KIZE ALVES ALMEIDA

RHIZOSPHERE AND SOIL MICROBIOME IN THE MANAGEMENT OF PLANT PATHOGENS AND DISEASES

LAVRAS – MG
2021

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Thesis presented to the Universidade Federal de Lavras, as part of the requirements of the Post Graduate Program in Agronomy/ Phytopathology, area of concentration in Phytopathology, for the degree of Doctor in Philosophy.

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2021

To my ancestors for their guidance, courage and strength and

to my family with love and gratitude,

I dedicate.

Kizzy (*Keisa*, in Mandinka)

means "***to stay put***"

"Uma chama não perde nada ao acender outra chama"

Provérbios africanos.

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RESUMO GERAL

ALMEIDA, K. A. Rhizosphere and soil microbiome in the management of plant pathogens and diseases 2021. **7**0 p. Tese (Doutorado) – Departamento de Fitopatologia, Universidade Federal de Lavras, Lavras, 2021.

As doenças das plantas são uma das principais causas de perdas na agricultura. O solo é um ecossistema diversificado que abriga microrganismos que contribuem para a saúde das plantas, constituindo sua primeira linha de defesa. Métodos alternativos ao uso intensivo da terra, como a rotação de culturas, o plantio direto e a indução de supressividade a patógenos têm sido explorados para aumentar a qualidade do solo e consequentemente a produtividade das plantas. O milho e a soja são os grãos mais cultivados no mundo, enquanto o coco é uma cultura importante para os países tropicais. O estímulo da microbiota do solo, seja pelo uso do solo ou pela indução de supressão, são aliados a um modelo de agricultura mais sustentável. Nesse contexto, no primeiro artigo estudamos o impacto da rotação do milho e da soja na diversidade microbiana do solo e no inóculo de *Fusarium* spp. produtores de fumonisina (*F. verticillioides e F. proliferatum*), patógenos de milho. Os experimentos foram realizados em um período de quatro safras, de 2016/2017 a 2019/2020. Os tratamentos foram soja contínua, milho contínuo e rotação de culturas nas primeiras três safras enquanto plantamos milho em área total na quarta safra para avaliar o impacto dos usos da terra na diversidade microbiana de solo sob cultivo de milho e na sanidade das plantas e inóculo de *Fusarium* spp. que infectam milho. Amostras de solo foram coletadas na colheita de cada estação, enquanto amostras adicionais da rizosfera foram coletadas na safra 2019/2020 em dois momentos: pendoamento do milho e colheita. Além disso, caules de milho foram deixados no campo durante os ciclos das plantas para avaliar a influência do uso da terra na multiplicação do patógeno. Amostras de solo foram sequenciadas utilizando a região marcadora 16SrRNA para Bacteria e Archaea. Os solos foram submetidos a qPCR para quantificação do patógeno utilizando o gene *fum1*, envolvido na produção de fumonisina em *Fusarium verticillioides* e *F. proliferatum*. As sequências resultantes foram agrupadas em unidades taxonômicas operacionais (OTU) a 97% de similaridade. Usamos o valor de Ct (cycle threshold) como medida quantitativa para o inóculo de *Fusarium* spp. *fum1* no solo e nos colmos de milho deixados na superfície do solo durante a safra. Nossos resultados mostraram que a rotação de culturas de três anos não aumentou a diversidade do solo em comparação com o milho ou soja contínua e a precipitação foi responsável pela formação das comunidades nas três primeiras safras. *Fusarium* spp. *fum1* diminuiu sua concentração em solos sob sistema de rotação, embora sua ocorrência tenha diminuído em colmos de milho deixados no campo sob cultivo contínuo de milho. Em acordo, os solos sob cultivo contínuo de milho diminuíram a ocorrência de doenças em plantas de milho cultivadas na última safra. No segundo artigo, avaliamos a natureza da supressividade do solo a *Ceratocystis paradoxa,* fungo causador da resinose do coqueiro. Classificamos 54 solos em níveis de supressividade. Cinco solos supressivos e cinco solos conducivos foram contrastados para determinar a natureza da supressividade. Análises físico-químicas e biológicas do solo foram analisadas para dissecar os principais fatores envolvidos na supressividade. Carbonato de cálcio e microbiota supressora foram testados e comparados quanto ao controle de *C. paradoxa* usando iscas e medindo a colonização e número de peritécios. Populações bacterianas cultiváveis totais foram a principal propriedade biológica implicada na supressividade. O perfil taxonômico do solo

mostrou Actinobacteria, Proteobacteria, Firmicutes e Chloroflexi como os filos mais abundantes em solos contrastantes embora Actionobacteria esteja em maior abundância em amostras supressivas. A microbiota supressiva reduziu a colonização do patógeno em 93,8% em comparação com o controle. Dentre as propriedades físicas e químicas, o teor de areia, pH do solo, cálcio, magnésio, soma de bases, capacidade efetiva de troca catiônica, saturação por bases e alumínio foram maiores em solos supressivos, enquanto o teor de argila e ferro caracterizam os não supressivos. O carbonato de cálcio foi eficaz em reduzir a colonização do patógeno quando comparado às outras fontes. O pH resultante dos tratamentos com cálcio e microbiota variou a neutro e diminuiu significativamente a recuperação do patógeno em iscas de banana. Nosso trabalho contribui para a compreensão do fenótipo supressor em solos brasileiros a *Ceratocystis paradoxa* e sugere o microbioma do solo, o carbonato de cálcio e o pH como os principais determinantes desse fenômeno.

Palavras chave: Microbioma do solo, *Fusarium verticillioides*, *Ceratocystis paradoxa*, 16SrRNA, rotação de culturas, supressividade do solo

GENERAL ABSTRACT

ALMEIDA, K. A. Rhizosphere and soil microbiome in the management of plant pathogens and diseases 2021. **70** p. Tese (Doutorado) – Departamento de Fitopatologia, Universidade Federal de Lavras, Lavras, 2021.

Plant diseases are one of the most causes of loss in agriculture. The soil is a diverse ecosystem which harbor microorganisms that contribute to plant health, being its first line of defense. Alternative methods to intensive land use such as crop rotation, no-till and induction of suppressiveness to pathogens have been explored to increase soil quality and consequently plant productivity. Maize and soybean are the most cultivated grains in the world, while coconut is an important crop for tropical countries. Stimulation of soil microbiota, either by land uses or suppression induction are allies of a more sustainable agriculture model. In this context, in the first paper we studied the impact of maize and soybean rotation in soil microbial diversity and the inoculum of *Fusarium* spp. fumonisin producers (*F. verticillioides* and *F. proliferatum*), a maize pathogen, in the system. We conducted the experiments during seasons 2016/2017 to 2019/2020. Treatments were continuous soybean, continuous maize and rotation as land uses for the first three years and maize in total area in the fourth with the aim to evaluate the impact of land uses on maize soil diversity and pathogen incidence. Bulk soil samples were collected in the harvest of each season, while additional rhizosphere samples were collected in season 2019/2020 in two time points: blooming and harvest. In addition, maize stalks were left in the field during plant cycles to evaluate the influence of land uses on pathogen multiplication. Soil samples were sequenced targeting the 16SrRNA for Bacteria and Archaea and soils were submitted to qPCR targeting the gene *fum1* of *Fusarium verticillioides* and *F. proliferatum*. The resulting sequences were clustered into operational taxonomic units (OTU) at a 97% of similarity threshold. We used cycle threshold as quantitative measure for *Fusarium* spp. *fum1* inoculum. Our results showed three-year crop rotation did not increase soil diversity compared to continuous maize or soybean and precipitation was responsible for shaping communities in the first three seasons. *Fusarium* spp. *fum1* decreased its concentration in soils under rotation systems although its occurrence decreased in maize stalks left in the field under continuous maize. In line, soils under continuous maize have decreased disease occurrence in maize plants cultivated in the last season. In the second paper, we evaluated the nature of soil suppressiveness to *Ceratocystis paradoxa*, a fungus that causes stem bleeding in coconut trees. We classified 54 soils into levels of suppressiveness. The five suppressive and conducive soils were contrasted to determine its nature. Soil physicochemical and biological analyses were analyzed in order to dissect the main factors involved in suppressiveness. Calcium carbonate and suppressive microbiota were tested and compared to the control of C. paradoxa by using baits colonization and number of perithecia. Total cultivable bacterial populations were the main biological property implicated in suppressiveness. Soil taxonomic profile showed Actinobacteria, Proteobacteria, Firmicutes and Chloroflexi as the more abundant phyla in contrasting soils although Actinobacteria showed highest abundance in suppressive samples. Suppressive microbiota reduced pathogen colonization in 93.8% compared to the control. Among physical and chemical properties, sand content, soil pH, calcium, magnesium, sum of bases, effective cation exchange capacity, base saturation, and aluminum were higher in suppressive soils while clay content and iron characterize non-suppressive. Calcium carbonate was effective in reduce pathogen colonization when compared to the other sources. The resulting pH from calcium

and microbiota treatments ranged to neutral and significantly decrease pathogen recovery on banana baits. Our work contributes to the understanding of suppressive phenotype in Brazilian soils to *Ceratocystis paradoxa* and suggest soil microbiome, calcium carbonate and pH as the main drivers to this phenomenon.

Key-words: Soil microbiome, *Fusarium verticillioides*, *Ceratocystis paradoxa*, 16SrRNA, crop rotation, soil suppressiveness

SUMMARY

FIRST PART

1 GENERAL INTRODUCTION

1.1 Soil microbiome

Soil is a complex system composed by minerals, gases, water, organic matter and living organisms. They are critical service providers to humankind and its management is one of the major challenges we have to deal with. Soil microorganisms have been explored in agriculture for years to promote plant growth and to increase productivity by reducing plant diseases, weeds and pests and also helping with extreme weathers (BERG, 2009; CHAPARRO et al., 2012; DÖRING et al., 2020; MENDES et al., 2011). Besides the crucial role microorganisms provide the environment such as nutrient cycling, decomposition and geochemical cycles, just recently they have been recognized as part of soils (VAN ES, 2017). It is estimated that one gram of soil contains a large amount of microbial cells and viruses (MENDES; GARBEVA; RAAIJMAKERS, 2013).

Soil microbiomes consist of a set of microorganisms, their genomes and the theatre of activity in a well-defined environment (WHIPPS; LEWIS; COOKE, 1988; BERG et al., 2020). Some microorganisms can cause plant diseases and food contamination while others can be plant beneficial promoters (MENDES; GARBEVA; RAAIJMAKERS, 2013). Plants recruit their microbiomes according to genotype and age and use them to defend themselves against pathogens, insects and to survive in extreme conditions (ARAUJO et al. 2019; PEIFFER; LEY 2013; MENDES et al. 2014; MENDES et al. 2011; MENDES et al. 2018; CHAPELLE et al. 2016). On the other hand, microorganisms take advantage of plant exudates for their development (MENDES et al., 2014).

The study of soil microbiomes became crucial to many areas, including agriculture, once it can address important questions related to microbial composition and function, resilience to biotic and abiotic disturbances and also to elucidate microbial suppressive or conducive patterns in soil (BERG et al. 2020; LEE et al. 2020; MENDES; RAAIJMAKERS 2015; MENDES et al. 2011). As an analogy to the immune system of mammals, soil demonstrated its own memory as a defense system to limit reinfections by some pathogen (BERENDSEN et al., 2018). Identifying these patterns linked to plant health and disease is the goal of many projects.

1.2 Suppressive soils in plant disease management

In the past years, plant diseases were defined as an interaction among three main factors: a) favorable environment, b) virulent pathogen and c) susceptible host (BAKER; COOK, 1974). On the other hand, with the advance in high-throughput sequencing, microbiomes have been proposed to disrupt this triangle (CHIARAMONTE; MENDES; MENDES, 2021). Disease suppressive soils are those in which "the pathogen does not establish or persist, establishes but causes little or no damage, or establishes and causes disease for a while but thereafter the disease is less important, although the pathogen may persist in the soil" despite the optimal conditions for disease occurrence (BAKER; COOK, 1974).

