pathogens (Verhulst et al., 2010). However, Govaerts et al., 2006 suggested that long-term CA practices provide greater conditions to the natural development of antagonists and predators. Then after a period of practice, this study described that CA improvement of soil structure, water retention, and nutrient supply gave higher crop productivity compared to the conventional system, even with root diseases (Govaerts et al., 2006; Verhulst et al., 2010). However, even though several studies have described the positive effect of CA practices in beneficial microorganisms for crop production, it is depended on location geography, soil profile, and previous practices. Thereby it is necessary more studies to better describe how CA practices impact soil microorganisms' structure and abundance.

1.6.2 Microbial activity, processes, and nutritional dynamics

As described previously, biological activity and metabolites are important for improving soil quality. Besides, crop management implies changes in microbial activity and functionality, that directly affect how microorganism's performance in the soil system. Cover crop and residues retention are described as improving soil microbial activity due to the higher organic matter supply and water available (Doran, 1980; Zuber et al., 2015; Zuber and Villamil, 2016; Somenahally et al., 2018). CA system impact in soil chemistry and physic forming several highly heterogenous environments in terms of oxygen, food, and water content, that result in hot spots of microbial activity (Beare et al., 1995; Gupta, Roper and Thompson, 2020). On the other hand, intense tillage and agricultural practices negatively affect microbial activity, continuously destroying soil stability and living sources supply (Dilly et al., 2003; Balota et al., 2004; Roldán et al., 2005; Madejón et al., 2007; Melero et al., 2011; Álvaro-Fuentes et al., 2013; Willekens et al., 2014). Soil activity is measured using soil enzyme quantification as a soil quality indicator (Sparling, 1997; Nannipieri, Kandeler, and Ruggiero, 2002). Soil enzymes are known to play a key role in catalyzing soil reactions and actively regulate soil organic matter decomposition and nutrient cycling (Dick, 1994; Gallandt, 2005; Verhulst et al., 2010; Tabatabai, 2018).

Soil management also affects soil microbial diversity and functionality, which are all signs of soil health (Wang and McSorley, 2005; Liu et al., 2020). Several studies describe higher microbial richness, evenness, and functional diversity under a no-till system compared to conventional tillage (Verhulst et al., 2010; Liu et al., 2020). Govaerts et al., 2007 added that even with zero tillage, without the retention of crop residues in the soil surface, the functional diversity decreases. Crop rotation also provides diversified organic material and exudates release in soil, selecting divergent microbial communities (Verhulst et al., 2010). These mean CA practices may promote microbial diversity and maintain soil function stability and health. A high soil functionality and diversity buffer microbial community against declining vital processes, proving guarantees that important functions will maintain occurring even in stressed periods (Allison and Martiny, 2008; Li et al., 2020). Cropland practices form different microhabitat in the soil system, causing complex and variables relationships among microbial organisms and how functionally respond. Understanding the effect of crop management on soil microbial community and dynamics is essential to design potential practices capable to restore or enhance soil diversity and functionality (Li et al., 2018).

Microorganisms play an important role in nutrient cycling and availability for plant growth and development (Vargas Gil et al., 2009; Fierer, 2017; Liu et al., 2019). CA practices of diversified cultures, protection of soil surface, and input of SOM provide a beneficial environment to maintain soil community and continuous supply of nutrients (Maron et al., 2011; Bardgett and Van Der Putten, 2014). As described previously, residue maintenance and reduced tillage increase SOM content and consequently, the nutrient source for soil living organisms and microbial activity. SOM decomposition by soil microorganisms allows the release of essential nutrients to the soil solution. Bacteria and fungi can promote nutritional availability through functions such as N fixation, P and K mobilization by producing organic acids and siderophores (Rashid et al., 2016). These functions' efficiency may be limited by soil historical input of organic matter in the soil system, which could have decreased microbial diversity (Gupta, Roper, and Thompson, 2020). After a few years of CA practices, it is expected the recovery of microbial functional diversity and the increase of organic matter decomposition rate (Adl, 2003). However, the organic matter quality and quantity can vary decomposition flow and drive microbial functions, defining nutrient release to soil solution and plant absorption (Gupta, Roper and Thompson, 2020).

1.7 Soil nitrogen and phosphorus dynamics under conservation agriculture system

Nitrogen (N) and phosphorus (P) are the most required nutrients for plant growth and soil living development (Brooker et al., 2015). Both availabilities are correlated to soil organic matter amount and microbial decomposition processes (Egamberdiyeva and Höflich, 2004; Caesar-Tonthat et al., 2014; Leifheit, Verbruggen, and Rillig, 2015). However, these nutrients supply in the soil system is dependent on soil organic matter quality (Gupta, Roper, and Thompson, 2020). Under conservation system, the residues retention in soil surface or incorporated in the soil profile is, in most of the cases, composed of high C: N ratio materials. This is due to lignified fragments of roots and shoots. Such materials could stimulate some nutrients immobilization and a temporary lower soil nutritional availability (Boddey et al., 2010; Verhulst et al., 2010; Bhardwaj et al., 2011; Palm et al., 2014). On the other hand, Palm et al., 2014 described the increase of P and K in the first 10 cm of soil under CA practices, while no difference in Ca and Mg availability. This shows each element may contain different nutritional behavior under CA management. Thus, it is described below two important functions of N and P supply in soils, describing how conservation system impacts and can promote such processes.

1.7.1 Biological N fixation

N is essential for plants and microbial development, participating in vital physiological processes (Galloway et al., 2008). On natural terrestrial ecosystem, N enters in soil by multiple sources, such as the organic matter decomposition, atmospheric deposition, and biological fixation, this last corresponding to 97% of N inputted in the soil system (Galloway et al., 2008). Organisms involved in the biological N fixation (diazotrophs) include Bacteria and Archaea domains and can be found as free-living or associated and protected with plants (Cleveland et al., 1999; Smercina et al., 2019). Diazotrophs are very diverse, including members of α -proteobacteria, β -proteobacteria, δ -proteobacteria, γ -proteobacteria, Firmicutes, Cyanobacteria, and others (Gaby and Buckley, 2015). This diversity shows the importance of N in ecosystem services and its continuous activity even if other functions fall (Smercina et al., 2019).

N biological fixation is an anaerobic process of converting N_2 gas into ammonia (NH_3) via nitrogenase enzyme activity (Vitousek et al., 1997, 2002; Smercina et al., 2019). This reaction is very expensive energetically, consuming 16 ATPs for each N_2 molecule reduced into two NH_3 (Smercina et al., 2019). The high cost of N fixation indicates its dependency on a proper C nutritional supply to efficient activity. Several studies demonstrate the absence of N fixation in limited C availability, valid for both free-living or symbiotic N fixers (Carnahan et al., 1960; Stacey, Burris, and Evans, 1992). Oxygen is also an important factor controlling N fixation (Smercina et al., 2019). Oxygen acts irreversibly inhibiting nitrogenase activity, even in oxygen-dependent organisms (Smercina et al., 2019). Diazotrophs employ several mechanisms to maintain N fixation reaction protected from oxygen inactivation. These include oxygen avoidance via growth strategy, nitrogenase temporal or special isolation from oxygen, and producing biofilms as diffusion barriers (Dixon and Kahn, 2004; Reed, Cleveland, and Townsend, 2011). Although oxygen concentration is a limiting factor for N fixation, diazotroph diversity present in soil provides different mechanisms to the function continue happening (Smercina et al., 2019). As an illustration, Inomura et al., 2017 described diazotrophs species whose optimal oxygen concentrations were around 3%, 5%, and 20%.

Free-living N fixation can be altered by several biotic and abiotic factors, as well as crop management. As an expensive reaction, soil N content regulates N fixation driving soil N available use rather than N fixing from the atmosphere (Reed, Cleveland, and Townsend, 2011; Smercina et al., 2019). Therefore, free-living N fixation is stimulated by low N availability. N fixation process is also influenced by soil P availability, mainly serving for energy storage, in form of ATP (Reed, Cleveland, and Townsend, 2011, 2013). Some studies have described the increase of N fixation efficiency by the application of P solubilizers microbes as biofertilizers (Zaidi and Khan, 2006; Singh, Pandey and Singh, 2011; Rashid et al., 2016). Further, soil agriculture management modulates the soil free-living N fixation activity by organic matter material input rate in soil, the use of fertilizers, and the presence of different plant species (Mirza et al., 2014).

As described previously, the organic matter cover quality and quantity influence the soil functions and activities. CA organic matter continuous input directly affects soil diazotrophic community by two important factors: a. the diazotrophs use the organic matter resources to attend their energy demand for N fixation; and b. the high availability of C support the maintenance of the high respiration rate, and consequently decrease the oxygen concentration, prompting the nitrogenase activity (Levy-Booth, Prescott and Grayston, 2014; Mirza et al., 2014). Considering the majority of crops retained in soil by CA management is composed of a high C:N ratio, some studies explain that residues covering soil could stimulate the biological N fixation (Poly et al., 2001; Mirza et al., 2014). High C:N organic material environment may promote a competitive advantage for organisms that can capture N directly from the atmosphere. However, there is still a gap in the diazotrophs activity knowledge related to the organic matter quality and the effect of land-use change on this community structure and functional efficiency. The N biological fixation has significant participation in the soil N input and the knowledge about the behavior and metabolism of such microbes are important to design crop practices to improve agriculture production and sustainability.

1.7.2 P solubilization

P is the second most required nutrient by plants, essential for physiological processes as energy transfer and molecular structural composer (Azziz et al., 2012; Krishnaraj and Dahale, 2014). The main P source in the soil system is the organic matter, where P is mostly found in the inositol phosphate form (soil phytate) and represents up to 50% of the total soil P (Shen et al., 2011; Alori, Glick and Babalola, 2017). However, in the soil solution, P can

follow two ways, it can be absorbed by plants and microorganism's development, or it can be strongly adsorbed on soil colloids (Wang et al., 2010). P release from inorganic to available form is mediated by P solubilizing microorganisms (PSM) (Alori, Glick, and Babalola, 2017). PSM is composed of a large number of species of soil fungi and bacteria that can solubilize P from inorganic reservoirs or mineralize it from organic stocks (Sharma et al., 2013; Alori, Glick, and Babalola, 2017). These organisms can increase P availability and have been studied as having great potential as bio-fertilizers (Alori, Glick, and Babalola, 2017).

P solubilizing mechanisms can occur in two processes, inorganic P solubilization and P mineralization (Alori, Glick, and Babalola, 2017). The main mechanism of inorganic P solubilization is based on the solubilizers compounds production, like organic acids, siderophore, which, together with carboxyl and hydroxyl ions, release P from mineral adsorption by the pH reduction or cations chelate (Seshachala and Tallapragada, 2012; Sharma et al., 2013). On the other hand, P mineralization process is defined as the P release from organic material. This mechanism uses P-hydrolyzing enzymes, such as phytases and phosphatases, to solubilize organic P from the organic matter, releasing it to soil solution (Halvorson, Keynan and Kornberg, 1990; Sato et al., 2015; Alori, Glick and Babalola, 2017). This is an essential activity once SOM is the major source of organic P in soil (Alori, Glick, and Babalola, 2017). Apart from external P input (i.e., chemical fertilization), microbial P-solubilization and P-mineralization is the only process capable to increase P in soil solution (Alori, Glick and Babalola, 2017). Microorganisms play a key function in P cycling and further availability for plant growth.

P solubilization process is, however, influenced by soil biotic and abiotic factors, such as SOM, soil nutritional availability, temperature, and moisture (Seshachala and Tallapragada, 2012). Generally, it is considered the higher soil organic matter contents, the better the environment for microbial growth and, therefore, P solubilization microorganisms (Alori, Glick and Babalola, 2017). It is expected that CA practices show higher P-release activity compared to the conventional system. SOM quality can also influence the P solubilization processes. Different content of C and N can drive the types of organic acids and P solubilizing products released by the microbial community (Narsian and Patel, 2000). High C:P organic material may increase microbial production of organic acids, while various C:N and N:P materials will affect microbial growth rate (Stuart Chapin, Matson, and Vitousek, 2012). Thus, CA practices of increasing crop and SOM diversity may promote microhabitats which can enhance and maintain P solubilization activity. PSM has huge importance in the soil ecological system and agronomical activities. However, crop management impact in this particular function is still unclear. CA approach aims to explore biological resources in an ecologically feasible and sustainable way. Then, knowing about PSM is fundamental to increase soil system and efficiency.

1.8 Conclusion

Agriculture has been required to increase food and energy production using fewer natural resources for the exponentially growing world population. Such necessity has implied changes from conventional agriculture to conservation agriculture as an alternative to sustainable production. CA practices of minimum soil disturbance, permanent soil cover, and crop rotations have the potential to promote soil health and quality by affecting physical, chemical, and biological properties. Soil structure enhanced by CA provides soil stability and protection for soil water storage, nutrient efficient use, and biological functions. CA increases SOM and SOC mitigating C loss and providing several environmental services. Further, the increase and high diversification of SOM boost soil diversity, providing efficient and resilient functionally of the soil system. Although the impact of CA is also affected by

external factors, such as climate, topography, and crop historical practices, its study is essential to design more efficient crop management. Particularly, the soil microbiome presents several potential benefits to promote plant performance from nutrient mineralization (e.g., N and P) to plant drought resistance under the CA system. This review highlighted the importance of CA practices, and the need for further studies to better understand and apply a more sustainable and productive system.

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2 THE ROLE OF THE *Urochloa* SPP. INTERCROPPING IN COFFEE PLANTATION UPON BACTERIAL COMMUNITY AND FUNCTIONAL GENES

Abstract

Intercropping is a growing agricultural practice to improve soil quality, crop sustainability, and plant performance. However, little is known about the intercropping influence on the bacterial community. Here, we investigated the effect of 3 years old *Urochloa* spp. (formerly *Brachiaria*) intercropping with coffee on bacterial community comparing to conventional cultivation. We evaluated bacterial communities from topsoil to 15 cm depth in four soil layers, being them 0 – 2.5, 2.5 – 5, 5 – 10, and 10 – 15 cm depths. Our results showed that intercrop improved soil organic matter from 2.5 to 10 cm, β -glucosidase activity in 2.5 – 5 cm, and acid-phosphatase activities in 0 – 2.5 and 5 – 10 cm depth. Intercrop presented a higher abundance of nitrogen fixation gene (*nifH*) in 5 – 10 cm, whereas there was no difference in phosphorus solubilizing marker (*phoD*). Bacterial richness and diversity, determined from *16S rRNA* sequencing, were similar between the two crop systems, but PCoA analysis showed structural differences in response to distinct practices. Network analysis and topological parameters demonstrated that intercrop promoted higher and more intimate connections between the active nodes. In addition, we identified higher competitiveness and modular structure under intercrop than conventional coffee. Taken together, our results indicate intercrop system may provide a more quality and stable environment for soil bacterial communities functioning. Moreover, intercrop improved bacterial interactions and may contribute to ecosystem services and efficient resources use in agriculture.

Keywords: *nifH*; *phoD*; network co-occurrence

2.1 Introduction

The combined cultivation of more than one crop in the same field is a growing practice to increase production yield and sustainability in several agricultural systems (Robinson et al., 2015; Van Tan et al., 2020). This strategy focuses on altering soil quality, reducing environmental stresses, and promoting several benefits, such as more efficient nutrient cycling (Brooker et al., 2015). Given the global climate scenario, intercropping is a practice that could minimize the effects of climate change through its potential to modify soil microclimate (Brooker et al., 2015; Moreira et al., 2018). Several studies have described efficient intercrop practices in coffee production, a highly affected plant to climate variations (Harelimana et al., 2018; Moreira et al., 2018; Van Tan et al., 2020). Coffee is a rising commodity, cultivated in more than 70 countries, with 11 Mha of harvested area in 2019 (FAO, 2019). However, Arabica coffee (*Coffea arabica* L.), responsible for 60% of global production, has a limited potential to adapt in front of global climate change (Aerts et al., 2017). Coffee production in general is most affected by water, light, and temperature variables which can be modified by intercrop practice (de Camargo, 2010). Understanding sustainable principles with prospective benefits should be further conducive to develop better strategies for agricultural management.

Intercropping can provide better efficiency in soil resource use, achieving food security and less external input dependence (Brooker et al., 2015). Mixtures of species in the field produce on average of 1.7 times higher biomass compared to monoculture (Cardinale et al., 2007). This, increase in soil organic carbon, is a source of nutrients for plant and soil living organisms (Oldfield, Wood and Bradford, 2018). However, the effectiveness of nutritional supply and crop acquisition of limiting resources is dependent on which and how plants are combined.

Plants species with large and ramified root system (i.e., grass) can boost soil organic matter increase and soil structure stability (Graham and Haynes, 2006; Calonego et al., 2017). Austin et al., 2017 described that, compared to aboveground plant residues, root system is more efficient in storing carbon in the soil. Roots have a particular influence in soil for releasing plant exudates interfering in soil microbial activity (Six et al., 2006; Austin et al., 2017; Faucon, Houben, and Lambers, 2017). Stimulating microbial activity is intended to promote soil fertility or suppress pathogenic organisms (Bennett, Daniell, and White, 2013). Thus, the intercropping system combining plentiful root plants can potentialize the soil benefits and fertilization.

High biomass and plant diversity stimulate the promotion of soil microbial community, their abundance, and diversity (De Deyn, Cornelissen, and Bardgett, 2008; De Deyn et al., 2011; Li and Wu, 2018). Microbial community plays a key role in nutrient cycling processes, organic matter decomposition, and plant growth promotion (Lalitha, 2017; Li and Wu, 2018; Kour et al., 2020). Microbial functionality and community structures respond directly to soil environment, including nutritional supply and organic matter content (Trivedi et al., 2015; Zheng et al., 2018; Chen et al., 2020). Further, microorganisms can closely interact with plant by direct and indirect mechanisms, from manipulating plant hormonal signaling to increase soil nutritional bioavailability (Van Der Heijden, Bardgett and Van Straalen, 2008; Mendes, Garbeva and Raaijmakers, 2013; Jacoby et al., 2017; Bargaz et al., 2018). Recent studies highlighted the link between the increase in soil diversity and higher plant productivity in intercropping systems (Van der Putten et al., 2013; Brooker et al., 2015). Plant diversity and high carbon (C) and nitrogen (N) input via well-ramified root biomass in soil depth have a positive influence on microbial functional diversity, increasing complementary functional groups (Mueller et al., 2013). This functional diversity can represent a more stable and connected environment and better use of nutritional sources (Morriën et al., 2017). However, the impact of plant diversity in microbial community is dependent on several factors and should be further studied to understand the effects on plant production.

The most common limiting nutrients in agricultural systems are N and phosphorus (P) (Brooker et al., 2015). Thus, beneficial microorganisms that could address these nutrients limitations have been extensively studied (i.e., N fixing and P-solubilizing/mobilizing groups) (Bargaz et al., 2018). Different grasses have been identified in association with *Azospirillum*, an associative symbiotic nitrogen fixer with plant growth-promoting (PGP) properties (Bashan and de-Bashan, 2010; Glick, 2014; Kour et al., 2020). Further, several studies have also described grass association with PGP non-symbiotic nitrogen fixers groups (Kennedy, Choudhury and Kecskés, 2004; Bargaz et al., 2018). However, N fixation is a highly energetically expensive reaction, dependent on P-containing molecules, adenosine triphosphate (ATP), and adenosine diphosphate (ADP) (Krishnaraj and Dahale, 2014). Phosphorus is a scarce nutrient in soil solution requiring specialized procurement and transportation mechanisms to provide efficient acquisition for vital physiological processes (Krishnaraj and Dahale, 2014; Bargaz et al., 2018). Nevertheless, several microorganisms are capable to mineralize P from organic matter and enhance its bioavailability in soil (Bargaz et al., 2018). Organic P can represent up to 50% of total soil P, which places its mineralizers organisms as essential in vital P-cycling (Shen et al., 2011).

The importance of these two functional groups, and the whole microbial community, in the soil system and agricultural management, evidence the necessity of studying them for designing a better crop strategy. However, to comprehend soil N-fixing and P-solubilizing groups' behaviors, it is also necessary to access microbial diversity indexes, as well as how their members interact with each other. Thus, here we aimed further investigate the effect of *Urochloa* spp. (formerly *Brachiaria*) intercropping with coffee cultivation in the bacterial community. We sampled soil in four layers, from 0 – 15 cm in the background of coffee treetop in conventional coffee cultivation and an

intercropping system. The project monitored how bacterial richness, diversity, and assembly changed in intercropping, as well as the relative abundance of N-fixation and P-solubilization functional genes. We highlighted important aspects that may help to understand and further design microbial aspects under the intercropping system.

2.2 Methodology

2.2.1 Site description, experimental design, and sampling

The study areas were sampled at the city of Araguari, Minas Gerais State, Brazil, one of the most important cities in the Minas Triangle region, where coffee is the main cultivated crop. Araguari climate is tropical with dry winter (Aw by Köppen classification), with 21.2 °C annual average temperature and 1566 mm of annual average precipitation. We selected two neighboring coffee fields, both with soil classified as Oxisol Udox according to USDA soil taxonomy (USDA, United States Department of Agriculture, 1998), in conventional coffee cultivation and a 3 years old intercropping coffee with grass (*Urochloa* spp. – formerly *Brachiaria*). Soil was sampled in five random points on each field, at least 30 meters distance, right on the background of coffee treetop, along with four depth layers as follows: 0–2.5 cm, 2.5–5.0 cm, 5–10 cm, 10–15 cm. The soil sampled for chemical and biological analysis was properly stored and frozen at – 20°C after sampling.

Figure 1. Coffee crop productions in two different agricultural management, a conventional crop and coffee cultivation with *Urochloa* spp. (formerly *Brachiaria*) consortia. The red arrows indicate the points where the soil was collected, right on the background of the coffee treetop.

2.2.2 Soil chemical analysis and enzyme quantification

Soil chemical properties were determined at Laboratory of Soil Analysis of Luiz de Queiroz College of Agriculture (ESALQ/USP) according to Raij methodology (Raij, B. van; Andrade, J.C. de; Cantarella and J.A., 2001). There were determined soil pH and potential acidity ($H+Al^{+3}$), the amount of soil organic matter (OM), phosphorus (P); potassium (K), calcium (Ca), magnesium (Mg), and ammonia nitrogen ($N-NH_4^+$). Then, we calculated the soil sum of bases (SB), base saturation (V), and cation exchange capacity (CEC).

β -glucosidase and Acid-phosphatase enzyme activities were measured using colorimetric determination on 1g of fresh soil with 1 mL of p-nitrophenyl- β -D-glucopyranoside (0.025 M) and p-nitrophenyl phosphate disodium hexahydrate (0.05 M) substrates, respectively, added with hydroxymethyl buffer solution following Tabatabai methodology (Tabatabai, 2018a).

2.2.3 Soil DNA extraction and real-time PCR amplification

DNA was extracted from soil (0.4 g) using Powersoil DNA Isolation Kit (MoBio Laboratories a QIAGEN company, Carlsbad, CA, USA) following the manufacturer's protocols. The final product was diluted in 100 μ L of elution buffer, checked in 1.5% (p/v) agarose gel 1 x TAE cap solution running, and stored at -20°C .