There are two types of suppression, general and specific (COOK; BAKER, 1983). The general suppression is the first line of plant defense against pathogens and it is associated to soil microbial diversity and also can be increased by adding organic carbon on soil (DAVEY et al., 2019; TOMIHAMA et al., 2016) while the specific is driven by groups of microorganism recruited by plant rhizosphere to protect them against pathogen infection (MENDES et al. 2011; LEE et al. 2020) and can be transferred to conducive soils (ELHADY et al. 2018; MENDES et al. 2011). However, soil suppression can be triggered also for soil physicochemical parameters such as pH, aluminum, iron and sand content (LEE et al., 2020; MEYER, 1991).

The biological nature of disease suppressive soils has been studied with the goal to identify the patterns and groups of microorganisms that govern this phenomenon. For example, a disruption in abundance of Actinobacteria and Firmicutes in soils with *Ralstonia solanacearum* resulted in diseased phenotype of tomato plants (LEE et al., 2020). The same pattern has been shown for *Fusarium* wilt, in which Actinobacteria play an important role in suppression (CHA et al., 2016). Nevertheless, the secondary metabolites produced by fluorescent *Pseudomonas* are responsible for the decline of all disease caused by the fungus *Gaeumannomyces graminis* var *tritici* in wheat (SCHLATTER et al., 2017).

1.3 Crop rotation effects on soil diversity and suppressiveness

Crop rotation consists in planting different crops (usually legumes and grasses) annually in the same area with the goal to improve nutrient cycling, disrupt pathogens life cycle and stimulate soil diversity. It has been postulated that aboveground diversification enriches soil

microbial communities and stimulates a diverse functional services provided to the ecosystem (NIELSEN; WALL; SIX, 2015; TIEMANN et al., 2015). Simplified cropping in agriculture leads to the disease outbreaks because of the high inoculum pressure of the pathogen. In the *Fusarium verticillioides*-maize pathosystem chemical control is widely used despite the ineffectiveness of this type of management when adopted alone (GUIMARÃES et al., 2020). Long term rotational systems showed that prnD (pyrrolnitrin) genes increased in the most diverse rotational system compared to maize monoculture (PERALTA et al., 2018). A meta-analysis containing 27 studies showed that increasing diversity in rotation systems resulted in an increase in 15.11% in microbial richness and 3.36% in diversity (VENTER; JACOBS; HAWKINS, 2016).

1.4 *Fusarium verticillioides*

Fusarium verticillioides (synonym, *Fusarium moniliforme*) is a plant pathogenic fungus within the monophyletic group *Fusarium fujikuroi* species complex (FFSC) that causes damage in a variety of crops, including maize (*Zea mays* L.). This model maize pathogen has been linked to the poor quality of grains and depletion in maize productivity regarding to the ear and stalk rot and seedling blight diseases (ALMEIDA et al. 2002; BLACUTT et al., 2018; STUMPF et al., 2013). Other *Fusarium* spp. within the FFSC such as *F. proliferatum* and *F. subglutinans* and *F. graminearum* from *Fusarium graminearum* species complex (FGSC) have been associated to maize ear rots in Brazil while FFSC accomplish for 96% all kernels contamination with *F. verticillioides* representing 76% within the species complex (STUMPF et al., 2013). The same pattern has also been found in other countries (DORN et al. 2009; FANDOHAN et al. 2003).

Despite its lifestyle, *F. verticillioides* is found in soil and associated with maize residue while the second niche has been recognized as the major source of inoculum in the field (MUNKVOLD, 2003). It can survive for a long period of time on maize stalks in the ground or buried samples, important both for conidia production and maintenance in the system (MUNKVOLD, 2003). Although less competitive in soil, it has been shown that at high inoculum potential, pathogens can cause seedling blights in maize and also affect soybean germination (PEDROZO; LITTLE, 2017) which have serious impacts in no till systems and rotational cropping. *Fusarium verticillioides* conidia was reported infecting maize roots at a rate of $6x10^4$ CFU/g (PEREIRA et al., 2007). Secondary roots, stem or ear wounds and also insects play an important role in the pathogen infection (BLACUTT et al., 2018). *Fusarium verticillioides* is more

likely to dry seasons and can infect at different plant stages showing or not symptoms (BLACCUT et al., 2018). It has been shown that fumonisins, mycotoxins produced by this fungus, play an important role in pathogen virulence and are also dependent on climatological conditions (BLACUTT et al., 2018; MUNKVOLD, 2003).

1.5 *Ceratocystis paradoxa*

Ceratocystis paradoxa (anamorph *Thielaviopsis paradoxa*) is a polyphagous fungus belonging to the *Ceratocystis paradoxa* species complex that affects crops of great economic importance such as coconut (*Cocos nucifera* L.) (MBENOUN et al., 2014). This pathogen produces two types of asexual conidia and resistant structure, chlamydospore, that can cause infection in plants mainly through natural or mechanical wounds or survive in soil for long periods (CHAPOLA et al., 2014) causing vascular infection and plant death (WARWICK, R. N.; PASSOS, E.M. 2009). Temperature of 28 °C and pH ranging from 5 and 7 are the optimal conditions to mycelial growth and conidia production (COSTA E CARVALHO et al., 2011).

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SECOND PART - PAPERS

PAPER 1 Crop rotation does not increase soil diversity but contributes to the occurrence of maize ear and stalk rots after three-year land use

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Abstract

Crop rotation has been widely adopted as alternative to the conventional planting system in order to minimize the impacts of intensive land use avoiding soil exhausting and reducing the use of pesticides. In Brazil, most part of rotation systems include maize and soybean crops because they are the most grains planted in the country and their rotation and/or succession improve the nutrient cycling in the soil and can impact on plant health. In this study we evaluated the diversity of bacterial communities using target metagenomics sequencing (16SrRNA, V3-V4 region) in continuous cropping and rotation systems and the impact of these land uses in *Fusarium verticillioides* fumonisin producers (*fum1*) inoculum and disease occurrence. Field experiments were conducted at Universidade Federal de Lavras, Brazil during four seasons (2016/2017 to 2019/2020). Bulk soil samples were collected at the end of each season, while rhizosphere was collected just in the season 2019/2020 in two time points: blooming and harvest. In addition, maize stalks were left in the field during plant cycles to evaluate the influence of land uses on pathogen

multiplication. The resulting sequences were clustered into operational taxonomic units (OTU) at a 97% of similarity threshold. Our results showed three-year crop rotation did not increase soil diversity compared to continuous maize or soybean and precipitation was responsible for shaping communities in the first three seasons. Actinobacteria was more abundant in the driest season compared to the regular ones. Bulk soil and rhizosphere communities were influenced by the previous three-year land uses and, in general, continuous soybean showed the highest diversity indexes. In addition, continuous soybean and rotation were the most dissimilar communities' structures. *Fusarium* spp. *fum1* decreased its concentration in soils under rotation systems although its occurrence decreased in maize stalks left in the field under continuous maize cropping suggesting specific suppression to pathogen multiplication. In line, soils under continuous maize have decreased disease occurrence in maize plants cultivated in the last season. In conclusion, crop rotation did not increase soil diversity and soils under continuous soybean in previous years showed higher microbial diversity. Rotation can contribute to decrease pathogen inoculum in soil although in maize stalks, continuous maize showed better control and suggest the induction of suppression to pathogen multiplication. Also, disease occurrence was lower in maize plants cultivated under continuous maize.

Key-words: maize, soybean, rotation, *Fusarium verticillioides*

1 Introduction

Rotational planting (versus simplified cropping systems) and no till systems are widely used worldwide as a strategy to improve soil quality, to control weed and pests and to increase plant productivity (TIEMANN et al., 2015). It is well known that crop rotation can also impact belowground diversity increasing resilience and assisting plants with extreme weathers such as drought (DE VRIES et al., 2020) or triggering soil suppressiveness through both composition or abundance of key microorganisms (PERALTA et al., 2018).

Soybean and maize represent the most important grains produced in Brazil. Moreover, they are cultivated mostly in succession and in rotation (each season) due to the high profits and also because of favorable climate. The cropland extension for these and other commodities is increasing

quickly mainly in biomes such as Amazon, Caatinga and Cerrado (ZALLES et al., 2019). According to Companhia Nacional do Abastecimento (Conab), season 2018/2019 had an increase of 4.7% (120.4 t millions) and 2.7% in production and extension area, respectively, while maize decreased 3% (97.5 t millions) in production and an increase in cropland rising 1.2%.

Crop rotation and no tillage are some of the strategies used in agriculture to minimize soil exhausting, increasing nutrient cycling and diminishing pests. Despite the benefits, rotational systems associated to no till can increase pathogen inoculum because of the culture stubbles (MUNKVOLD, 2003). Some works have shown rotation as an ally of microbial diversity and induction of suppressiveness to pathogens (PERALTA et al., 2018) while others oppose this idea.

Fusarium verticillioides (Sacc.) Nirenberg represents a serious economic threat to maize production and grains quality (BLACUTT et al., 2018). Furthermore, at high inoculum potential can influence germination of soybean seeds (PEDROZO; LITTLE, 2017). It can survive in soil and maize residues, increasing inoculum potential each season. *Fusarium verticillioides* can infect maize plants through seeds, wounds, or natural openings (silk stage) where it causes ear rot and stylar canal resulting in starburst patterns on kernels (BLACCUT et al., 2018).

Soil microorganisms are capable to protect plants against diseases. Soil microbiomes are shaped according to soil gradients such as pH, water content, soil texture and organic carbon (DAVEY et al., 2019) but also the management system that can trigger specific suppressiveness to pathogens (PERALTA et al., 2018). Some works have shown that as more diverse is the system aboveground as more belowground, which is associated with soil and plant health (NIELSEN; WALL; SIX, 2015).

In our work, we hypothesized a three-year crop rotation can contribute to increase soil microbial diversity and influence on *Fusarium verticillioides* (*FUM1*, mycotoxin producer) inoculum potential in soil and maize stalks. We believe this knowledge will be important to *F. verticillioides* management in agricultural systems. The aim of this work was to evaluate the soil diversity in rotational and continuous systems as well study the impact of crop rotation in *F. verticillioides* inoculum.

2 Material and Methods

2.1 Site description and experimental design

Field experiments were carried out at Centro de Desenvolvimento Científico e Tecnológico em Agropecuária, University of Lavras, Brazil. Area chosen for implementation has a history of about 10 consecutive years of maize cropping and incidence of ear and stalk rots evaluated by PINTO (2016). Prior to the installation, samples of maize stalks were collected in the whole area to confirm *Fusarium verticillioides* incidence and etiology. Land uses were continuous maize, continuous soybean and rotation for the first three years (2016-2017 to 2018-2019) and a fourth year (2019-2020) with maize cultivated in the whole area, all under no tillage system. The experiment was designed in four complete blocks and 15 m^2 plots with five rows 0.6 m apart. Maize hybrid DKB290 VTPRO3 and soybean NS5700 IPRO were planted in a stand of 65,000 and 300,000 plants per hectare all seasons, respectively.

2.2 Soil sampling

Soil samples were taken at harvest time point (here defined by the humidity of maize grains and soybean at 22% and 18%, respectively). We sampled bulk soil at harvest time point the four seasons 2016-2017 to 2019-2020 with an additional rhizosphere sample at both blooming (VT) and harvest time points for season 2019-2020. The three-year rotation started with soybean in 2016-2017 and rotated yearly. Maize was planted in the whole experimental area in the fourth season to compare the effect of three-year land uses on soil diversity and *Fusarium* spp. (*fum1*) inoculum causing ear and stalk rots. With exception of the last season, ten randomized samples were taken per each plot (three central rows, 7.2 m^2) and mixed into a composite sample totaling 36 for the first three years. In the fourth season, we sampled 12 points per plot (for bulk and rhizosphere) and combined each four into three composite samples, totaling 72 for bulk and 72 for rhizosphere at blooming and harvest time points, respectively.

2.3 Soil DNA extraction and sequencing

Genomic DNA was extracted from 0.5 g of soil using DNeasy PowerSoil Kit (Qiagen Inc.) according to manufacturer's instructions and quantified using dsDNA HS Assay Kit (Invitrogen) on Qubit 2.0 Fluorometer (Life Technologies, Grand Island, NY). Libraries were prepared according to Illumina's protocol and analyzed using the V3 kit for Illumina MiSeq platform (2x300

bp). The set of primers Bakt341F and Bakt805R targeting the hypervariable V3-V4 region of 16S rRNA gene were used. Soil DNA samples were diluted to concentration of 10 ng/µl and sent to Psomagen (Maryland, USA) for sequencing.