The *16S rRNA*, *nifH*, and *pboD* genes were quantified targeting the number of copies of the bacterial marker, N-fixation, and P-mineralization functions, respectively. The genes were determined via real-time PCR (qPCR) using Real-Time PCR System StepOne (Applied Biosystems) equipment and the SYBR Green I fluorescent detection technology. We used primer pairs of P1/P2 (Muyzer, De Waal and Uitterlinden, 1993), for *16S rRNA* amplification in a reaction of 20 μ L final volume with 10 μ L Syber, 0.5 μ L of each primer (5.56 μ M) and 0.3 mg mL⁻¹ of bovine serum albumin (BSA). Reaction conditions followed: 10 min at 95 $^{\circ}\text{C}$, and 35 cycles of 30 s at 94 $^{\circ}\text{C}$, 30 s at 55 $^{\circ}\text{C}$ and 30 s at 72 $^{\circ}\text{C}$. Gene *nifH* was target by FGPH19/PolR pair of primers (Simonet et al., 1991; Poly, Monrozier, and Bally, 2001), in a reaction of 25 μ L final volume with 12.5 μ L Syber, 0.4 μ L of each primer (100 μ M) and 0.6 mg mL⁻¹ of BSA. Amplification conditions followed: 15 min at 95 $^{\circ}\text{C}$, and 40 cycles of 1 min at 95 $^{\circ}\text{C}$, 27 s at 55 $^{\circ}\text{C}$ and 1 min at 72 $^{\circ}\text{C}$. Similarly, the gene *pboD* was amplified using primer pairs of ALPS-F730/ALPS-R1101 (Sakurai et al., 2008) in a reaction of 20 μ L with 10 μ L Syber, 0.5 μ L of each primer (100 μ M) and 0.5 mg mL⁻¹ of BSA. The reaction conditions followed: 10 min at 95 $^{\circ}\text{C}$, and 40 cycles of 30 s at 95 $^{\circ}\text{C}$, 1 min at 57 $^{\circ}\text{C}$ and 30 s at 72 $^{\circ}\text{C}$. *16S rRNA*, *nifH*, and *pboD* amplifications generated fragments of 193, 429, and 371 bp, respectively. All the reactions were checked to confirm amplified fragment purity and their data were expressed as log numbers of copies of gene per gram of soil.

2.2.4 16S rRNA gene sequencing and data analysis

Bacterial community was amplified targeting the V4 region of *16S rRNA* gene (Caporaso et al., 2012) and PGM Ion Torrent Technology (Thermo Fisher Scientific, Waltham, MA, USA). PCR amplification used 25 μ L final volume composed by 4 μ L of 10 $\times$ High Fidelity PCR buffer, 2 mM of MgSO₄, 2U of Taq Polymerase, 0.2 mM of dNTP Mix, 25 μ g of Ultrapure BSA (Invitrogen, Carlsbad, CA, USA), 0.1 μ M of each primer 515F and 806R (Caporaso et al., 2012), and $\sim$ 50 ng of DNA template. Reaction conditions followed: 5 min at 94 $^{\circ}\text{C}$, and 30 cycles of 45 s at 94 $^{\circ}\text{C}$, 45 s at 56 $^{\circ}\text{C}$ and 1 min at 68 $^{\circ}\text{C}$. PCR products were purified and quantified using Agencourt® AMPure® XP Reagent (Beckman Coulter, Brea, CA, USA) and Qubit Fluorometer kit—DNA High Sensitivity Assay kit (Invitrogen, Carlsbad, CA, USA). Further, the product was combined in equimolar ratios and sequenced using Ion PGM™ Sequencing 400 kit on an Ion PGM™ System, using the Ion 318™ Chip v2.

Raw sequencing reads were pre-processed using USEARCH version 7.0.1090 (Edgar, 2010) and QIIME ver. 1.9.1 (Caporaso et al., 2010). Raw data were processed using QIIME and UPARSE standard pipeline57, following the Brazilian Microbiome Project (Pylro et al., 2014). In short, reads were clustered using the UPARSE

method at 97% similarity cutoff, to access OTU files, and aligned against Greengenes database version 13.8 (DeSantis et al., 2006) reference database (version 13.8). After, unassigned reads were removed, and the dataset was rarified to further analysis.

2.2.5 Statistical analysis

Data from chemistry, enzyme, and gene amplifications were analyzed using t-test ($P \geq 0.05$) to compare the two coffee systems' significant difference. Considering the data difference in each system along the four depths, we used one-way analysis of variance (ANOVA) in the mixed procedure of SAS Software (SAS, 2018). It was performed ante-dependence covariance-structure and Tukey test at 0.95 significance to evaluate the difference among soil layers in each system and for each variable.

Bacterial richness and diversity were analyzed using one-way ANOVA t-test in Microbiome Analyst (Dhariwal et al., 2017) by calculating Chao1 and Shannon-H indexes. We performed PCoA to access microbial structure using PAST 3 Software (Hammer, Harper, and Ryan, 2001). The analysis explored the bacterial differences among soil layers and between conventional and intercropping coffee systems. To access the effect of intercropping system in soil bacterial interaction, we calculated co-occurrence network with Sparcc (Friedman and Alm, 2012) based on assigned OTUs. Correlations were performed considering their strength considering a magnitude of $p > 0.6$ or $p < -0.6$ and a significance of $p < 0.03$. We calculated topological properties of the network using Gephi version 0.9.2 software (Bastian, Heymann, and Jacomy, 2009).

2.3 Results

2.3.1 Soil chemical and biological activity

Soil chemical analysis indicated that intercropping system showed little difference in soil chemical properties comparing to conventional coffee (Table 1). There were no significant differences in soil P, K, Ca, and N-ammonium content, potential acidity, sum of bases, CEC, and base saturation from topsoil to 15 cm. However, intercropping increased soil OM and Mg from 2.5 cm to 10 cm depth, besides soil pH in 5 to 10 cm layer.

Soil β -glucosidase and acid-phosphatase enzyme activities presented some differences under distinct crop systems. We found higher values of β -glucosidase activity between 2.5 – 5 cm, and higher acid-phosphatase activity in 5 – 10 cm in intercrop compared to conventional. On the other hand, comparing enzyme activities of soil layers into each system, β -glucosidase presented the same pattern from 0 to 15 cm in conventional and intercrop coffee, with higher activity in 0 – 2.5 cm. However, acid-phosphatase activity presented a different response to conventional and intercrop coffee along with the soil layers. For conventional, there was no difference of acid-phosphatase from 0 – 15 cm, although, under intercrop, its activity was higher in the first 2.5 cm of soil with similar values from 2.5 – 15 cm (Figure 2).

Figure 2. a. β -glucosidase and b. acid-phosphatase activity along soil 0–2.5, 2.5–5, 5–10 and 10–15 cm depths under conventional and intercrop system. Different capital letters in the column present significant difference between the crop system in each layer (t-test $P < 0.05$), and small letters show significant difference among the layers in each system (Tukey test $P \geq 0.05$).

Table 1. Soil chemical properties of different crop systems for 0–2.5, 2.5–5, 5–10, and 10–15 cm depths.

Treatment	pH	OM	P	K	Ca	Mg	H+Al ³⁺	SB	CEC	V	N-NH ₄ ⁺											
	CaCl ₂	g.dm ⁻³	mg.dm ⁻³	mmolc.dm ⁻³						%	mg.kg ⁻¹											
0 – 2.5 cm																						
Conventional	4.63	a	50.00	a	175.3	a	7.53	a	37.67	a	6.67	a	68.33	a	51.87	a	120.2	a	44.67	a	92.67	a
Intercropping	4.70	a	38.67	a	38.00	a	3.97	b	25.33	a	8.67	a	52.33	a	37.97	a	90.30	b	41.67	a	78.67	a
<i>P value</i>	0.40		0.18		0.12		0.02		0.07		0.15		0.18		0.08		0.03		0.39		0.27	
2.5 – 5 cm																						
Conventional	3.90	a	31.67	b	63.33	a	2.87	a	11.00	a	1.67	b	107.7	a	15.53	a	123.2	a	13.33	a	95.00	a
Intercropping	4.37	a	36.00	a	17.00	a	2.07	a	15.00	a	4.33	a	67.67	a	21.40	a	89.07	a	25.67	a	103.0	a
<i>p</i>	0.10		0.02		0.12		0.13		0.29		0.036		0.07		0.26		0.057		0.13		0.39	
5 – 10 cm																						
Conventional	3.73	b	25.00	b	21.00	a	1.77	a	5.33	b	1.33	b	81.33	a	8.43	b	89.77	a	9.33	b	91.33	a
Intercropping	4.33	a	31.67	a	18.00	a	1.33	a	13.67	a	4.67	a	66.33	a	19.67	a	86.00	a	24.00	a	64.33	a
<i>p</i>	0.03		0.03		0.37		0.17		0.045		0.012		0.21		0.04		0.40		0.048		0.21	
10 – 15 cm																						
Conventional	3.83	a	20.33	a	8.00	a	1.37	a	4.67	a	1.67	a	66.33	a	7.70	a	74.03	a	10.33	a	91.33	a
Intercropping	4.10	a	22.67	a	19.50	a	1.03	a	7.33	a	3.33	a	64.00	a	11.70	a	75.70	a	16.00	a	83.33	a
<i>p</i>	0.10		0.25		0.21		0.17		0.15		0.056		0.45		0.13		0.46		0.14		0.31	

The values are means and different letters in the column present significant differences. *P values* were calculated using *t*-test ($P \geq 0.05$).

2.3.2 Gene abundance of *nifH* and *phoD* functional genes

NifH and *phoD* genes were quantified and evaluated considering their abundance to the number of copies of *16S rRNA* gene. The relative abundance of *nifH* presented a higher value under intercrop compared to conventional in 5 – 10 cm layer, whereas no difference was found for *phoD*. Comparing into each system, *nifH* and *phoD* relative abundances presented no difference from topsoil to 15 cm under the conventional system. However, intercropping showed higher *nifH* relative abundance in 5 – 10 and 10 – 15 cm depths, although 10 – 15 was similar to 0 – 2.5 and 2.5 – 5 cm depths. Intercrop presented higher *phoD* abundance in 5 – 10 cm and 2.5 – 5 cm depths, whereas 2.5 – 5 cm showed similar values with 0 – 2.5 and 10 – 15 cm (Figure 3).

Figure 3. a. *nifH* and b. *phoD* genes relative abundance along soil 0–2.5, 2.5–5, 5–10, and 10–15 under conventional and intercrop coffee systems. Different capital letters present significant difference between the crop systems in each layer (t-test $P < 0.05$), and small letters show significant difference among the layers in each system (Tukey test $P < 0.05$).

2.3.3 Bacterial community richness, diversity, structure, and composition

A total of 655,222 high-quality reads were obtained from 24 samples for *16S rRNA* gene sequencing (on average 27,342 reads per sample), from which it was identified 4,815 OTUs. Richness and diversity indices were calculated and presented no difference between conventional and intercrop coffee systems within each soil depth analyzed (Figure 4). However, comparing soil layers into each system, conventional presented bacterial richness differences along the depths analyzed (Figure 4).

Figure 4. **a.** Bacterial richness and **b.** diversity along with soil 0–2.5, 2.5–5, 5–10, and 10–15. Red and green contour represent conventional and intercrop coffee systems, respectively. Different capital letters present significant difference between the crop systems in each layer (t-test $P < 0.05$), and small letters show significant difference among the layers in each system (Tukey test $P < 0.05$).

PCoA plot indicated structural differences in bacterial communities comparing conventional and intercrop systems in the four layers analyzed. The two axes PCoA1 and PCoA2, explained 63.3%, 63.4%, 71.5%, and 65.8% of total community structural variation in 0 – 2.5, 2.5 – 5, 5 – 10 and 10 – 15 cm, respectively (Figure 5). In addition, considering changes observed in each crop system, PCoA encompassing samples from conventional coffee showed a clear separation of the community structure of each layer, explained by the two axes with 63.3% of total variation (Figure 6a). On the other hand, samples from intercropping system showed a progressive overlap of community structure from the 0 – 2.5 to 10 – 15 cm, being the deepest, the most isolated among the others (Figure 6b). Intercrop PCoA explained 61% of total variation of community structure.

Figure 5. PCoA plot of bacterial community structure in each soil depth under conventional and intercrop coffee system. Red and green contour represent conventional and intercrop coffee systems, respectively. **a.** 0 – 2.5, **b.** 2.5 – 5, **c.** 5 – 10, and **d.** 10 – 15 cm depth layers.

Figure 6. PCoA plot of bacterial community structure along soil depths in two coffee crop systems. **a.** conventional coffee, and **b.** intercrop coffee.

2.3.4 Network co-occurrence analysis

Co-occurrence analysis and network construction were performed to access bacterial interactions and community topological properties based on genera level, which are summarized in Table 2 and plotted in Figures 7 and 8. Overall, comparing conventional to intercrop coffee, the last presented a higher number of nodes and competitiveness in all layers analyzed. From 2.5 cm further, intercrop also showed higher average connectivity per node and a higher number of edges. There were closer connections between nodes under the intercrop system from 5 to 15 cm depth. Comparing

in each system along with the soil layers, the conventional showed more similar patterns along depth, whereas intercrop presented more distinct values.

Table 2. Bacteriall network properties under conventional and intercrop coffee system for 0–2.5, 2.5–5, 5–10 and 10–15 cm depth layers.

	0 – 2.5 cm		2.5 – 5 cm	
	Conventional	Intercropping	Conventional	Intercropping
Number of nodes	1004	1201	925	1069
Number of edges	7700	7351	5974	8433
Positive correlations (%)	78.83	59.86	79.86	67.59
Negative correlations (%)	21.17	40.13	20.14	32.41
Positive/negative ratio	3.72	1.49	3.97	2.09
Avg degree	15.34	12.24	12.91	15.78
Graph density	0.015	0.01	0.014	0.015
Avg clustering coefficient	0.26	0.23	0.25	0.26
Modularity	0.88	2.58	1.03	1.45
Number of modules	155	175	153	134
Average path length	4.76	4.72	4.36	4.44
Diameter	10	10	11	11
	5 – 10 cm		10 – 15 cm	
	Conventional	Intercropping	Conventional	Intercropping
Number of nodes	488	955	528	748
Number of edges	1972	7428	3151	9866
Positive correlations (%)	80.07	63.49	73.12	65.89
Negative correlations (%)	19.93	36.51	26.88	34.11
Positive/negative ratio	4.02	1.74	2.72	1.93
Average degree	8.08	15.56	11.94	26.38
Graph density	0.017	0.016	0.023	0.035
Average clustering coefficient	0.265	0.351	0.366	0.642
Modularity	0.92	1.68	1.06	0.72
Number of modules	114	188	84	221
Average path length	4.57	3.85	3.53	2.79
Diameter	10	11	12	12

Figure 7. Network topological proprieties plot under conventional and intercrop coffee system in each depth. **a.** 0 – 2.5, **b.** 2.5 – 5, **c.** 5 – 10, and **d.** 10 – 15 cm depth layers.

Figure 8. Network topological proprieties plot along soil depths in two coffee crop systems. **a.** conventional coffee, and **b.** intercrop coffee.

Figure 9. Network of co-occurrence bacterial genera based on correlation analysis along soil depths in two coffee crop systems. **a.** conventional coffee, and **b.** intercrop coffee.

2.4 Discussion

2.4.1 Bacterial activity and functional gene abundance

Soil microbial composition, functionality, and community structure are known to have different responses to environmental changes, as related to agricultural management, either to local and small scales (Bailey et al., 2013; Kuzyakov and Blagodatskaya, 2015; Gupta, Roper and Thompson, 2020). These complex microbial properties result from ecological associations and environmental stimulus, which may imply beneficial or deleterious outcomes for soil quality (Lidicker, 1979; Gupta, Roper, and Thompson, 2020). In this study, we used soil chemical attributes, microbial activity indicators, the abundance of genes related to key processes, and community indices to identify the alterations and potential benefits of coffee intercropping with *Urochloa* spp. to soil bacterial community. Further, we evaluated topological properties of co-occurrence-based network to explore the community assembly and interactions changed by different crop system.

Intercropping is described as environment-friendly practice for nutritional supply, microclimate conditions, and microbial expansion (Carter, 1992; Salinas-García et al., 2002; Brussaard, 2013; Palm et al., 2014). Some studies reported intercrop systems promoting soil chemical properties such as C, N, and pH (Morris and Garrity, 1993; Fan, Chen, and Li, 2006; Bedoussac and Justes, 2010; Liu et al., 2014). Here, we found that intercropping did not change soil P, K, and N content. However, evaluating soil OM content and microbial β -glucosidase and acid-phosphatase activities as soil quality indicators, our results showed improvement in soil quality promoted by intercrop management. Intercrop system presented an increase of soil OM in 2.5 – 5 and 5 – 10 cm depth compared to conventional, and simultaneous increase of β -glucosidase activity in 2.5 – 5 cm, and acid-phosphatase activity in 0 – 2.5 and 5 – 10 cm depth. Soil OM and enzymes indicate soil health, fertility, and functioning (Dick, 1994; Gallandt, 2005; Verhulst et al., 2010; Turmel et al., 2015; Tabatabai, 2018b). β -glucosidase is involved in the final step of plant residues decomposition and is important for maintaining soil labile carbon, that directly influences soil stability and energy supply (Acosta-Martínez et al., 2003; Haynes, 2005; Murphy, 2014; Furtak and Gałazka, 2019). Phosphatase is a biological indicator of soil activity involved in organic P hydrolyzation and soil P bioavailability (Bargaz et al., 2018). Although β -glucosidase is widely dependent on substrate supply, some studies have reported its activity decrease with soil pH increase from 4.3 to 8.5 (Adetunji et al., 2017). Our results presented an increase of soil pH under intercrop in soil 5 – 10 cm depth with the mean value of 4.3, which may explain why we have found no difference of β -glucosidase activity in 5 – 10 cm even with the increase of soil OM. In the same way, acid-phosphatase has an optimal response under an acid environment and decreases its activity as the pH increase (Adetunji et al., 2017). Here, however, the increase of soil pH under intercropping system in 5 – 10 cm did not affect decreasing soil acid-phosphatase activity.

Changes in soil management and crop practices imply changes in microbial functionality and nutrient dynamics in soils (Wang and McSorley, 2005; Liu et al., 2020). We evaluated the abundance of *nifH* and *phoD* genes, responsible for N-fixing and P-solubilizing, respectively, to access key soil functions of the most required nutrients for soil living organisms. Our results showed higher *nifH* relative abundance in 5 – 10 cm depth under intercropping compared to conventional crops. Interestingly, although the relative abundance of *phoD* gene presented no difference between crop systems, *nifH* relative abundance was higher in the same depth where it was found higher acid-phosphatase

enzyme activity. N-fixing is essential for supplying new N in the soil system, widely dependent on microbial community (Smircina et al., 2019; Arantes et al., 2020). Several studies described N-fixing been controlled by P availability as a key nutrient for its energetically expensive reaction (Reed et al., 2007; Reed, Cleveland, and Townsend, 2013; Smircina et al., 2019). Zheng et al., 2016 described a similar increase of free-living N fixers with N or P additions, which suggests that P limitation has stronger control in N fixing than N availability. In the present study, although we found no significant difference in N and P total content between crop systems, there were an increase in OM and higher activity of mineralizing P enzyme in 5 – 10 cm. This may have stimulated N-fixing abundance, at least on small scale, through the high C:N rate of *Urochloa* spp.'s material and its mineralization.

2.4.2 Soil bacterial community diversity, structure, and assembly

Intercropping and combined cultivation of different cultures in the same field are known to enhance soil diversity of substrates and change microbial community properties (Liu et al., 2020). Here, we accessed the effect of coffee crop intercropped with *Urochloa* spp. in bacterial diversity and structure. Although our results expressed no significant difference in bacterial richness and diversity indices, PCoA of bacterial community showed clear separation between conventional and intercrop systems in all the layers analyzed. Conventional and intercrop systems have several differences which might impact bacterial behavior. Coffee intercropping with well-ramified root plant, such as *Urochloa* spp., provide a higher number of substrates exuded to soil than conventional crop (Balota and Auler, 2011). Besides, several studies have reported lower temperature amplitude under intercrop system through shading effect (Campanha et al., 2004; Morais et al., 2006; Siles, Harmand and Vaast, 2010; Pezzopane et al., 2011; Moreira et al., 2018). Indeed, Alster et al., 2016 showed that soil temperature has strong control of soil microbial structure, which could explain the differences between the crop systems. Considering our PCoA results of each crop system along with the layers, we found a clear separation of bacterial structure among the layers in conventional system. On the other hand, intercropping presented an overlap of communities' structures from 0 – 2.5 cm to 10 – 15 cm. In these senses, we speculate that the mitigation of extreme temperatures and well ramification of *Urochloa* spp. root system in intercropping may contribute to soil stability and smaller changes of bacterial structure along with the soil depth.

Due to limiting information demonstrated by diversity indices, we further investigated bacterial co-occurrence network effects under the two crop systems. Network co-occurrence has been currently used as a soil quality indicator to assess soil practices effects (Karimi et al., 2017). We found that intercrop provided a higher number of nodes in all the layers, a higher number of edges from 2.5 – 15 cm, higher connectivity in 0 – 2.5 and 5 – 15 cm, and lower average path length in 0 – 2.5 and 5 – 15 cm depths compared to conventional system. The number of nodes indicates the significantly active taxon in the network, while the number of edges and average degree show how connected these nodes are among each other. The simultaneous determination of high connectivity and lower path length demonstrate that the community is highly connected. It has been described that a highly diverse and connected community may be less susceptible to pathogen invasion (Latz et al., 2012; Mallon, Van Elsas and Salles, 2015). A highly connected community has been also correlated with more efficient nutrient use and stable environments (Morriën et al., 2017). Thus, in agreement with previous studies, we suggest that intercrop enhances soil quality for bacterial interactions and

efficient functioning. Besides, it may provide a more stable and valuable environment for plant growth and crop performance.

Further based on co-occurrence analysis, we identified higher competitiveness under intercrop compared to conventional coffee crop. Also, we found a more modular structure and connected community. It is expected that the competitiveness increase as microbial growth and produce biomass requiring more space and nutrients (Ghoul and Mitri, 2016). Zelezniak et al., 2015 described that phylogenetic distance may determine microbial competitiveness for similar niches. However, intense competition for resources has been reported in highly abundant and diverse communities, and, as well as community connectivity, enhance soil suppression of pathogen invasion (Wei et al., 2015). Poudel et al., 2016 suggested that high modularity implies common nodes resources requirements or even niche overlap. In this sense, modular structure determines the presence of different clustered groups named modules, with highly connected nodes (Newman, 2006). This is in accordance with our results, in which we also found a higher average clustering coefficient under intercrop system from 2.5 cm further. Clustering coefficient described how well the nodes are connected with their neighbors (Deng et al., 2012). Such parameter is used to measure module extension and the degree they tend to cluster. We propose that modular structure combined with high clustered nodes and low average path may imply in prompter community response to soil disturbances. It suggests that under intercrop system the bacterial community might have less damage or more efficient and faster reaction to disturbances and invasion.