2.4 Environmental data

Precipitation (mm) data were collected for 14, 10, 7 and 5 days prior to the soil sampling time point at Universidade Federal de Lavras for the first three experimental years (2016-2017 to 2018-2019) and submitted to principal coordinate analysis (PCoA). No data were available for season 2019-2020. Soil samples for each plot were sampled at 0-10 depth and mixed into composite samples for each land use for analysis.

2.5 Bioinformatics and diversity analysis

Sequences were trimmed of primers using Cutadapt (MARTIN, 2011) and truncated at 244-233 and at 250-217 for forward and reverse reads, first and second datasets, respectively, using FIGARO (WEINSTEIN et al., 2019). We merged paired-end reads using USEARCH and processed by using QIIME2 (BOLYEN et al., 2019) and DADA2 plugin (CALLAHAN et al., 2016). Classification into operational taxonomic units (OTUs) was done using RDP classifier (WANG et al., 2007). Diversity analyses were performed using vegan: Community Ecology Package version 2.5-6 (OKSANEN et al., 2019) on R software version 3.6.1 (https://www.R-project.org/). Alpha and beta diversity were estimated by richness, Shannon diversity index and Bray-Curtis dissimilarity. Data were rarefied to get the exact number of counts per sample before diversity analyses. We used the resources from the High Performance Computing Center (HPCC), Michigan State University, USA.

2.6 Quantification of *Fusarium* spp. fumonisin producers in soil and maize stalks

We used maize stalks from the previous sampling (abovementioned, 2.2 Methods) to assess the inoculum of *Fusarium* spp. mycotoxin producers on soil and on maize stalks left in the field during the whole season. We used the set of primers Verpro-F (5’ GCCATGCGTCACGGCCAC

3') and VERTI-R (5' GGAGTAGACAGGGTATTTGC 3') targeting a conserved region in the polyketide synthase gene *fum1,* which is involved in the biosynthesis of fumonisin and specifically detects isolates from the fumonisin producing *F. verticillioides*, *F. proliferatum*, *F. nygamai* and *F. globosum* (Waalwijk et al. 2008). However, *F. verticillioides* and *F. proliferatum* are the major species associated to maize ear and stalk rot and potentially fumonisin producers in Brazil although *F. verticillioides* is the major concern (STUMPF et al., 2013). Ten maize stalk fragments (20-30 cm) were put into nylon bags with 2 mesh holes and left on the soil surface of each land use experimental plots, totaling 12 bags per season. Once the plant cycle has completed, stalks were fragmented into 1-2 cm pieces, grounded, freeze dried and incubated to -20 ° C until DNA extraction. For maize stalks, genomic DNA was extracted from 40 mg of sample material using Wizard® Genomic DNA Purification kit (Promega Corporation) according to the manufacturer's protocol. Quantification was performed in the Rotor Gene 6000 thermocycler using SYBR Green PCR Mix (Qiagen) with a final volume of 25µl reaction and 2µl of template. Amplification cycle consisted of 38 cycles at 95ºC for 5 min, denaturation to 95ºC for 25 secs, annealing 58ºC for 30 sec and extension at 72ºC for 30 sec. Six standard curves were built from a $\log_{10}$ serial dilution starting at 0.2 pg/µl of *Fusarium verticillioides* DNA (isolate FV425), a fumonisin (*fum1*) producer for soil although it started at 0.2 fg/µl for maize stalks. For soil samples, we used the soil DNA extracted for taxonomical diversity experiments (item 2.3, Methods). All samples were analyzed in duplicate, including the standards and negative controls using water. Melting curve was assessed for each run to confirm the primer specificity. Absolute quantifications were calculated based on cycle threshold (Ct) that is the cycle number in what the fluorescent signal of a PCR product can be detected above the threshold line (background). The efficiency, threshold and R^2 of each run are shown on Table S1 (support material) and the undetermined cycle threshold were excluded of statistical analysis.

2.7 Quality of maize grains and stalk rot evaluation

The incidence of *Fusarium verticillioides* was assessed using a blotter test according to recommendations of Manual de Análise Sanitária de Sementes (MAPA, 2009). Maize seeds were surface sterilized using ethanol 70% for 30 secs, hypochlorite 2.5% for 3 min and three successive washes in sterilized water to avoid the superficial contaminants. A total of 400 grains from each plot was distributed over Petri dishes (146x21mm) using 25 grains per plate. The evaluation was

performed in a stereoscopic microscope (Zeiss Stemi DV4) with augmentation 30-80x to identification of pathogen characteristics. For maize stalk rot evaluation, we sampled 10 maize plants for each plot area of 7.2 m^2 (three central rows) and checked stalks rot by pressing the stalks in the first and second buds and them submitting them to humid chamber for five days in the dark. Recovery of *Fusarium verticillioides* from infected stalks was confirmed by isolation in potato dextrose agar. The number of stalks infected were converted to percentage and submitted to statistical analysis.

3 Results

3.1 Three-year crop rotation does not increase microbial diversity

Alpha diversity metrics, richness (Chao1), evenness (Pielou's) and Shannon index showed no difference between continuous soybean, continuous maize and rotation for the first two seasons (Figure S1). However, richness was higher for continuous soybean compared to continuous maize and rotation cropped with annual soybean in the third season (Figure 1). Evenness and Shannon index showed no difference among land uses ($p>0.05$). Relative abundance at phylum level showed Actinobacteria, Proteobacteria and Acidobacteria as the most abundant phyla representing 71.1%, 81.2% and 70.7% of all phylum for all seasons at harvest time point (Figure 2). Actinobacteria substantially increase its abundance in the second season while the minor phyla Verrucomicrobia, Gemmatimonadetes, Bacteroidetes and WPS-1 have decreased (Figure 2). Communities' structure showed no difference according to three-year land uses (Figure S2) although differences those dissimilarities could be explained by precipitation (mm) (Figure 3). Results showed higher similarity between communities from the first and third seasons while very distinct from the second (Figure 3). Mean precipitation for 5, 7, 10 and 14 days prior to the time point ranged from 0.2 to 3 mm in the driest year (second) while it ranged from 3.2 mm to 123 mm in the 14 days evaluated for first and third seasons. No precipitation data was available for the fourth and last season.

3.2 Rotation does not increase soil diversity both in bulk soil or maize rhizosphere

In an attempting to investigate if the shift in communities according to land uses were masked by the lower number of replicates sampled in the first three years, we increased the number for 12. Also, as the rhizosphere communities respond faster than bulk soil, we hypothesized three-year land uses could affect maize rhizosphere communities in the fourth year and these differences would be visible both at blooming or harvest time. Results showed three-year of continuous soybean increased richness and diversity of microbial communities in maize rhizosphere during blooming of the fourth year (Figure 4A) although bulk soil was not affected (Figure 4B) in the blooming (maize tasseling). In contrast, we observed no differences for rhizosphere communities at harvest while the diversity in maize bulk soils cultivated with continuous soybean were higher compared to the other land uses (Figure 5). We performed a beta diversity using Bray-Curtis dissimilarity to assess shifts in communities between soil compartments at harvest and blooming (maize tasseling) time points. The three-year land uses did not affect bulk soil communities 'structure at harvest ($p > 0.05$) although in the rhizosphere, continuous soybean and rotation showed the most dissimilar structures ($p=0.003$) compared to continuous maize and continuous soybean or continuous maize and rotation ($p=0.032$) (Figure 6). In the blooming time, samples showed high dissimilarity both considering bulk soil or rhizosphere compartments ($p<0.05$) (Figure 6). Proteobacteria, Actinobacteria, Bacteroidetes and Firmicutes were the most abundant phyla in rhizosphere samples in both time points, blooming and harvest. Proteobacteria, Bacteroidetes and Firmicutes increased their relative abundances in 6.3%, 10.3% and 5.2% at blooming time compared to rhizosphere although Acidobacteria increased its abundance in 7.2% for samples collected in harvest (Table 1). Bulk soil samples in both sampling times were very similar in abundance with exception of Actinobacteria that represented 27% of phyla in the blooming compared to 19.2% in harvest (Table1).

3.3 *Fusarium* spp. *fum1* inoculum depend on season and not on land uses

The plots cultivated with continuous maize showed the highest concentration of *Fusarium* spp. (*fum1*) in bulk soil samples for the first season while rotation (cultivated with annual soybean) and continuous soybean showed the lowest but no difference between them (Figure 7). In contrast, in the second season continuous soybean increased the survival of *Fusarium* spp. (*fum1*) together with continuous maize. We found no difference in pathogen survival according to land uses for

the third season ($p>0.05$) (Figure 7). When considering the fourth season, where maize was planted in total area to evaluate the residual effect of three-year land uses, rotation also performed better in controlling pathogen inoculum in bulk soil samples compared to continuous soybean or maize (Figure 8B). However, the concentration of *Fusarium* spp. (*fum1*) decreased better in plots under continuous soybean than rotation (annual soybean) when rhizosphere soils were considered (Figure 8A). The *Fusarium* spp. (*fum1*) quantification in maize stalks left in soil surface during the plant cycle showed no difference between land uses for the first season (Figure 9) and had most undetermined Ct values for the second (data not shown). Continuous maize performed better in decreasing pathogen concentration compared to rotation (annual soybean) and continuous soybean where pathogen inoculum has increased in the third season (Figure 9). Maize stalk rot increased with three-year rotation (47.5%) compared to continuous soybean (40%) and continuous maize (5%) (Figure 10A). Grains quality were assessed by blotter test and showed continuous maize decreased the percentage of rotten grains (and starburst pattern) (35%) compared to continuous soybean and rotation, which showed an average of 64.54% and 83.75% of rotten grains, respectively (Figure 10B).

4 Discussion

Soil biodiversity has been associated with soil quality, plant health and a greater resilience to biotic and abiotic stresses once microbial communities are responsible for several processes to edaphic ecosystem (NIELSEN et al., 2015). In agroecosystems, the intensive land use through simplified crops, conventional planting system and the indiscriminate use of chemical fertilizers and pesticides have shown to decrease soil biodiversity, functionality and plant yield once it is more susceptible to the attack of plant pathogens and pests (ALLAN et al., 2015; SOARES DE CARVALHO et al., 2016; TSIAFOULI et al., 2015). Many studies have shown crop rotation, cover crops and no tillage as alternatives to the intensive practices (PERALTA et al., 2018; TIEMANN et al., 2015) and also as allies to build soil suppression to pathogens (PERALTA et al., 2018). In this study, we hypothesized three-year crop rotation between maize and soybean would increase microbial diversity in soil and decrease the inoculum potential of *Fusarium verticillioides* here target by the gene of *fum1* (fumonisin production), a maize pathogen, in soil and also in maize stalks left in soil surface during all plant cycle. However, our results showed that rotation have no

effect on bacterial diversity at phylum level in the first two years of this study. On the other hand, richness and diversity were higher for continuous soybean compared to maize and rotation (annual soybean) in the third season (2018-2019). A recent study performed in southern Wisconsin found that rotational maize and soybean or continuous cropping did not affect richness and diversity in bulk soils, both for spring or fall seasons. Nevertheless, communities from continuous maize and continuous soybean showed great dissimilarity while it did not happen in rotational systems (CHAMBERLAIN et al., 2020).

Crop did not affect community structure in our study at phylum level, but precipitation showed to be crucial to shape microbial communities in soil. In general, the taxonomic profile and abundances were similar among seasons, with exception of an increase of 21% in Actinobacteria in the second and driest season compared to the other seasons. Abundance of Verrucomicrobia, Gemmatimonadetes, Planctomycetes and WPS-1 were lower in the driest season (4.5%) compared to a mean of 17.5% for the seasons where precipitation was higher. This finds corroborate with other studies that observed stimulation of Actinobacteria under controlled dry conditions and a reduction under rewetting controlled conditions in Chinese, Japanese and African forest soils (ZHOU et al., 2016). The same study also found a decrease in Proteobacteria although its abundance increased after rewetting. Actinobacteria are likely to be R strategist, characterized by fast growing and highly variable population size (HO; DI LONARDO; BODELIER, 2017). TAN et al. (2020) found mean annual precipitation and soil pH as the major drivers shaping microbial communities in maize soils. In the same study, more abundant phyla were Proteobacteria, Actinobacteria, Acidobacteria, Chloroflexi and Gemmatimonadetes and Deltaproteobacteria although with the increase in mean annual precipitation, the relative abundance of Proteobacteria increased while Gemmatimonadetes and Nitrospirae decreased. Gemmatimonadetes are frequently found in soil microbiomes and they seem to be more adapted to low moisture conditions (DEBRUYN et al., 2011). Planctomycetes have been found in a range of environmental conditions including marine and fresh water (BUCKLEY et al., 2006) as also Verrucomicrobia (FREITAS et al., 2012).

We assessed precipitation data 14, 10, 7 and 5 days prior to the sampling time point for each season and results showed a clear dissimilarity between first and third seasons compared to the second and driest one (2017-2018). Indeed, mean precipitation for first and third seasons ranged from 120-123 mm in during the 14 days prior to sampling while there was no or very low

precipitation (0-3 mm) in the same period for the second. Environmental parameters are of utmost relevance in the assemble of microbial communities. Some studies have found soil pH (de CARVALHO et al. 2016; TAN et al. 2020), organic carbon (DAVEY et al., 2019) and precipitation (TAN et al., 2020; ZHOU et al., 2016) as the most important drivers in community assembly.

In the fourth season (2019-2020), we planted maize in the whole experimental area to evaluate the effect of three-year land uses on maize bulk soil and rhizosphere diversity at blooming and harvest time. We also evaluated the impact of rotation and continuous cropping in *Fusarium* spp. *fum1* inoculum and the occurrence of maize stalk and ear rot in maize plants. The three-year land uses had no effect on bulk soil diversity (richness and Shannon index) during maize blooming in the season 2019-2020. However, maize plants growing in soils previously cultivated with continuous soybean showed higher species richness and diversity in their rhizosphere, followed by continuous maize and soybean. On the other hand, in the harvest time maize bulk soils under continuous soybean in the previous three seasons were more diverse than maize and rotation. In general, our results showed soils under soybean cultivation were more diverse and soils under rotation were the less diverse soil or rhizosphere ecosystems.

Plants recruit their microbiomes according to several aspects such as genotype and physiological stages or age (MENDES et al., 2014; PEIFFER et al., 2013). Soybean are legumes that establish a complex interaction with soil microorganisms, including nitrogen fixing bacteria such as those belonging to *Bradyrhizobium* spp., but also with other genera such as *Bacillus* and *Paenibacillus* (MARTÍNEZ-HIDALGO; HIRSCH, 2017). Plants shape their microbiome through the exudation of metabolites and sugars, what have been shown to be very distinct for maize and soybean (KUDJORDJIE et al., 2019; PASCALE et al., 2020). ZHOU et al. (2018) have found that diversity in legume system are greater than grass systems and also legumes enrich fungi populations more than grasses does. Although in the fourth year we planted just maize, soybean residues as well its root system may still in soil.

In general, we could notice the three-year land uses influenced in maize microbial communities in the fourth season, both in rhizosphere and bulk soil structures. Land uses planted with soybean were highly distinct from those under rotation in the previous seasons. Crop rotation have been linked to greater diversity in agroecosystems, however, this attribution is linked to more diversified rotational systems such as the five-crop in rotational systems (TIEMANN et al., 2015). In the last season, we also increased the number of replicates in our study with the aim to get better

resolution in communities. Difference in microbial structure in the last season can also be linked to the higher number of replicates and consequently the higher statistical power in analysis (KNIGHT et al., 2018; NANNIPIERI et al., 2019; TAN et al., 2020).

Finally, we studied the dynamics of *Fusarium* spp. *fum1*in soil and maize stalks for all four seasons and evaluated the disease incidence in maize plants influenced by the three-year land uses in the last experiment year. It is important to highlight that despite of the occurrence of other *Fusarium* fumonisin producers in maize such as *F. proliferatum*, the specie *F. verticillioides* answer for the greatest majority of maize ear and stalk rots in tropical regions (STUMPF et al., 2013). Rotation decreased the concentration of *Fusarium* spp. *fum1* on bulk soil in the first two seasons and also in the fourth although maize rhizosphere influenced by the three-year of continuous soybean showed a lower concentration of maize pathogen. Interestingly, in the second year of experiment (season 2017-2018), continuous soybean favored pathogen survival in soils. Some studies have shown that at higher concentrations *Fusarium verticillioides* can affect soybean seeds germination in soil (PEDROZO; LITTLE, 2017) although there is no evidence soybean can be a secondary host for this pathogen. Continuous maize increased pathogen concentration in soil in most seasons studied. On the other hand, when maize stalks left in soil surface were analyzed continuous maize decreased the concentration of *Fusarium* spp. *fum1* in the third season and also the disease incidence in maize stalk and grains in the fourth, that can be associated to pathogen virulence or suppression. Indeed, maize stubble is the major source for pathogen multiplication (BLACUTT et al., 2018; MUNKVOLD, 2003) although after consecutive years of cropping, maize stalks can develop suppression to pathogen multiplication and be a source of antagonists (KÖHL et al., 2015).

5 Conclusion

In general, our results showed that rotation did not increase soil diversity compared to continuous cropping and precipitation was the major environmental factor shaping bulk soil communities. With the increase in replicates, was possible to conclude that in general maize bulk soil and rhizosphere cultivated with soybean resulted in higher diversity. Although rotation have no impact on diversity, it was efficient in decreasing pathogen multiplication in soil. In contrast, continuous maize performed better in the controlling pathogen multiplication in maize residues

left in the field and also decreased the disease occurrence in maize plants which can be linked to a specific suppression to pathogen.

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7 List of figures

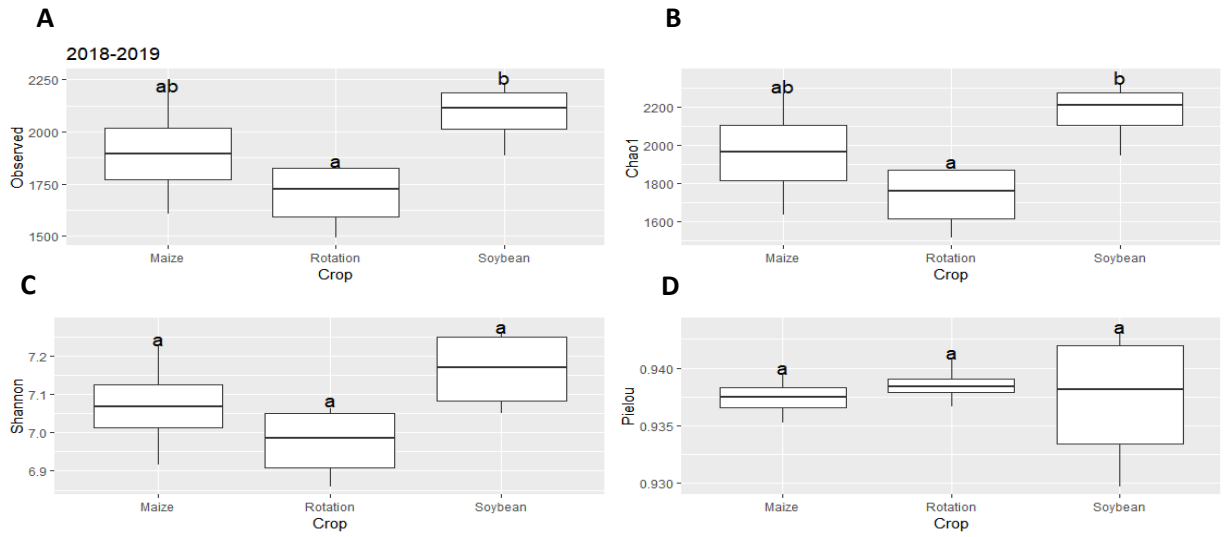

Figure 1 Alpha diversity metrics according to land uses for season 2018-2019. A) Richness (Chao1), B) observed number of taxa, C) Pielou's evenness and D) Shannon index. Differences were tested by Wilcoxon test ($p < 0.05$). Rotation = annual soybean.

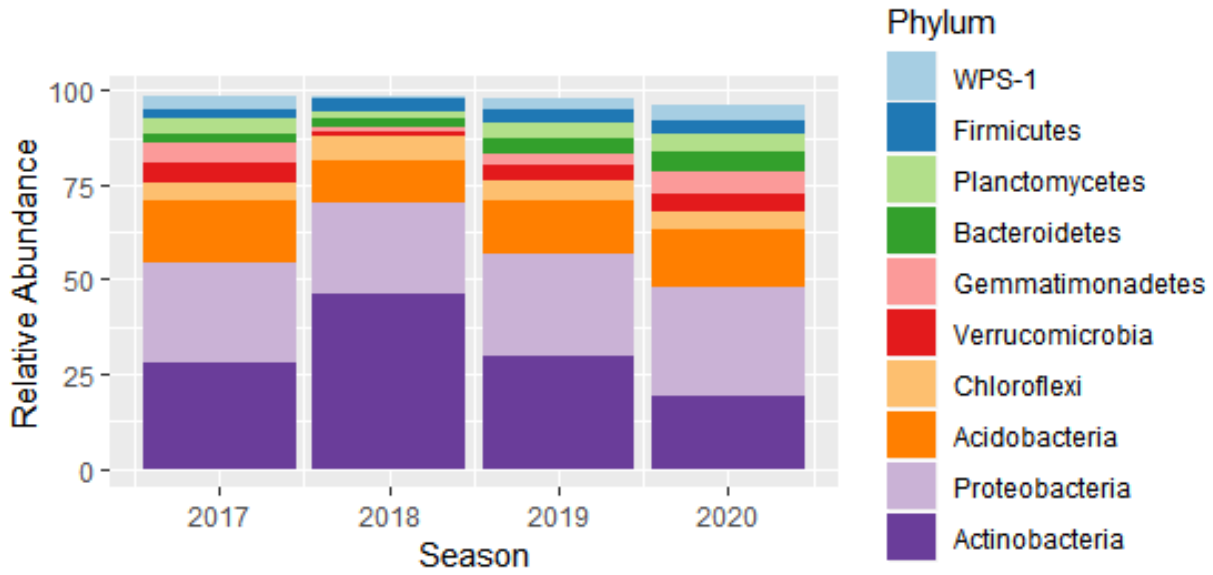

Figure 2 Relative abundance (%) of the top ten phyla according to seasons. From left to right, first (2016-2017), second (2017-2018), third (2018-2019) and fourth (2019-2020) seasons.

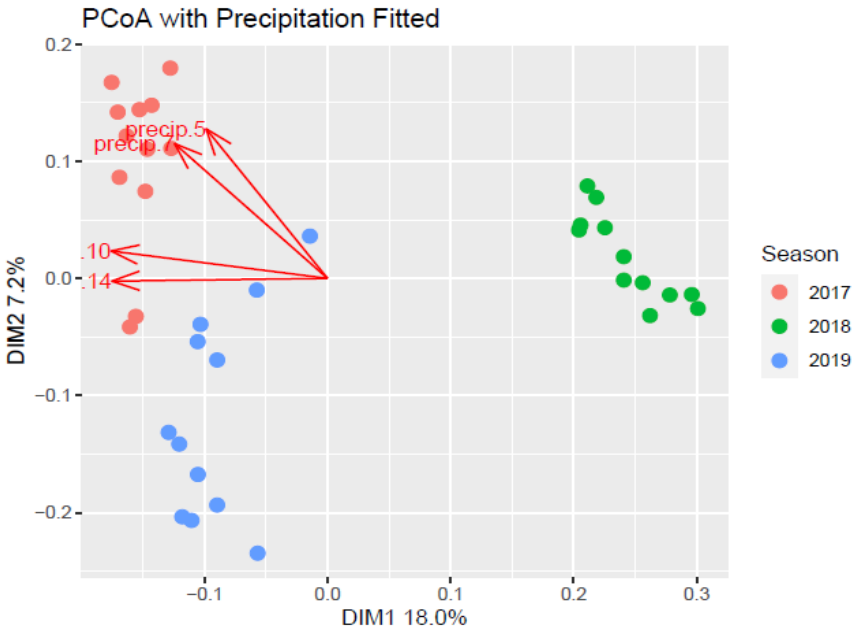

Figure 3 Precipitation (mm) driving shift in communities at 5, 7, 10 and 14 days prior to time point for the three-year land uses. First (2016-2017), second (2017-2018) and third (2018-2019) seasons. Crop rotation land use were annual soybean, annual maize and annual soybean for the three-year experiments, respectively.