2.5 Conclusions

The environmental benefits of intercropping have been widely explored in soil chemistry and physics, but poorly taking the microbial community into account. Here, we demonstrated in detail the effect of conventional and *Urochloa* spp. intercrop coffee system in bacterial activity, potential functions, diversity, and assembly in the first 15 cm of soil. Although it is needed more effort to define microbial quality parameters, we support that intercropping system provides a better environment for bacterial growth and activity, besides increase potential N-fixing. We also propose that intercropping enhance soil stability and bacterial community response to environmental disturbances, possibly including pathogen invasion. The use of co-occurrence analysis is essential to further assess how microbial community interacts and how crop management can influence its structure. Overall, our results reinforce the intercropping potential to enhance soil quality and crop sustainability.

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3 EFFECTS OF FOUR QUANTITIES OF SUGARCANE STRAW ON SOIL COVERAGE UPON BACTERIAL COMMUNITY AND FUNCTIONAL GENES

Abstract

Sugarcane straw accumulation in soil surface has been described as a promising practice to improve soil quality and crop sustainability. However, there is no consensus about how much straw is recommendable to provide soil benefits. Here, we investigate the effect of four levels of straw maintained in soil surface [total remove (TR), high remove (HR), low remove (LR), and no remove (NR)] on soil bacterial community after a six-year experiment. We evaluated bacterial community in five soil depths, being them 0 – 2.5, 2.5 – 5, 5 – 10, 10 – 15, and 15 – 20 cm depths. Our results showed that straw release increased soil organic matter compared to TR, especially NR, which presented the highest or the second higher values of soil OM in all the layers. NR presented the highest ammonium N value and potential nitrogen fixation (*nifH* gene relative abundance) from 10 to 20 cm depth. Bacterial richness and diversity, assessed by *16S rRNA* sequencing, showed that TR and NR presented similar bacterial diversity indices, although different structures in the first 5 cm soil depth. Network analysis and topological parameters presented that bacterial community under TR have high average path length and network diameter, while under NR showed lower modularity and higher density assembly. In addition, in 15 – 20 cm, NR presented higher connectivity and intimate between the nodes. Taken together, this study indicates that straw maintenance provides a more stable environment for bacterial behavior. Besides, NR may provide higher values of N supply from 10 cm further, thus contributing to the ecological system and crop fertility. In summary, the soil bacterial community interplay with the straw management, and its outcomes may potentialize beneficial functions and pathogen suppression to boost plant performance.

Keywords: *nifH*; *phoD*; bacterial assembly; co-occurrence analysis

3.1 Introduction

Sugarcane (*Saccharum officinarum* L.), a typical tropical and subtropical plant, is a highly important crop for producing food (sugar) and bioenergy (first and second ethanol generation and bioelectricity) (Oliveira et al., 2019). Sugarcane is a perennial C4 monocotyledonous plant, remaining in the soil for up to six annual harvesting cycles before replanting (Cerri et al., 2011; De Matos, Santos, and Eichler, 2019). Its high efficiency in converting solar energy in biomass and the economic importance of its sub-products has promoted the large cultivation of sugarcane in world tropical and subtropical regions (Cerri et al., 2011). At present, sugarcane is produced in more than 100 countries, with 26.8 Mha harvested around the world, mainly between 35° N and 35° S latitudes (FAO, 2019). Considering this, Brazil is positioned as the world's greatest producer area of sugarcane crop, with 10.1 Mha of area harvest and 74.7 t ha⁻¹ average yield, which is responsible for 38% of global production (FAO, 2019). Besides, this large area is projected to expand over degraded pastures to attend, mainly, the increasing demand for energy sources (Oliveira et al., 2019).

In recent years, sugarcane crop has been required to develop sustainable practices across all their production sections to decrease natural resources damaging (Souza et al., 2012). The usual manual harvest after crop burning has changed to mechanical system in the majority of Brazilian cultivated regions (Souza et al., 2012; Sousa Junior et al., 2018). Such innovation resulted in large amounts of crop residues on the field, covering soil surface around 10-20 Mg ha⁻¹ (dry mass) of straw each year (Menandro et al., 2017). Even though part of this straw could be removed for industrial uses

(i.e., bioethanol and bioelectricity production), its retention in the field provides several benefits for soil quality (Cherubin et al., 2018). Residual retention positively impacts carbon stock and nutrient cycling, storage, and availability through sustainable system (Cherubin et al., 2018). However, there is no consensus about the minimum straw level recommendable to provide such benefits, but indiscriminate straw removal may magnify soil damages (Carvalho et al., 2017; Cherubin et al., 2018).

Biology is the most sensitive soil property affected by agriculture change in soil management (Kaschuk, Alberton, and Hungria, 2011). Soil microbes, specifically, drive organic matter decomposition and soil nutrient cycling, that allow nutrient recycling to be absorbed over again by plant root (Cusack et al., 2011; Cherubin et al., 2018; Sauvadet et al., 2019). Several studies described that sugarcane straw increases microbial biomass and diversity in soil surface layers (Graham and Haynes, 2006; Souza et al., 2012; Liao et al., 2014; Paredes, Portilho, and Mercante, 2015). Microbial promotion is closely related to organic matter amount, then stimulated by straw maintenance. Microorganisms respond to organic matter composition and its dynamic of decomposition (Palm et al., 2014; Sauvadet et al., 2019). Sugarcane straw is composited by cellulose (40-44 %), hemicellulose (30-32 %), and lignin (22-25 %) (Meier et al., 2006; Robertson and Thorburn, 2007) with a high C: N ratio (70: 1 to 120: 1) (Gomez et al., 2010; Cherubin et al., 2018). These complex polymers act as barriers to microbial degradation and characterize a slow decomposition with disproportional and delayed nutritional release (Hagen-Thorn et al., 2004; Carvalho et al., 2017; Lorenz and Thiele-Bruhn, 2019).

Nitrogen (N) and phosphorus (P), the most required nutrients for plant growth, have their availability affected, at least in the short-term, by sugarcane straw composition and abundance (Cherubin et al., 2018). In superficial layers, or on the depth where crop residues are affecting soil, the high C: N material induces N immobilization (Trivelin et al., 2013; Karlen and Johnson, 2014; Meier and Thorburn, 2016). This possibly drives microbial selection to organisms that are capable of source N from other forms, such as from converting atmospheric N₂ into ammonia (i.e., N fixation) (Gentile et al., 2011; Smercina et al., 2019). Such a reaction is highly expensive, requiring energy from organic molecules oxidizing (Smercina et al., 2019). At the same time, P, abundantly contained in sugarcane straw (about 0.41 kg ha⁻¹ per ton) (Trivelin et al., 2013), is the key nutrient forming adenosine triphosphate (ATP), the energetic molecule required for N₂ fixation (de Souza, Ambrosini, and Passaglia, 2015; Smercina et al., 2019). P is mineralized from organic matter, or solubilized from inorganic stocks, through microbial mediated processes (Rashid et al., 2016; Grafe et al., 2018). Such as N fixers, P solubilizer organisms also respond to soil conditions and may present a living advantage in P scarce environment (Grafe et al., 2018). The link between N-fixation and P-solubilization is important to comprehend microbial association and assemble in different crop management. These directly contribute to environmental services and crop sustainability.

Thus, understanding the soil microbial behavior and functions mainly considering different levels of sugarcane straw is essential to design a better management practice for crop residues. This study aimed further investigate how four different levels of straw maintenance affect bacterial communities' composition and potential functions. The project analyzed a 6-years cumulative effect experiment testing 4 levels of sugarcane straw leftover soil. We sampled soil in five layers, from 0 – 20 cm to deeply monitor how top straw affects bacterial ecology. We assessed bacterial richness, diversity, abundance, and assembly as well as N-fixing and P-solubilization functional genes. Our project highlighted important factors that improve bacterial community dynamic and straw management comprehension.

3.2 Methodology

3.2.1 Site description, experimental design, and sampling

Sugarcane study area was a six years long experiment located at the Experimental Area of Iracema's sugarcane mill in the northeastern Piracicaba city, Sao Paulo State, Brazil. The climate is dry winter subtropical (Cwa according to Köppen classification), with an annual average temperature and precipitation of 20.4 °C and 1294 mm, respectively; and the soil is classified as Rhodic Eutrudox by USDA soil taxonomy (USDA, United States Department of Agriculture, 1998).

The experimental field was arranged in a randomized block design composed of four treatments and four replicates. Each of the 16 plots had an area of 120 m² (10 m long by 12 m wide) containing eight sugarcane rows and 1.5 m of space between them. To simulate different straw removal rates, it was established 15 Mg ha⁻¹ residue of dry straw as total residue amount naturally left on the soil surface. Then, the treatments were chosen as: a. total straw removal (TR); b. 5 Mg ha⁻¹ (high removal, HR); c. 10 Mg ha⁻¹ (low removal, LR); and d. 15 Mg ha⁻¹ of sugarcane straw topsoil residual (no removal, NR).

The soil was sampled along with five layers as follows: 0–2.5 cm, 2.5–5.0 cm, 5–10 cm, 10–15 cm, 15–20 cm; between sugarcane rows of each experimental treatment and replicates (Figure 10). The soil sampled on sugarcane experimental area for chemical and biological analysis (September 2019) was properly stored and frozen after sampling.

Figure 10. Sugarcane crop system arranged in a randomized block design experimental area containing four treatments (total sugarcane straw removal, high removal, low removal, and no removal) and four repetitions, where the dimensions of each block are 10 x 12 m, and the sugarcane rows are 1.5 m distance. The red arrow indicates the point where the soil was collected, in between the rows.

3.2.2 Soil chemical analysis

Chemical analysis was determined by Soil Department Laboratories of Luiz de Queiroz College of Agriculture (ESALQ) at the University of Sao Paulo using Raij methodology (Raij, B. van; Andrade, J.C. de; Cantarella and J.A., 2001). There were calculated active acidity (pH); soil organic matter (OM); phosphorus (P); potassium (K); calcium (Ca); magnesium (Mg); potential acidity ($H+Al^{+3}$); and N ammonium ($N-NH_4^+$). Considering these measurements, we calculated the values of the sum of bases (SB), base saturation (V), and cation exchange capacity (CEC).

3.2.3 Enzyme activity

Soil microbial activity was evaluated by β -glucosidase and acid-phosphatase enzyme activities using p-nitrophenyl- β -D-glucopyranoside (0.025 M) and p-nitrophenyl phosphate disodium hexahydrate (0.05 M) as substrates. These measurements were colorimetric determined on 1g of fresh soil sample incubated with 1 mL of respective substrates and 4 mL of Tris (hydroxymethyl) aminomethane buffer solution (pH 6.0 for β -glucosidase and pH 6.5 for acid-phosphatase activities) at 37 °C for 1 hour, following the Tabatabai protocols (Tabatabai, 2018).

3.2.4 Soil DNA extraction

Total genomic DNA was extracted from 0.4 g soil of each sample using Power soil DNA Isolation Kit (MoBio Laboratories a QIAGEN company, Carlsbad, CA, USA) according to manufacturer's instructions. The extraction product was checked and evaluated by 1.5% (p/v) agarose gel 1 x TAE cap solution run, using 1 kb GeneRuler DNA Ladder (Invitrogen). DNA extracted was qualified and quantified by Nanodrop 1000 (Thermo Scientific) and Qubit 2.0 (Invitrogen) equipment, respectively.