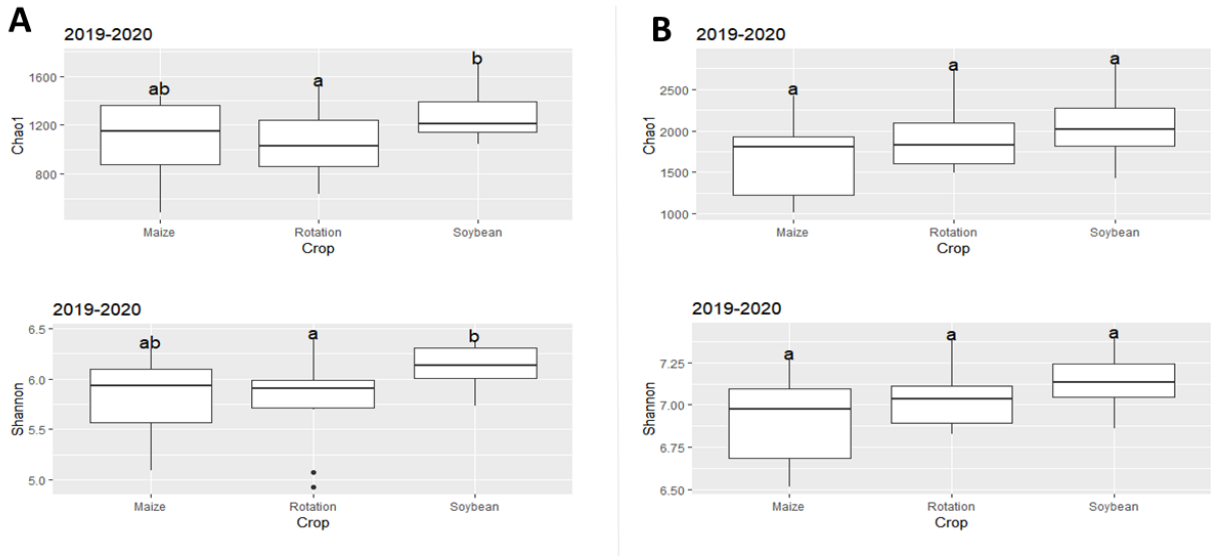

Figure 4 Effect of three-year land uses on alpha diversity metrics at blooming time point on A) maize rhizosphere and B) maize bulk soil. Differences were tested by Wilcoxon test ($p < 0.05$). Fourth season was cultivated with maize in the whole plots area.

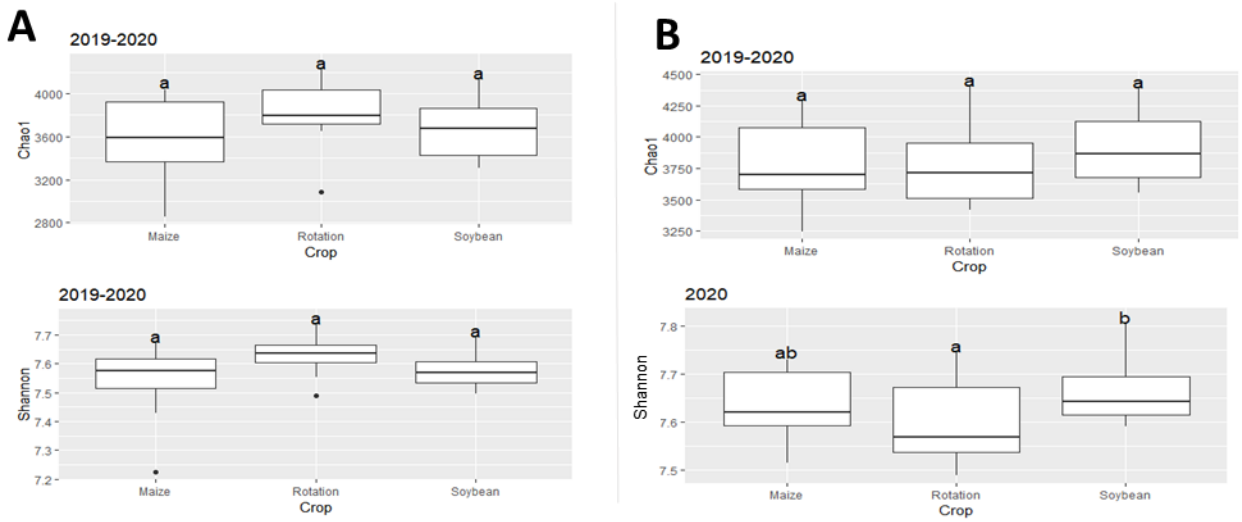

Figure 5 Effect of three-year land uses on alpha diversity metrics at harvest time point on A) maize rhizosphere and B) maize bulk soil. Differences were tested by Wilcoxon test ($p < 0.05$). Fourth season was cultivated with maize in the whole plots area.

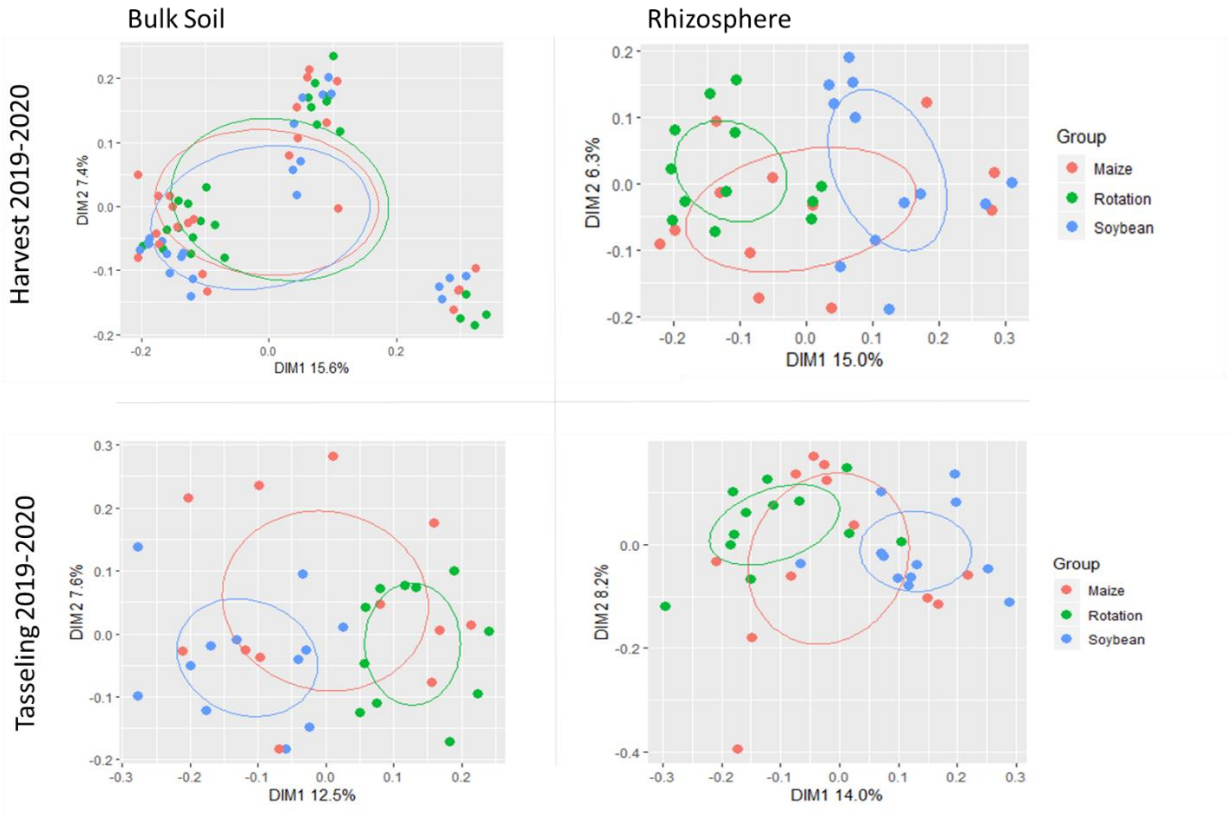

Figure 6 Principal Coordinate Analysis (PCoA) using Bray-Curtis dissimilarity by land uses, bulk soil and rhizosphere, on season 2019-2020 at harvest and maize blooming time (maize tasseling).

Table 1 Mean abundance of the top ten phyla in the rhizosphere and bulk soil at blooming (maize tasseling) and harvest time points for season 2019-2020 cultivated with maize in total area.

	Rhizosphere		**Bulk soil**	
Phylum	Blooming	Harvest	Blooming	Harvest
Proteobacteria	41.5	35.2	28.6	28.8
Actinobacteria	17.7	18.7	27	19.2
Bacteroidetes	19.1	8.84	3.79	5.77
Acidobacteria	4.62	11.8	12.6	15.1
Firmicutes	7.98	2.79	4.38	3.70
Chloroflexi	1.91	4.76	5.82	4.74
Verrucomicrobia	2.19	3.80	4.65	5.07
WPS-1	1.75	3.54	3.11	4.33
Gemmatimonadetes	1.37	3.58	5.32	5.35
Planctomycetes	1.17	3.58	2.90	4.27

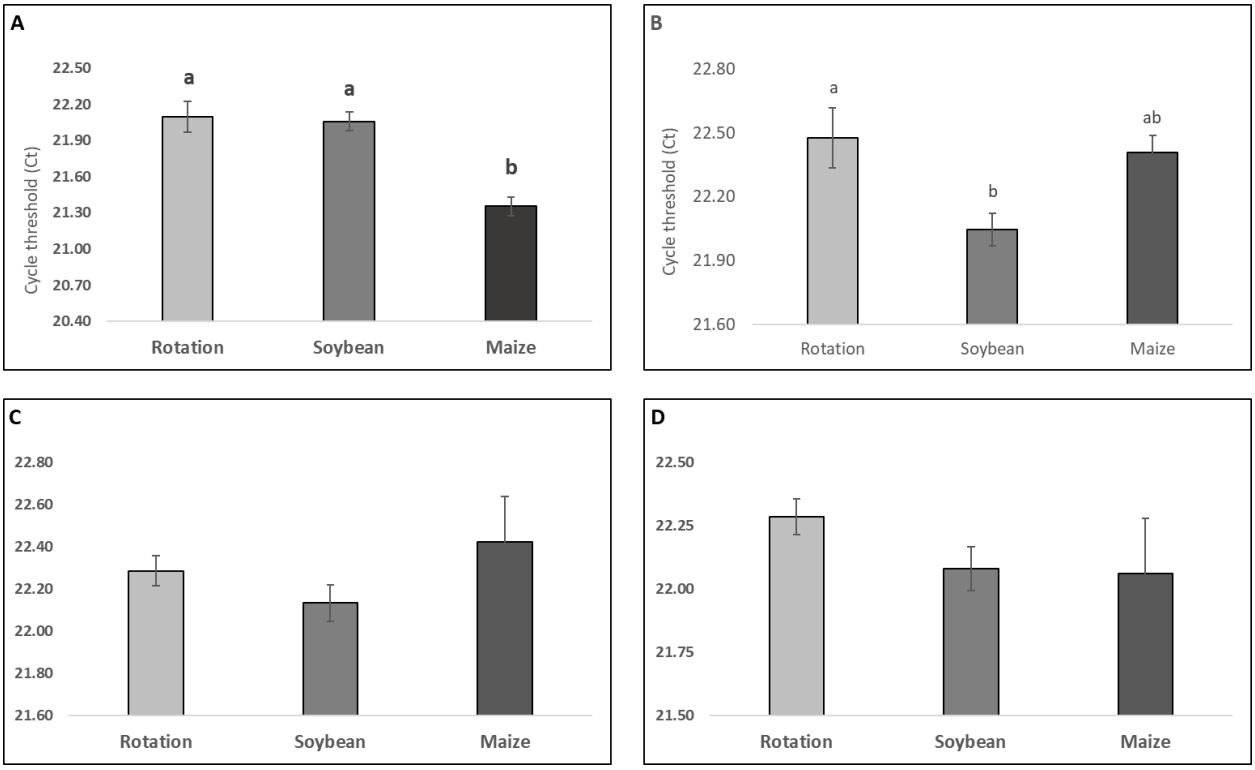

Figure 7 Quantification of *Fusarium* spp. *fum1* in bulk soil samples according to land uses on A) First season (2016-2017), B) second season (2017-2018), C) third season (2018-2019) and D) all seasons pooled. Means were submitted to Tukey test with $p < 0.05$.

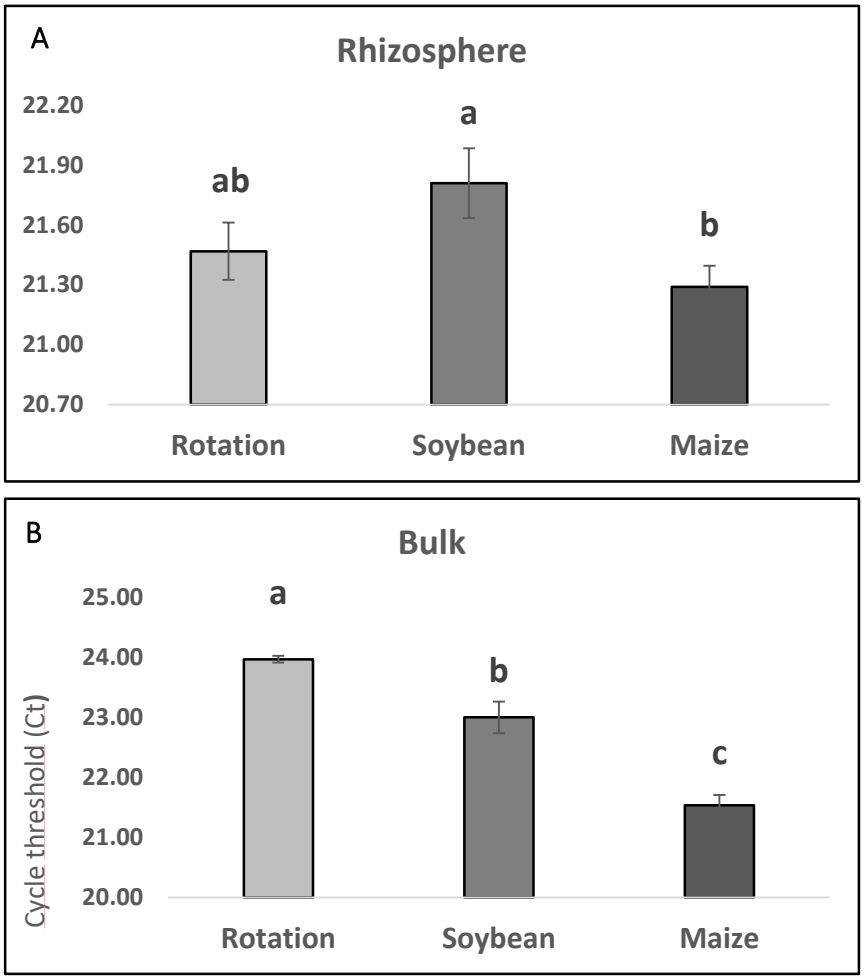

Figure 8 Effect of three-year land use on *Fusarium* spp. *fum1* quantification in A) maize rhizosphere and B) maize bulk soil in the fourth season (2019-2020) at harvest. Means were compared with Tukey test $p<0.005$.

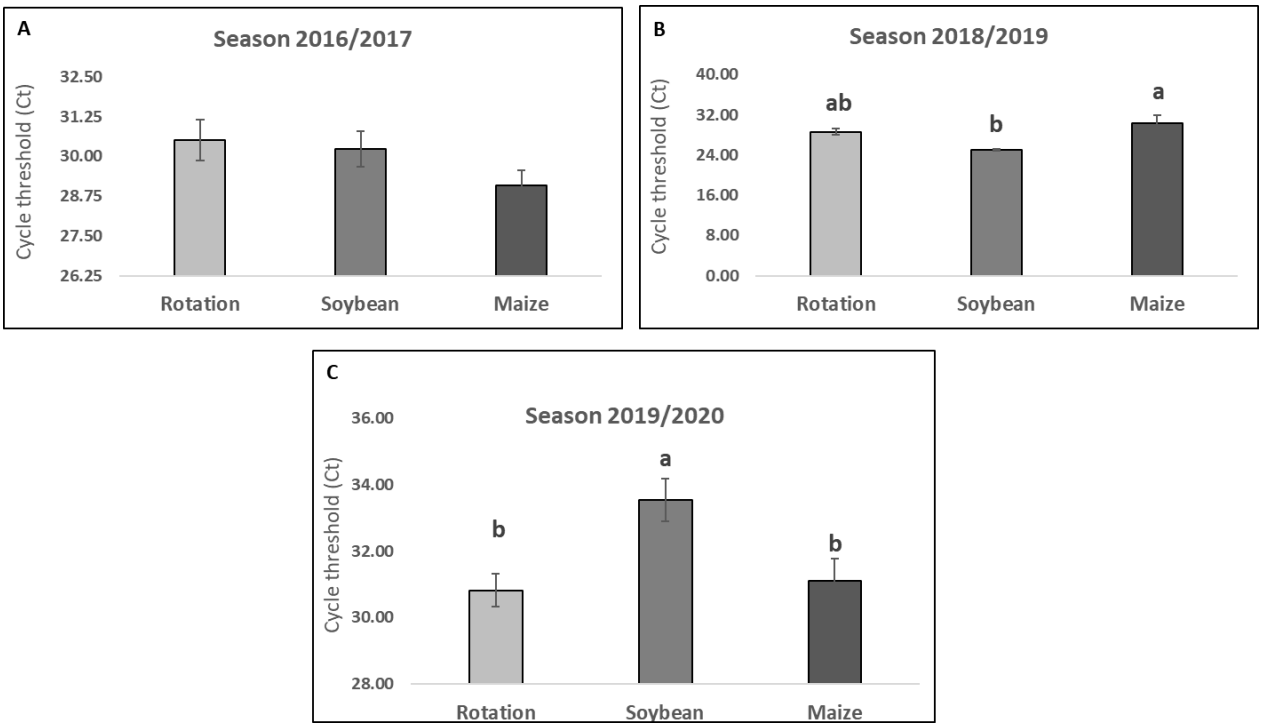

Figure 9 Survival of *Fusarium* spp. *fum1*in maize stalks left in the field during plant cycle according to land uses A) First season (2016-2017), B) Third season (2018-2019) and C) Fourth season (2019-2020).

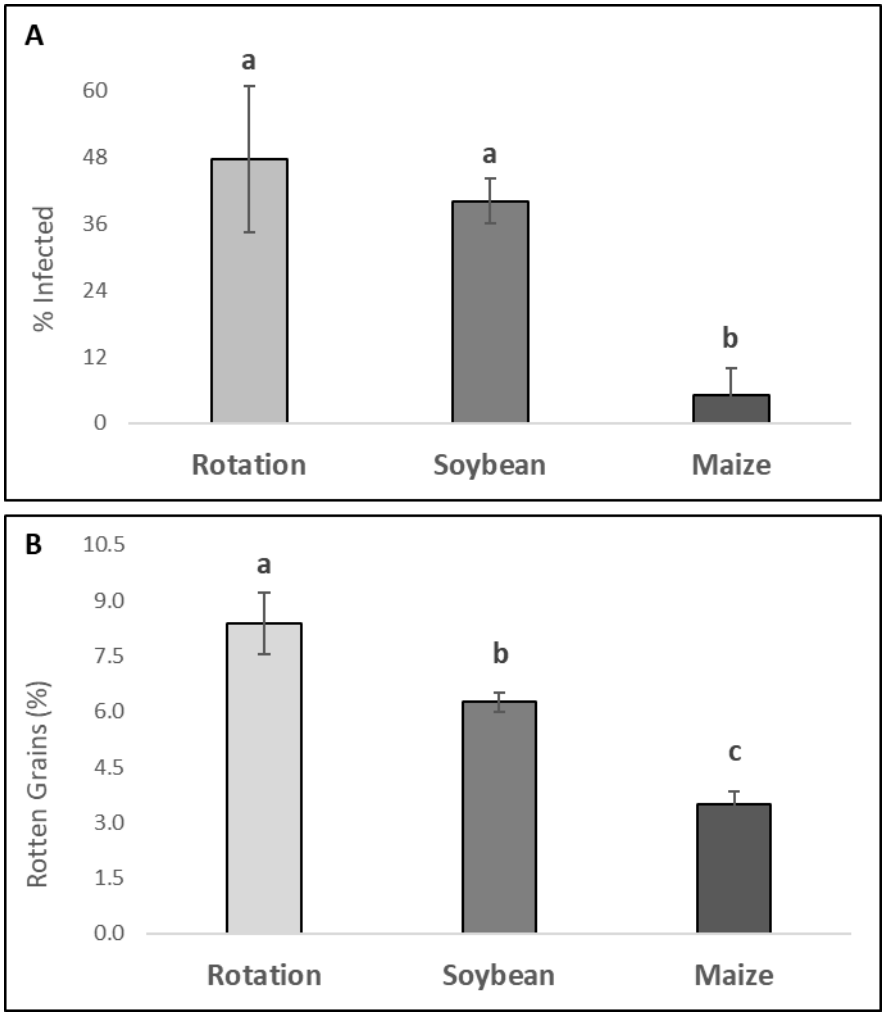

Figure 10 Effects of the three-year land uses in *Fusarium verticillioides* FV425 causing A) maize stalk rot and B) rotten grains (or starburst pattern) in the fourth season 2019-2020.

Appendix

Support material

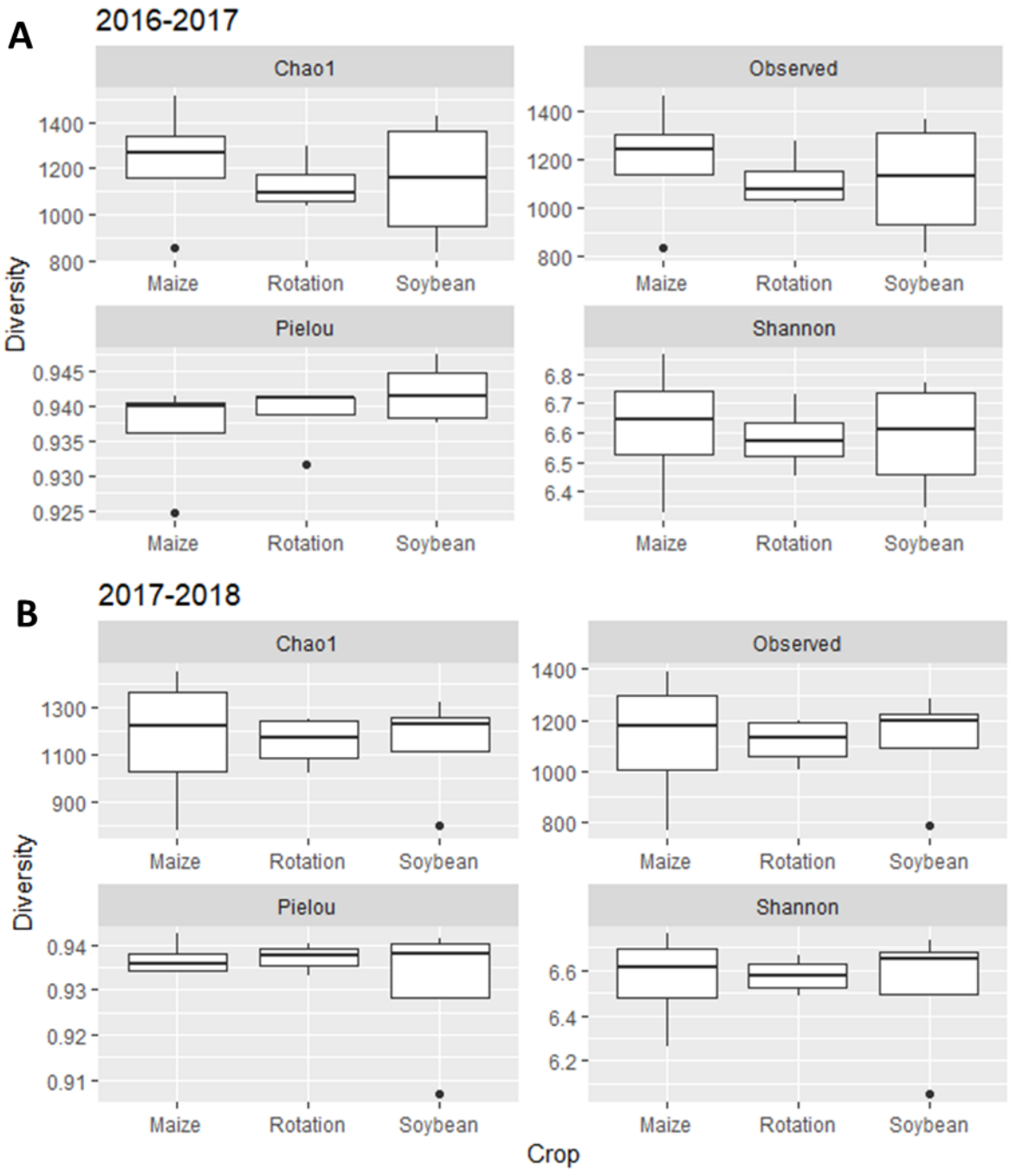

Figure S1 Alpha diversity metrics showed by Richness (Chao1), observed number of taxa, Pielou's evenness and Shannon index according to land uses for season A) 2016-2017 and B) 2017-2018. Differences were tested by Wilcoxson test ($p < 0.05$).