3.2.5 Real-time PCR analysis

Real-time PCR (qPCR) was performed to quantify the numbers of bacterial marker genes used as proxies for total bacteria (*16S rRNA*), markers of N fixation (*nifH*), and P mineralization (*phoD*) functions. The amplifications were performed with SYBR Green PCR master mix (Thermo Scientific) and StepOne Software v2.3. *16S rRNA* gene was targeted using 20 μ L reaction volume with 10 μ L Syber, 0.5 μ L of each primer P1 (5' CCT-ACG-GGA-GCG-AGC-AG 3') and P2 (5' ATT-ACC-GCG- GCT-GCT-GG 3') (5.56 μ M) (Muyzer, De Waal and Uitterlinden, 1993), and 0.3 mg mL⁻¹ of bovine serum albumin (BSA). *16S rRNA* reaction conditions followed: 10 min at 95 °C, and 35 cycles of 30 s at 94 °C, 30 s at 55 °C and 30 s at 72 °C. *NifH* gene was quantified using 25 μ L reaction volume with 12.5 μ L Syber, 0.4 μ L of each primer FGPH19 lab43 (5' TAC-GGC-AAR-GGT-GGN-ATH-G 3') (Simonet et al., 1991) and PolR (5' ATS-GCC-ATC-ATY-TCR-CCG-GA 3') (100 μ M) (Poly, Monrozier and Bally, 2001), and 0.6 mg mL⁻¹ of BSA. *NifH* reaction conditions followed: 15 min at 95 °C, and 40 cycles of 1 min at 95 °C, 27 s at 55 °C and 1 min at 72 °C. *PhoD* gene was

amplified using 20 µl reaction volume with 10 µl Syber, 0.5 µl of each primer ALPS-F730 (5' CAG-TGG-GAC-GAC-CAC-GAG-GT 3') and ALPS-R1101 (5' GAG-GCC-GAT-CGG-CAT-GTC-G 3') (100 µM) (Sakurai et al., 2008), and 0.5 mg mL⁻¹ of BSA. *P_hoD* reaction conditions followed: 10 min at 95 °C, and 40 cycles of 30 s at 95 °C, 1 min at 57 °C and 30 s at 72 °C. Each reaction generated amplicon sizes of 193, 429, 371 bp for *16S rRNA*, *nifH* and *p_hoD* markers. In all the amplifications the melting curve were performed to confirm the purity of the amplicon fragment and the data were expressed as log numbers of copies of gene per gram of soil.

3.2.6 *16S rRNA* gene sequencing and data processing and analysis

Bacterial community was amplified targeting V4 region of *16S rRNA* gene (Caporaso et al., 2012) and PGM Ion Torrent Technology (Thermo Fisher Scientific, Waltham, MA, USA). PCR amplification used 25 µL final volume composed by 4 µL of 10× High Fidelity PCR buffer, 2 mM of MgSO₄, 2U of Taq Polymerase, 0.2 mM of dNTP Mix, 25 µg of Ultrapure BSA (Invitrogen, Carlsbad, CA, USA), 0.1 µM of each primer 515F and 806R (Caporaso et al., 2012), and ~50 ng of DNA template. Reaction conditions followed: 5 min at 94 °C, and 30 cycles of 45 s at 94 °C, 45 s at 56 °C and 1 min at 68 °C. PCR products were purified and quantified using Agencourt® AMPure® XP Reagent (Beckman Coulter, Brea, CA, USA) and Qubit Fluorometer kit—DNA High Sensitivity Assay kit (Invitrogen, Carlsbad, CA, USA). Further, the product was combined in equimolar ratios and sequenced using Ion PGM™ Sequencing 400 kit on an Ion PGM™ System, using the Ion 318™ Chip v2.

Raw sequencing reads were pre-processed using USEARCH version 7.0.1090 (Edgar, 2010) and QIIME ver. 1.9.1 (Caporaso et al., 2010). Raw data were processed using QIIME and UPARSE standard pipeline57, following the Brazilian Microbiome Project (Pylro et al., 2014). In short, reads were clustered using the UPARSE method at 97% similarity cutoff, to assess OTU files, and aligned against Greengenes database version 13.8 (DeSantis et al., 2006) reference database (version 13.8). After, unassigned reads were removed, and the dataset were rarified to further analysis.

3.2.7 Statistical analysis

We performed one-way analysis of variance (ANOVA) to assess the difference in chemistry, activity of enzymes, and genes' quantification data among the four levels of straw. There were calculated ANOVA and Tukey test at 0.95 significance to compare the means using Sisvar software version 5.7 (Hammer, Harper and Ryan, 2001). To evaluate the data considering soil depth at the same treatment, there was analyzed the difference among the layers using ANOVA in a mixed procedure of SAS Software (SAS, 2018). We used ante-dependence covariance structure and compared the means differences using the Tukey test at 0.95 significance for each variable and each treatment.

We assessed bacterial richness and diversity calculating Chao1, Shannon-H, and Simpson indexes using one-way ANOVA t-test in Microbiome Analyst (Dhariwal et al., 2017). PCoA and Permanova (Anderson, 2001) were calculated using PAST 3 Software (Hammer, Harper, and Ryan, 2001). We calculated the bacterial differences among soil layers and into straw residue treatments. To assess bacterial interaction and community parameters, we calculated co-occurrence network using Sparcc correlation (Friedman and Alm, 2012). Such correlations were performed based on

their strength, magnitude of $p > 0.6$ or $p < -0.6$ and a significance of $p < 0.03$. Topological properties were then calculated using Gephi version 0.9.2 software (Bastian, Heymann, and Jacomy, 2009).

3.3 Results

3.3.1 Soil chemistry

In general, chemistry analysis indicated that different straw residue maintenance on soil surface changed soil chemical and nutritional availability in soil from 0 to 20 cm depth. TR showed the lowest value of OM in 0–2.5, 2.5 – 5, 5–10, and 15 – 20 cm soil depths, besides the lowest P content in all layers compared to straw residue maintenance. On the other hand, NR presented the highest amount of OM in 0–2.5, 5–10, 10 – 15, and 15 – 20 cm soil depths compared to other treatments. Between 5 and 15 cm, NR showed the highest CEC and lowest SB compared to other treatments. N ammonium was higher under TR from topsoil to 10 cm depth, followed by a higher value under NR treatment from 10 to 20 cm (Table 3).

Table 3. Soil chemical properties of different treatments of straw residue.

Treatments	pH		OM		P		K		Ca		Mg		H+Al ³⁺		SB		CEC		V		N-NH ₄ ⁺	
	CaCl ₂		g.dm ⁻³		mg.dm ⁻³		mmolc.dm ⁻³												%		mg.kg ⁻¹	
0 – 2.5 cm																						
TR	4.63	d	29.3	d	39.7	d	7.43	a	22.6	d	11.7	c	54.7	b	41.8	d	96.4	c	43.0	d	82.0	a
HR	4.73	b	32.3	c	65.3	a	4.16	d	32.3	c	13.7	b	46.7	c	50.2	c	96.8	b	52.3	b	70.3	c
LR	4.83	a	38.0	b	55.7	b	5.13	b	35.3	b	14.7	a	36.7	d	55.1	b	91.8	d	59.7	a	57.3	d
NR	4.67	c	40.3	a	54.7	c	4.23	c	38.6	a	14.7	a	62.3	a	57.6	a	119.9	a	48.0	c	80.7	b
p	<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001	
2.5 – 5 cm																						
TR	4.77	b	24.3	d	27.0	d	5.63	a	25.0	c	11.7	c	45.3	b	42.3	c	87.6	c	48.3	c	75.0	a
HR	4.90	a	26.3	a	79.3	a	4.17	c	38.7	a	15.3	a	38.3	c	58.2	a	96.5	a	60.3	b	45.7	d
LR	4.77	b	25.7	c	76.7	b	4.73	b	33.7	b	13.3	b	33.3	d	51.7	b	85.1	d	60.7	a	63.3	c
NR	4.50	c	26.0	b	44.3	c	3.63	d	24.0	d	10.0	d	58.0	a	37.6	d	95.6	b	39.3	d	67.0	b
p	<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001	
5 – 10 cm																						
TR	4.80	b	20.0	d	21.0	d	4.57	a	21.7	d	10.7	c	45.3	b	36.9	c	82.2	c	44.3	c	95.0	a
HR	5.07	a	23.0	b	92.3	a	3.37	c	39.7	a	14.7	a	34.3	c	57.7	a	92.0	b	62.3	a	62.0	d
LR	4.73	c	22.7	c	77.7	b	3.57	b	27.7	b	11.0	b	33.7	d	42.2	b	75.9	d	53.7	b	84.3	b
NR	4.50	d	26.0	a	45.7	c	3.57	b	22.3	c	8.7	d	59.3	a	34.6	d	93.9	a	37.7	d	71.3	c
p	<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001	
10 – 15 cm																						
TR	4.47	c	19.0	c	20.7	d	3.60	a	16.7	c	9.7	b	56.3	b	29.9	b	86.3	b	34.3	c	56.3	c
HR	4.73	a	19.3	b	35.0	a	3.00	c	26.3	a	11.0	a	38.3	d	40.3	a	78.7	c	48.3	a	56.3	c
LR	4.57	b	17.0	d	23.7	c	3.17	b	17.0	b	8.0	c	43.7	c	28.2	c	71.8	d	41.3	b	57.7	b
NR	4.23	d	20.0	a	24.0	b	2.67	d	15.0	d	6.3	d	64.7	a	24.0	d	88.7	a	28.3	d	71.3	a
p	<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001	
15 – 20 cm																						
TR	3.97	d	13.7	d	14.3	d	2.57	a	5.7	d	4.667	c	82.7	a	12.9	d	95.6	b	15.0	d	61.0	c
HR	4.57	a	15.7	c	16.3	c	2.50	b	16.0	a	8.333	a	39.0	c	26.8	a	65.8	d	39.7	a	62.3	b
LR	4.20	c	17.7	b	21.3	a	2.10	d	8.7	c	4.667	c	53.3	b	15.4	c	68.8	c	24.3	b	57.3	d
NR	4.27	b	19.3	a	17.3	b	2.43	c	11.7	b	5.667	b	82.7	a	19.8	b	102.4	a	23.7	c	75.0	a
p	<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001	

The values are means and different letters in the column present significant difference ($P < 0.05$) among the treatments in each soil layer. TR – Total Remove; HR – High Remove; LR – Low remove; NR – No Remove.

3.3.2 Enzyme activity

β -glucosidase and acid-phosphatase enzyme activities presented differences among the straw treatments in each layer analyzed. TR showed the lowest β -glucosidase activity in 0 – 2.5, 2.5 – 5, and 10 – 15 cm depth. Besides, TR presented the lowest acid-phosphatase activity in 0 – 2.5 and 15 – 20 cm depth. On the other hand, HR presented the highest β -glucosidase activity in the first 10 cm of soil, followed by NR as the second higher from 0 – 5, and third in 5 – 10 cm. In addition, we found that NR showed the highest or second higher value of acid-phosphatase activity in all the layers (Figure 11).

Further, into each treatment, enzyme activities showed differences among the layers, decreasing their activities as depth increased. β -glucosidase activity presented a slower decrease along with the layers with straw maintenance compared with TR. Moreover, NR presented a more subtle curve of fall activity compared to HR and LR, which is also demonstrated for acid-phosphatase activity. However, TR showed a slower decrease of acid-phosphatase activity along with the layers compared to HR and LR, which had higher activity in 0 – 2.5 cm layer and similar from 2.5 cm further (Figure 11).

Figure 11. a. β -glucosidase and b. acid-phosphatase activity along soil 0–2.5, 2.5–5, 5–10, 10–15, and 15–20 cm depths. Red, yellow, blue, and green contour represent TR, HR, LR, and NR treatments, respectively. Different capital letters in the column present significant difference between the crop system in each layer (Tukey test $P \geq 0.05$), and small letters show significant difference among the layers in each system (Tukey test $P \geq 0.05$).