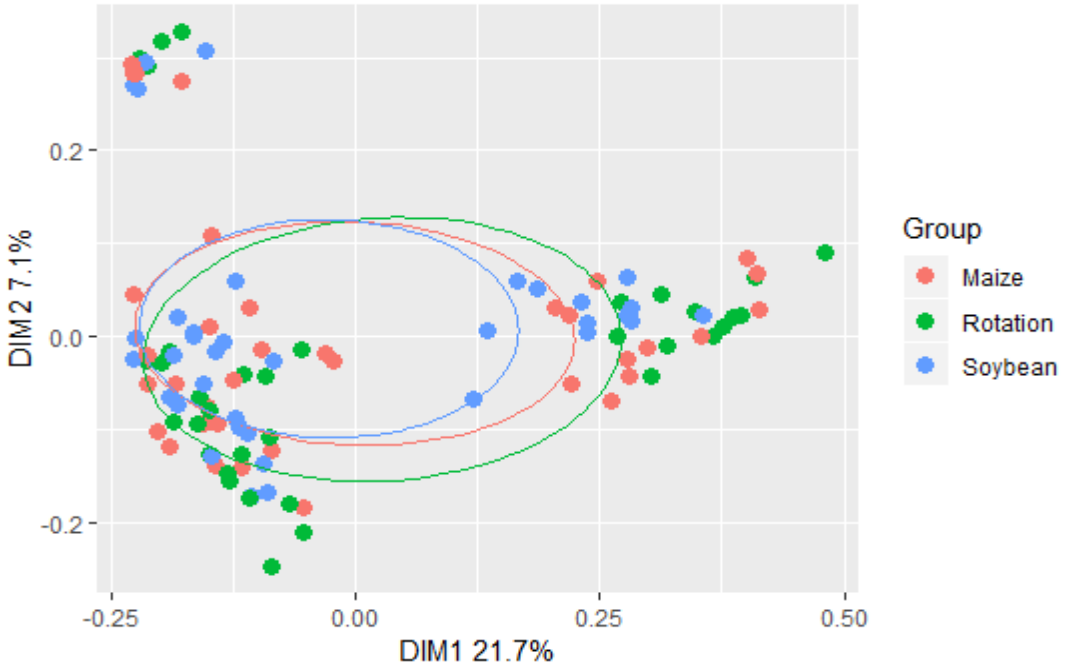

Figure S2 Similarity in soil microbial communities using Bray-Curtis distance according to three-year land uses and seasons pooled.

Table S1 Quality parameters for quantification of *Fusarium* spp. *fum1* (qPCR) in soil and maize stalks for all seasons.

Run number	Compartment	Threshold	R^2	Efficiency
1	Maize stalk	0.29	0.97	1.17
2		0.28	0.99	1.20
3		0.20	0.98	1.05
4		0.28	0.98	1.22
5		0.32	0.98	1.19
6		0.29	0.99	0.96
7		0.20	0.99	0.98
8		0.27	0.99	1.03
9		0.30	0.99	0.91
10		0.37	0.99	0.92
11		0.27	0.99	1.01
12	Bulk soil	0.28	0.99	1.07
13		0.22	0.99	1.03
14		0.15	0.99	0.97
15		0.19	0.99	1.04
16		0.17	0.99	0.96
17		0.21	0.96	1.17
18		0.19	0.96	1.17
19		0.19	0.99	0.95
20		0.16	0.99	0.79
21		0.14	0.99	0.99

Runs performed using rotor gene 6000 thermocycler.

PAPER 2 Linking soil biology and physicochemical parameters to soil suppression to *Ceratocystis paradoxa*

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[6]Center for Microbial Ecology, Michigan State University, MI, United States

Abstract

Disease suppressive soils have been mostly attributed to biological nature while soil physicochemical characteristics remain neglected. This study aimed to characterize soil suppressiveness to *Ceratocystis paradoxa* and determining the biological, chemical, and physical properties associated with this phenomenon. We classified 54 soils into levels of suppressiveness. The five most and least suppressive soils were contrasted to determine its nature. Total cultivable bacterial populations were the main biological property implicated in suppressiveness. Soil taxonomic profile showed Actinobacteria, Proteobacteria, Firmicutes and Chloroflexi as the more abundant phyla in contrasting soils. Suppressive microbiota reduced pathogen colonization in 93.8% compared to the control. Among physical and chemical properties, sand content, soil pH, calcium, magnesium, sum of bases, effective cation exchange capacity, base saturation, and aluminum were higher in suppressive soils while clay content and iron characterize non-suppressive. Calcium carbonate was effective in reduce pathogen colonization when compared to the other sources. The resulting pH from calcium and microbiota treatments ranged to neutral and significantly decrease pathogen recovery on banana baits. Our work contributes to the

understanding of suppressive phenotype in Brazilian soils to *Ceratocystis paradoxa* and pinpoint soil microbiome, calcium carbonate and pH as the main drivers of this phenomena.

Key words: *Cocos nucifera*, stem bleeding, 16SrRNA, suppressive soils, soil pH

1 Introduction

Ceratocystis paradoxa sensu stricto is a polyphagous soil pathogen infecting various plant species of great economic importance for the tropics. Hosts include pineapple (*Ananas comosus*), banana (*Musa* sp.), sugarcane (*Saccharum* sp.) and coconut (*Cocos nucifera*), where it causes the disease known as stem bleeding (MBENOUN et al., 2014). The disease was first reported in Sri Lanka, spreading later to several producing countries such as Indonesia, the Philippines and India. These countries are, along with Brazil, the world's leading coconut producers (FAO, 2014).

In Brazil, immature coconuts are produced in the north coast because of climate and soil conditions. In 2004, one of the most important regions for coconut production known as Platô de Neópolis (Sergipe) suffered with a disease outbreak resulting in death of 50 plants after four months (WARWICK; PASSOS, 2009). Interestingly, the occurrence of healthy plants in areas with high incidence of *Ceratocystis paradoxa* causing stem bleeding was first noticed in 2014, in the states of Sergipe and Pará (personal communication). This natural phenomenon, which acts against the establishment, survival of pathogens, and their pathogenic activities, is called suppressiveness (COOK; BAKER, 1983).

Suppressiveness to soil-borne pathogens might be either natural or induced by biotic and abiotic factors (MEYER; SHEW, 1991a; SILVA; MEDEIROS; CAMPOS, 2018). There are several components in soil reported as responsible to pathogen suppression, such as pH, macro and micronutrient levels, texture, organic matter, microbial activity and their interactions (DAVEY et al., 2019; LEE et al., 2020). The most studied factors that lead to soil suppressiveness to pathogens are the biological properties, which may reduce the chances of pathogen establishment by antibiosis, competition or other mechanisms (LEE et al. 2020; SCHLATTER et al., 2017). Therefore, plants explore the soil microbial consortia for protection after the attack by a root pathogen (LEE et al. 2020). Nevertheless, soil physicochemical properties can interfere with

suppressiveness favoring microbial establishment and activities and also interfering on the pathogen's life cycle (MEYER; SHEW, 1991b).

Stem bleeding has been of extreme concern among farmers, research institutions and technical assistance agencies because of its rapid dissemination. Although this disease has been associated with the occurrence of bark beetles (Coleoptera: Curculionidae: Scolytinae) in diseased coconut stems, there is no evidence they disseminate pathogen spores (GUZZO et al., 2018). This pathogen produces chlamydospores (resistance structures) ensuring high survival rates in soil (MBENOUN et al., 2014). Susceptible host, environmental stresses and mechanical damage are factors that predispose plants to the disease and pathogen establishment. Nevertheless, the lack of resistant varieties and the low efficacy of chemical fungicides further complicates the situation.

The study of the soil properties governing the phenomenon of suppressiveness may lead to the development of sustainable disease management practices through microbial consortia, microbiome engineering and/or soil management. We hypothesized that biological and chemical properties govern soil suppressiveness to *Ceratocystis paradoxa* in coconut soils from Sergipe and Pará, Brazil. The aim of this study was to examine soil attributes including their biological, chemical and properties in an attempt to determine their contribution to the suppressiveness to the stem bleeding pathogen.

2. Methods

2.1 Site description and sampling

Two coconut production areas under the occurrence of stem bleeding (*Ceratocystis paradoxa*) located in Sergipe and Pará, Brazil, were investigated. A total of 54 soil samples were collected in the vicinity of healthy coconut plants at 0-10 cm depth by discarding the leaf litter layer with the aim of screening for suppressiveness. Samples from Sergipe were collected at Platô de Neópolis and were coded from T1 to T25 while those from Pará were sampled coded from T26 to T54, totaling 25 and 29 samples, respectively. Five randomized points under the canopy projection were mixed to assemble a composite sample, totaling 500 g of soil per plant. Samples were air dried and sent to Biological Control Laboratory at Universidade Federal de Lavras where they were maintained at 4 °C until processing.

2.2. Screening for suppressiveness

Samples were screened for suppressive capacity by adding the pathogen to the soil and quantifying its recovery on banana peel as baits. Subsamples of 30 g of soil were transferred to 90 mm Petri dishes and 8 mL of pathogen suspension at the concentration 10^5 conidia/mL added. *Ceratocystis paradoxa* isolate TCTL003 from the mycological collection of Embrapa Tabuleiros Costeiros was used in all experiments. Plates were sealed and distributed in a randomized design, with five replicates and incubated at 28 ± 2°C for 7 days. After incubation, 13 pieces of banana peel with 1 cm^2 each (ripe fruit of cultivar Prata) were evenly distributed on the surface of the infested soil. Plates were again sealed and maintained under the same conditions for additional 7 days, when the percentage of baits colonization was evaluated (adapted from Ferreira et al., 2010). Control samples were 1) Subsoil classified as Brazilian latosols (Oxisols) collected from a ravine located at Universidade Federal de Lavras hereafter named TS; and 2) coarse sand used for construction never deliberately cropped to any plant species (TA). Controls were processed by sieving and sterilization in autoclave for three days 1 hour at 121°C. Percentage data was transformed to arcsin (p / 100), where p is the percentage of colonized baits to comply with the normality test. Means were compared by the Scott-Knott test (p = 0.05).

2.3 Biotic nature

Ten soils selected in the previous analysis, hereafter classified as suppressive (T13, T8, T18, T6 and T2) and conducive (P19, T32, T53, T47 and T52) were tested in a second assay for baits colonization with four replicates (item 2.2, Methods). Subsoil (TS), classified as conducive, was also included in the analyses. Samples of the 11 soils above-mentioned were divided in two groups: 1) autoclaved three times for 1h at 121°C; and 2) non-autoclaved. The efficacy of sterilization was confirmed by the absence of microbial growth on PDA (Potato Dextrose Agar) seeded with the autoclaved soil samples. Results were analyzed by the t test for paired data (autoclaved and non-autoclaved).