3.3.3 Gene abundance of *nifH* and *phoD* functional genes

The number of copies of *nifH* and *phoD* genes were normalized to the number of copies of *16S rRNA* gene, considering the functional genes' relative abundance in each treatment and layer. Both relative abundances presented differences due to the amount of straw residue in each depth analyzed. We found a higher relative abundance of *nifH* gene under TR compared to others in 0–2.5 and 5–10 cm layers. On the other hand, in 2.5–5 and 10–15 cm, NR showed more abundance of *nifH* gene. Relative abundance of *phoD* presented higher values in LR from topsoil to 20 cm, whereas the lowest abundances were in TR in 0–2.5 cm, NR from 2.5–15 cm, and TR in 10–20 cm (Figure 12).

Comparing the relative abundances among the layers into each treatment, *nifH* and *phoD* genes presented different patterns in each treatment along with the soil depth. The *nifH* relative abundance showed a similar pattern in TR, HR, and NR with no difference from 0–20 cm depth. On the other hand, LR presented a slow decrease of *nifH* abundance along with the increase of soil depth (Figure 12a). The gene *phoD* presented different patterns of relative

abundance in each treatment. NR presents a slower decrease of *phoD* relative abundance compared to other treatments, although all the treatments show *phoD* abundance decrease with increasing soil depth (Figure 12b).

Figure 12. a. *NijH* and b. *PhoD* genes relative abundance along with soil 0–2.5, 2.5–5, 5–10, 10–15, and 15 – 20 cm depths. Red, yellow, blue, and green contour represent TR, HR, LR, and NR treatments, respectively. Different capital letters in the column present a significant difference between the crop system in each layer (Tukey test $P \geq 0.05$), and small letters show significant differences among the layers in each system (Tukey test $P \geq 0.05$).

3.3.4 Bacterial community richness, diversity, structure, and composition

A total of 2,558,890 high-quality reads were obtained through *16S rRNA* sequencing (on average 31,986 reads per sample), from which it was identified 19,468 OTUs. Chao1 and Shannon analysis showed a difference of bacterial richness and diversity in 0 – 2.5 cm, 2.5 – 5 cm depth, and only diversity change in 15 – 20 cm comparing among soil treatments (Figure 13). Into system treatments, TR and HR presented a change in soil richness and diversity among soil

layers, LR changed just bacterial diversity, and it was found no significant difference of community richness and diversity into NR system (Figure 13).

Figure 13. a. Bacterial richness and b. diversity along with soil 0–2.5, 2.5–5, 5–10, 10–15, and 15 – 20 cm depths. Red, yellow, blue, and green contour represent total remove, high remove, low remove, and no remove treatments. Different capital letters in the column present significant differences between the crop system in each layer (Tukey test $P \geq 0.05$), and small letters show significant differences among the layers in each system (Tukey test $P \geq 0.05$).

In agreement with richness and diversity analysis, PCoA and Permanova presented bacterial structure differences in 0 – 2.5 and 2.5 – 5 cm depth comparing straw residual levels. It is indicated that the community structure is most affected in the first 5 cm of soil for topsoil residues (Figure 14). However, in each system, there were found significant differences in bacterial structure under TR, HR, and LR comparing the 5 layers, whereas there was no change in the NR system (Figure 15). PCoA suggests that from TR to NR, the bacterial community structure gradually approaches until they become similar.

Figure 14. PCoA plot of bacterial community structure in each soil depth under four straw residue treatments. **a.** 0 – 2.5, **b.** 2.5 – 5, **c.** 5 – 10, **d.** 10 – 15, **e.** and 15 – 20 cm depth layers.

Figure 15. PCoA plot of bacterial community structure along soil depths in four straw residue treatment. **a.** Total remove, **b.** high remove **c.** low remove, and **d.** no remove.

3.3.5 Network co-occurrence analysis

To evaluate bacterial community interactions under different straw residual treatments in each soil layer, we calculated bacterial genera co-occurrence and topological parameters which are summarized in Table 4 and plotted in Figure 16 and 17. In general, TR and NR showed more similarity in topological properties along with the depth compared to HR and LR. TR presented a gradual decrease of bacterial competition as depth increased, which were distributed in other straw treatments.

Comparing the effect of different levels of straw released in soil surface in each depth, it is shown that as depth increases, topological parameters tend to be more similar among the treatments. We found higher competitiveness in LR compared to other treatments in layers 0 – 2.5, 2.5 – 5, and 15 – 20 cm. HR presented higher average connectivity from topsoil to 15 cm. TR presented higher average path length in 0 – 2.5, 2.5 – 5, and 10 – 15 cm, describing a more distant correlation among the nodes. TR also showed higher network diameter in 0 – 2.5, 2.5 – 5, 5 – 10, 10 – 15 cm, and the second higher from 15 – 20 cm, next to HR. In addition, we found that from 15 – 20 cm, LR and NR showed a higher number of edges and competitiveness.

Table 4. Bacterial network properties under four treatments in each 0–2.5, 2.5–5, 5–10, 10–15 and 15 – 20 cm soil depths.

	0 – 2.5 cm				2.5 – 5 cm			
	TR	HR	LR	NR	TR	HR	LR	NR
Number of nodes	1687	1582	1925	1585	1464	1564	1372	1159
Number of edges	16165	24870	25614	17121	14113	24865	17864	13092
Positive correlation (%)	61.9	64.4	58.9	63.6	64.2	66.8	56.9	80.3
Negative correlation (%)	38.1	35.6	41.1	36.4	35.8	33.2	43.1	19.7
Positive/negative ratio	1.62	1.81	1.43	1.75	1.79	2.01	1.32	4.07
Avg degree	19.16	31.44	16.61	21.60	19.28	31.80	26.04	22.59
Graph density	0.011	0.02	0.014	0.014	0.013	0.02	0.019	0.02
Avg clustering coefficient	0.338	0.492	0.381	0.355	0.378	0.414	0.47	0.362
Modularity	2.152	1.424	2.648	1.925	1.718	1.149	2.955	0.752
Number of modules	140	199	145	167	175	196	240	122
Average path length	4.71	4.119	4.178	4.518	4.534	4.079	4.031	4.389
Diameter	15	12	10	13	13	12	12	13
	5 – 10 cm				10 – 15 cm			
	TR	HR	LR	NR	TR	HR	LR	NR
Number of nodes	1671	1570	1933	1841	1943	1792	1859	1773
Number of edges	16427	30964	20001	24675	16149	22115	16155	20972
Positive correlation (%)	67.3	57.2	65.6	65.7	67.1	64.2	67.0	70.7
Negative correlation (%)	32.7	42.8	34.4	34.3	32.9	35.8	33.0	29.3
Positive/negative ratio	2.06	1.34	1.90	1.92	2.04	1.79	2.03	2.41
Avg degree	19.66	39.45	20.69	26.81	16.62	24.68	17.38	23.66
Graph density	0.012	0.025	0.011	0.015	0.009	0.014	0.009	0.013
Avg clustering coefficient	0.358	0.501	0.331	0.343	0.317	0.363	0.357	0.356
Modularity	1.582	3.07	1.578	1.477	1.685	1.659	1.619	1.246
Number of modules	174	109	90	152	102	111	132	160
Average path length	4.41	3.67	4.73	4.58	4.83	4.34	4.52	4.46
Diameter	11	12	12	13	12	12	10	12
	15 – 20 cm							
	TR	HR	LR	NR				
Number of nodes	1368	1087	1654	1357				
Number of edges	15353	1244	18114	17229				
Positive correlation (%)	74.9	77.3	63.3	72.6				
Negative correlation (%)	25.1	22.7	36.7	27.4				
Positive/negative ratio	2.99	3.40	1.72	2.65				
Avg degree	22.45	22.90	21.90	25.39				
Graph density	0.016	0.021	0.013	0.019				
Avg clustering coefficient	0.357	0.415	0.386	0.41				
Modularity	1.117	0.946	1.802	1.118				
Number of modules	117	139	163	174				
Average path length	4.48	4.21	4.51	4.28				
Diameter	13	14	12	13

Figure 16. Network topological proprieties plot under four treatments in each soil depth. **a.** 0 – 2.5, **b.** 2.5 – 5, **c.** 5 – 10, **d.** 10 – 15, **e.** and 15 – 20 cm depth layers.

Figure 17. Network topological proprieties plot along soil depths in four straw residue treatments. **a.** Total remove, **b.** high remove **c.** low remove, and **d.** no remove.

Figure 18. Network of co-occurrence bacterial genera based on correlation analysis in four straw residue treatments. **a.** Total remove, **b.** high remove **c.** low remove, and **d.** no remove.

3.4 Discussion

Crop residue maintenance in soil surface is a key factor for proper soil functioning and nutritional dynamics (Cherubin et al., 2018). Plant residues are nutrients sources for soil living organisms and subsequent crops, enhancing soil organic material and, as a consequence, soil quality (Palm et al., 2007; Verhulst et al., 2010; Cherubin et al., 2018). Simultaneously, the adoption of crop harvested maintenance promotes soil protection against erosion and extreme temperature, promoting environmental stability (Ward et al., 2013; Rabot et al., 2018). Soil microbial community is very sensitive to soil management and it is known to respond differently to environmental changes and external practices (Gupta, Roper, and Thompson, 2020). Microbes are widely involved in nutrient cycling, soil quality, and plant development, thus important property to evaluate soil management practices (Vargas Gil et al., 2009; Fierer, 2017; Liu et al., 2019). The main goal of this study was to evaluate the effect of four levels of sugarcane straw left on the soil surface in bacterial community, activity, N fixing, and P-solubilizing functions. Besides, we studied the effect of straw residues in bacterial assembly and topological parameters to further assess changes in bacterial structure and co-occurrence.

3.4.1 Effects of straw residue on bacterial activity and functional gene abundance

Straw residues maintenance provides continuous input of OM and is described as enriching nutrients in the soil system (Turmel et al., 2015; Cherubin et al., 2018). Soil OM is an indicator of soil quality and directly impacts soil stability and nutrient availability (Verhulst et al., 2010). Our results showed that the maximum level of sugarcane straw presented the highest or the second higher values of soil OM in all the layers analyzed. However, our results showed that N-ammonium presented the highest values under TR in the first 10 cm depth, and under NR from 10 cm further. It is expected that intense decomposition of high C:N ratio material, as sugarcane straw, may induce N immobilization and decrease its availability (Trivelin et al., 2013; Karlen et al., 2014; Ferreira et al., 2016; Meier and Thorburn, 2016). The results of soil enzymes activity, particularly β -glucosidase, showed that straw maintenance treatments presented a higher mineralization rate comparing to TR from 0 – 5 cm. β -glucosidase is an indicator of soil activity and OM mineralization, involved in the final step of plant residues decomposition (Acosta-Martínez et al., 2003; Haynes, 2005; Murphy, 2014; Adetunji et al., 2017). However, from 5 cm further, β -glucosidase activity did not impact N availability. We found high β -glucosidase activity in high N content layers, especially from 10 – 20 cm, where N and β -glucosidase presented the highest values under NR. We suggest that from up to 10 cm depth, topsoil residues do not induce N immobilization, even though increasing soil OM content and mineralization activity. On the other hand, acid-phosphatase activity presented variable results among treatments in soil layers. Acid-phosphatase is widely involved in P mineralization and directly contributes to soil P supply (Alori, Glick and Babalola, 2017; Bargaz et al., 2018). However, our results showed that even though the enzyme did not present the lowest activity under TR from 2.5 – 10 cm, TR presented the lowest P values in all the layers analyzed. Organic P can represent up to 30% of total P in the soil, which indicates that microbial action and organic material are essential for P bioavailability and cycling (Shen et al., 2011). Thus, we speculate that under low OM conditions, acid-phosphatase activity does not reflect in total P availability, especially for crop supply.

We further investigated N-fixing (*nifH*) and P-solubilizing (*pboD*) genes abundances under different straw levels to assess these functions in soil. It is well known that crop practices impact microbial functionality and nutritional cycling (Wang and McSorley, 2005; Liu et al., 2020). We found higher *nifH* relative abundance under TR in 0 – 2.5 and 5 – 10 cm layer, in which we have the highest N and lowest OM values. These are not a common result and further investigation is needed to understand them. We expected that high N availability and no sugarcane straw would decrease N-fixation stimulus. N-fixing is an energetically expensive reaction, requiring carbon resources and ATP (Hill S., 1992; Smercina et al., 2019). Besides, N-fixing is closely related to C:N ratio of OM material (Roper and Gupta, 2016). The mineralization of high C:N material can promote N-fixing population and functionality in order to increase N supply, while available ammonium N is assimilated by other organisms (Kavadia et al., 2007). N-fixing is mediated via nitrogenase enzyme, a strongly sensitive molecule to oxygen presence (Reed, Cleveland and Townsend, 2011; Smercina et al., 2019). Although N-fixing organisms have mechanisms to protect the rapidly and irreversibly inhibition of nitrogenase by oxygen, in aerated soils, aggregates are essential to provide multiple oxygen conditions to proper soil functioning system (Reed, Cleveland, and Townsend, 2011; Roper and Gupta, 2016). These factors highlight the importance of environmental conditions in N-fixers composition and functionality (Reed, Cleveland, and Townsend, 2011; Roper and Gupta, 2016; Smercina et al., 2019). Contrasting with TR, our results showed that NR and LR presented the second and third higher values of *nifH* in 0 – 2.5, 5 – 10 layers, in addition to being the highest values in 2.5 – 5 cm depth. In these senses, the high OM content and mineralization, previously described by enzymatic activities under NR and LR, may have stimulated N-fixing gene abundance, and potential functionality.

From 10 cm further, straw residues treatments showed different influences in *nifH*. NR continued presenting high amounts of *nifH* in 10 – 15 and 15 – 20 cm depth, whereas LR showed the lowest relative abundance values. LR showed the lowest OM and high ammonium N values in 10 – 15 cm, and high OM and the lowest ammonium N in 15 – 20 cm. Although it is expected contrasting responses of *nifH* relative abundance between the two environmental conditions, we found the lowest β -glucosidase activity under LR in 15 – 20 cm. This can suggest a low decomposition rate, and consequently, a low requirement for N-fixing. However, using the maximum level of sugarcane straw, we found high *nifH* relative abundance with the highest OM and ammonium N values. Thus, we suggest that NR promotes a high potential N-fixing from topsoil to 20 cm depth, which reflects in the highest N availability from 10 cm further compared to other treatments. In addition, potential N-fixing promotion keeps happening even though we found higher values of ammonium N in soil under NR.

P is a key nutrient in energy production as ATP constituent, and it is known to directly influence N-fixation (Reed, Cleveland, and Townsend, 2011, 2013). Some studies indicate that P limitation may have more impact in N-fixing than N availability, strongly driving the reaction (Reed et al., 2007; Reed, Cleveland, and Townsend, 2013; Zheng et al., 2016). Thus, we investigated soil *pboD* gene abundance, which assists P-mineralization and release for soil functioning (Kageyama et al., 2011). Here, *pboD* gene relative abundance presented the highest values under LR in all the layers. It is expected that P availability decreases phosphatase encoding gene abundance (Arantes et al., 2020). However, under LR, all the layers presented high values of available P. Gene *nifH* presented high abundance under LR from 0 to 10 cm depth, whereas showed the lowest abundances between 10 – 20 cm, as described previously. In our study, *pboD* gene abundance is not directly related to *nifH*. We found the lowest *pboD* relative abundance under TR in 0 – 2.5 cm and 15 – 20 cm, and NR from 2.5 – 15 cm. As noted previously, soil OM is essential for soil P supply and cycling. Evaluating the values of

OM, P, and acid-phosphatase activity in the same samples we measured the lowest *phoD* gene abundances, TR was the only treatment in which we found the four variables with the lowest values. Thus, we suggest that TR system may be exhausting soil available P for soil functioning, especially for N-fixing, which presented the highest potential in 0 – 2.5 cm. On the other hand, high OM mineralization and acid-phosphatase activity can suppress P demanded to N-fixing and other functions, particularly under NR, even though it is not seen *phoD* gene potential.

3.4.2 Effects of straw residue on bacterial community diversity, structure, and assembly

Straw retention and continuous input of soil OM are known to enhance soil richness, evenness, and diversity (Verhulst et al., 2010; Liu et al., 2020). However, several studies described the impact of straw maintenance in soil surface being restricted on shallow layers, especially in the first 10 cm of soil (Graham and Haynes, 2006; Souza et al., 2012; Rachid et al., 2013, 2016; Liao et al., 2014; Paredes, Portilho and Mercante, 2015). Here, different levels of straw altered bacterial community richness in 0 – 2.5 and 2.5 – 5 cm, and diversity index in 0 – 2.5, 2.5 – 5, and 15 – 20 cm depth. A microbial-rich and diverse environment is more likely to perform key functions in soil and fulfill required ecosystem services and nutrient cycling (Peter et al., 2011). Our results showed similar richness and diversity in opposite straw treatments in the first 5 cm of soil. It is expected that straw maintenance provides continuous substrate supply for soil health and favors specific groups, enhancing microbial diversity and abundance (Liu et al., 2020). However, crop management impacts on soil microorganisms are complex and diverse, often presenting ambiguous responses (Bünemann, Schwenke and Van Zwieten, 2006; Nelson and Spaner, 2010; Hartmann et al., 2015). Some studies described differences in microbial structure and interactions, which may yield decrease microbial diversity even in rich and diversified environments (Chen et al., 2018). PCoA analysis presented bacterial structure differences in the first 5 cm depth. It can suggest that even though TR and NR presented similar diversity metrics, their community might be structured differently in response to different management practices.

Evaluating microbial diversity throughout the soil profile, some studies have described little variation in microbial richness and diversity along with soil depth (Zhang et al., 2019). Here, our results showed that NR was the only treatment with no change in bacterial richness, diversity, and community structure along the layers analyzed. Straw retention is an important factor protecting soil surface from temperature and moisture variation while increase soil OM supply (Balota et al., 2003; Helgason, Walley and Germida, 2010a, 2010b; Sapkota et al., 2012; Liu et al., 2016). PCoA results showed a significant difference in communities' structure among the layers under TR, HR, and LR. Besides, we found a progressive overlap of communities' layers structures comparing from TR to NR. It is clear that as the straw level increases, the community structures of each layer get closer until total overlap in NR. Although NR did not present higher diversity indices in the first 5 cm depth compared to other treatments, we suggest that total straw retention provides a more stable and nutritive environment for the bacterial community from soil surface to 20 cm. Soil stability is essential to maintain soil proper functioning and deliver environmental services (Allison and Martiny, 2008; Li et al., 2020). This may provide a more resilient and resistant community to stresses and environmental changes (Mariotte et al., 2018).

Network co-occurrence analysis was performed at genera level to further assess bacterial community assembly and interactions. Network is a good parameter to deeply evaluate soil quality and health (Karimi et al., 2017). Here, the number of edges and connectivity were the lowest, or second lower, under TR than under straw use treatments. Moreover, TR presented a high average path length and network diameter, representing a more distant connection between nodes. Low connectivity and high average path length indicate the community is slightly connected. Several studies have linked a highly connected community to environmental stability, efficient use of nutrients, and pathogen suppression (Latz et al., 2012; Mallon, Van Elsas and Salles, 2015; Morriën et al., 2017). Our results showed that TR presented high modularity in the first 15 cm depth and low clustering coefficient in 0 – 2.5, 2.5 – 5, 5 – 10, and 15 – 20 cm. Modularity represents nodes' niche overlap, resources use similarity, or common environmental preferences (Montoya, Yallop, and Memmott, 2015; Poudel et al., 2016). A low clustering coefficient indicates that nodes are partially connected to their neighbors (Deng et al., 2012). In these senses, we suggest that no straw maintenance implies less stability and more vulnerability for the soil environment, committing vital functions and ecological services. The low clustering and high average path length suggest a slower response to environmental stress and soil disturbances.

HR, LR, and NR did not present linear changes in bacterial assembly in response to the progressive increase of the amount of straw released on soil surface. HR presented the highest number of edges and connectivity combined to a lower number of nodes and average path length from topsoil to 15 cm depth. It means that under HR, the bacterial community may have a prompter response to environmental changes, although less diversity of active organisms. NR presented lower modularity and higher density assembly compared to other treatments in the first 15 cm depth. As previously described, it is suggested that low modularity implies in a low similarity of resources requirement or habitat preferences (Poudel et al., 2016). Continuous OM supply, and consequent soil improvement, provide different and multiple microhabitats that may promote microorganisms with different or ample conditions of survival. Additionally, from 15 – 20 cm, NR presented. Simultaneously, a high number of edges, competitiveness, connectivity, density, clustering coefficient, modularity, and low average path length. We found the highest competitiveness in straw treatments in all layers. Competitiveness is a natural response in environments with nutritional supply and increase of microbial biomass (Ghoul and Mitri, 2016). Wei et al., 2015 described that intense competition may be an important factor enhancing soil suppression of pathogens. Overall, even though it is needed more studies to define community structure parameters, we propose that straw use provides a more stable and beneficial environment for bacterial functioning and service delivery. Moreover, we suggest that NR is the best treatment for providing a more quality environment in from 15 – 20 cm. Such benefit may create, in-depth, positive feedbacks, especially for plant performance.

3.5 Conclusion

In summary, we illustrated that sugarcane straw release on soil surface increased soil quality and prompted distinct bacterial communities' structures. Total straw maintenance may improve soil ammonium N and potential N-fixing from 10 cm further. However, the impact of different levels of straw on bacterial community diversity and structure were not linear and more studies are needed to better understand their dynamics under different practices. Co-occurrence analysis is essential to further investigate bacterial interactions under crop practices. Our results propose that

NR provides a more stable and nutrient-rich environment for bacterial living, especially from 15 – 20 cm. Overall, this study reinforces the importance of straw maintaining in soil surface for enhancing microbial dynamic and soil quality.

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4 FINAL CONSIDERATIONS

Permanent soil coverage is essential to promote soil health and quality, improving soil physics and chemistry, protecting soil from erosion, temperature variation, water loss, and providing a more stable environment for soil living organisms. The survey presented in this dissertation has been through the role of soil coverage upon bacterial communities structuring and functioning, highlighting its effect along with soil depth. This attempt aims to connect improvements in microbial parameters with beneficial ecological services, which is fundamental to provide an efficient and resilient system for plant growth. Although it is needed more studies to further investigate microbial quality parameters, our project supports that soil coverage may provide a more stable and healthy system for microbial growth.

Results from distinct crops (coffee and sugarcane) were congruent in several points, for example, the increase of soil OM, different bacterial community structures, and a more stable environment under soil cover. Indeed, due to particularities, *Urochloa* spp. coverage is a living system compared to dead sugarcane straw residues released in soil surface, other parameters analyzed were contradictory. For instance, total sugarcane straw maintenance presented high values of ammonium N from 10 cm further, whereas coffee systems presented no difference. Intercrop system showed higher and more intimate connected community in all the layers, although it is identified under sugarcane NR in 15 – 20 cm. The same difference is observed considering bacterial competitiveness, an important factor for evaluating microbial behavior and response to environmental conditions.

In summary, we propose that crop residues and cover crop improve bacterial community that can benefit and protect plant from pathogen invasion and poor fertility. We reinforce the importance of co-occurrence analysis to deeply assess bacterial assembly and interactions. Overall, our project was essential and contributed to advance in understanding the microbial behavior under different crop managements. This study might help in future design of more productive and sustainable agriculture system.