2.4 Abiotic nature

Soil physicochemical analyses were performed for the selected suppressive and conducive samples. To dissect the possible factors involved on suppression, we proceeded with comparisons of means of different soil parameters. The obtained data were transformed to comply with the normality test of Shapiro-Wilk and means were compared by using the t test for paired data ($p<0.05$). Statistical analysis was performed with the software R (R Development Core Team, 2009).

2.5 Isolation of microorganisms in contrasting soils

Selected microbial populations (total fungi, *Trichoderma* spp., total bacteria, *Pseudomonas* spp., fluorescent *Pseudomonas* and *Bacillus* spp.), were determined by plating serial dilutions. Samples of 10 g of each soil were placed in Erlenmeyer flasks containing 90 mL of sterile saline solution (0.85% NaCl), stirred for 30 min at 25°C and 100 µl aliquots of each dilution prepared in 10X steps were plated in three replicates onto the following media: 1) PDA amended with 2.50 mg/L of chloramphenicol for total fungal population; 2) *Trichoderma* Selective Medium-Lincoln University (TSM-LU) to determine the populations of *Trichoderma* spp. (MCLEAN et al. 2005); 3) Nutrient Agar - IN (LEVINE, 1954) with 10 mg/L cycloheximide to determine the density of total bacteria; 4) King's B medium (KING et al., 1954) supplemented with 10 mg/L cycloheximide to quantify *Pseudomonas* spp. and fluorescent *Pseudomonas*; 5) ATCC 573 medium (SNEATH, 1986) was used to determine *Bacillus* spp. populations. Before plating on ATCC 573 medium, the dilutions were incubated at 80°C for 10 min (JOHNSON et al., 1982). Bacterial populations were assessed after 48 h of incubation at 25°C, while total fungal populations were determined after five days of incubation at 25°C and *Trichoderma* spp. after 7 days of incubation at 20°C in the dark. For statistical analysis, data were transformed to $\log (1 + x)$, where x is the number of CFU. Paired data were compared by using the t test ($p \leq 0.05$).

2.6 Calcium supplementation and the resulting pH on suppressive capacity

The structure and abundance of soil microorganisms are affected by several parameters such as pH, water content, and nutrient availability and can result or not in suppression to pathogens (DÖRING et al., 2020; LEE et al., 2020; TAN et al., 2020). Preview soil physicochemical analysis showed several chemical parameters potentially influencing on

suppression (Table 3) (PEREIRA, 2015) in which we can manipulate by adding calcium sources such as pH and aluminum saturation. To study the role of calcium and pH on suppressive capacity we supplemented soil with calcium carbonate ($CaCO_3$), calcium sulfate ($CaSO_4$) and calcium chloride ($CaCl_2$) as calcium sources at five concentrations ranged from 0 to 3 mg/dm^3 and 3.5 mg/dm^3, simulating the concentrations of suppressive samples (Table 3). Supplementation with $CaCO_3$ and $CaSO_4$ were made 50 days prior to the experiment installation to allow reactions in soil. Subsoils (TS) was used as substrate for all treatments. We designed a complete randomized experiment with five replicates using the same scheme of banana baits detailed in item 2.2 (Methods). Data were submitted to non-linear regression test according to their best fit. Percentage of colonized baits, number of perithecia and the resulting pH were evaluated. Biotic and abiotic properties were later correlated using Pearson's correlation coefficient by linear correlations.

2.7 Transferring soil microbiota

The transferability of suppressive capacity was tested by washing the microbial communities from soil T2 (suppressive) and inoculating into a new raw autoclaved subsoils (TS), previously classified as conducive. A 10x dilution (10 g of soil in 90 mL of sterile 0.85 % NaCl solution) was blended at high speed for 60s and the supernatant passed through a 5 μm sieve to remove remaining particles or debris. Microbes were pelleted for 10 min at 4000 g and re-suspended in 60 ml sterile ultrapure water (ELHADY et al., 2018). Each 30 g soil plate received 4 ml of soil microbial suspension, corresponding to 70% of subsoil (TS) water capacity. The transplanted microbiomes were established for seven days and after that, pathogen was added in a concentration of 10^5 conidia/mL and maintained for additional seven days. We used banana peels on soil surface as baits for pathogen colonization as described above (item 2.2, Methods). Treatments were a) extracted suppressive microbiota; b) calcium carbonate; c) extracted suppressive microbiota and calcium carbonate; d) subsoils (TS) and e) suppressive soil (T2). Calcium carbonate (1mg/dm^3) was applied to the soil surface 50 days before the installation of the experiment and maintained at 60% of soil water capacity to allow calcium and soil reactions. Data were analyzed by ANOVA and previously submitted to normality (Shapiro-Wilk) and Homogeneity (Bartlett) tests, after transforming them by $\sqrt{x}$, where x was treatments' mean.

Percentage of colonized baits, perithecia number and the resulting pH were grouped according to pos-hoc Tukey test ($p<0.05$).

2.8 Soil DNA extraction, sequencing and bioinformatics analysis

Contrasting soil samples, suppressive (T2) and conducive (T19), were chosen for DNA extraction and metataxonomic analysis. The set of primers, Bakt341F and Bakt805R, ranging the hypervariable V3-V4 region of 16S rRNA gene were used. Genomic DNA was extracted from 0.5 g of soil using the DNeasy PowerSoil Kit (Qiagen Inc.) according to manufacturer's instructions. Nucleic acidic quantification was performed with dsDNA HS Assay Kit (Invitrogen) on Qubit 2.0 Fluorometer (Life Technologies, Grand Island, NY). DNA samples were sent to Psomagen Inc. (Rockville, Maryland, United States). Libraries were prepared according to Illumina's protocol and analyzed using the V3 kit for Illumina MiSeq platform (2x300 bp). Sequences were trimmed of primers using Cutadapt (MARTIN, 2011) and truncated at 244-233 and at 250-217 for forward and reverse reads, first and second datasets, respectively, using FIGARO (WEINSTEIN et al., 2019). We merged paired-end reads using USEARCH and processed by using QIIME2 (BOLYEN et al., 2019) and DADA2 plugin (CALLAHAN et al., 2016). The classification into operational taxonomic units (OTUs) was done using RDP classifier (WANG et al., 2007). Diversity analysis were performed on R software version 3.6.1 (https://www.R-project.org/) with vegan: Community Ecology Package version 2.5-6 (OKSANEN et al., 2019). We used the resources from the High Performance Computing Center (HPCC), Michigan State University, USA.

3. Results

3.1 Suppressiveness is not linked to sampled sites

The percentage of baits colonized by *Ceratocystis paradoxa* varied from 4.6 % to 98.5 % in the 56 different soil samples (including controls) used in this study (Figure 1). Analyses of the mean number of colonized baits with the Scott-Knott test ($P = 0.05$) resulted in seven significantly different groups of soils. Groups depicted with letters e, f, and g were considered conducive and

those with a, the most suppressive. These groups were not associated with the areas from where the samples were collected (Figure 1).

3.2 Total bacteria are higher in suppressive soils

Among the 10 soils chosen to perform a detailed characterization, the five most suppressive were from Sergipe (T13, T8, T18, T6 and T2) while four of five conducive were from Pará (T32, T53, T47 and T52, barring P19). After autoclaving, seven of them showed a significant reduction in suppressiveness, indicating its biological nature. This reduction was observed for both suppressive and conducive soils, but it was higher for soils T2, T18 and T6 in comparison with soils T32, T47, T19, and T53 (Table 1). Baits colonization in soils T8 (suppressive), and T52 (conducive) were not influenced by autoclaving, whereas soil T13 showed an increase in baits colonization in the raw soil as compared to the sterilized sample (Table 1). Bacterial and fungal population densities were similar in suppressive and conducive soils, with exception of total bacterial populations that tended to be higher on average in suppressive soils (Table 2; Table 3). Fluorescent *Pseudomonas* occurred only in soil T13 (Table 2).

3.3 Soil physicochemical parameters are different between suppressive and conducive soil phenotypes

A paired t test analysis was performed between the mean values of the suppressive and conducive soils to determine their chemical, physical and biological properties that may be involved in the suppressive phenotype to *C. paradoxa*. Ten of the 25 variables tested were significantly different and may be linked to suppressiveness. Total cultivable bacterial populations, pH in water, calcium, sum of bases, effective cation-exchange capacity, base saturation, and sand content were on average 8%, 12.2%, 58.7%, 32.7%, 57.4%, 13.2%, and 17.7% higher in the suppressive soils. On the other hand, iron and clay content were 95,2% and 66% higher in conducive (Table 3).

3.4 Calcium carbonate and neutral pH decrease pathogen colonization in banana baits

In an attempting to elucidate calcium and pH effect on triggering suppressiveness to *Ceratocystis paradoxa*, we performed an experiment testing three calcium sources at increasing doses. Calcium carbonate reduced baits colonization by 41% when dose 1 mg/dm^3 was applied compared to the control without calcium supplementation, reaching 1% of baits colonization at dose 3 and 3.5 mg/dm^3 (p=0.04) (Figure 2C). Increasing doses have no influence on colonization for calcium sulfate or calcium chloride (Figure 2C). The same pattern was observed for perithecia number where counts in the first dose (1 mg/dm^3) dropped 95,89% compared to the control (p=0.04) (Figure 2B). In contrast, pH was significant at increasing doses when soils were supplemented with calcium sources (Figure 2A). As expected, calcium carbonate increased pH from 5.20 (control) to 7.09 in the first dose, rising 7.36 in the concentration of 3.5 mg/dm^3 (p=0.03) while for calcium sulfate, we observed a decrease in pH when first dose was applied (4.73) and an average of 5.41 for the following concentrations managed (p=0.05) (Figure 2A). For calcium chloride, the mean pH for all four doses was 4.21 (p=0.05). Pearson's correlation showed a negative interaction for pH and both baits colonization (r=-0.87) and number of perithecia (r=-0.80). Instead, a positive correlation was observed when using calcium chloride, r=0.76 and r=0.66 for baits colonization and counts of perithecia, respectively (Support figure S1).

3.4 Suppression can be transferred

We compared extracted microbiota from the suppressive soil T2 with calcium carbonate at the concentration of 1 mg/dm^3. Results showed a reduction of 80% in baits colonization and 95.37% in perithecia number when the suppressive microbiota was compared to calcium carbonate ($p<0.05$) (Figure 3). Number of perithecia showed no difference between calcium carbonate and control TS (Subsoils) while it decreased (68.5%) in the original soil T2 (Figure 3), classified as suppressive (Figure 1 and Table 1). Suppressive soil (T2) and extracted microbiota from T2 sample showed a decrease up to 93.8% on number of pathogen structures (perithecia) when compared to the control TS and have no difference between them while the original soil was slightly higher. Interestingly, when calcium carbonate and extracted soil microbiota were combined the number of perithecia was three, four and 67 folds higher than calcium carbonate only, control TS and extracted microbiota only (Figure 3). Soil pH tended to neutral when calcium was added or microbial suspension was inoculated on soil (Support figure S2).

3.5 Actinobacteria is more abundant in suppressive soil

The taxonomic profile of contrasting soils reveled *Actinobacteria*, *Proteobacteria*, *Firmicutes* and *Acidobacteria* as the major phylum in the top ten, corresponding to 80.7% and 82.2% for conducive and suppressive, respectively (Figure 4). Actinobacteria was the most abundant in the suppressive soil corresponding to 28.6% against 22.4% in conducive. Other representatives were *Chloroflexi*, *Planctomycetes*, *Verrucomicrobia*, *Bacteroidetes*, *Gemmatimonadetes* and WPS-1.

4. Discussion

Suppressiveness is a phenomenon where disease or pathogen establishment is limited by soil attributes such as biology, physics or chemistry, even under the optimal conditions to disease occurrence (BAKER; COOK, 1974). It has been mostly attributed to microorganisms (LEE et al., 2020; OU et al., 2019; PENTON et al., 2014) although there are some physicochemical such as soil pH, aluminum, iron, sand and organic carbon contents associated with suppression to soilborne pathogens (MEYER; SHEW, 1991a; MEYER; SHEW, 1991b; DAVEY et al., 2019). A well-known example of suppression is take-all on wheat, disease caused by *Gaeumannomyces graminis* var. *tritici*, where the increase in fluorescent *Pseudomonas* spp. secondary metabolites limit or reduce disease.

Indigenous communities use of several strategies to suppress pathogen infection or disease development on plants. As example, synthesis of 2,4-diacetylphloroglucinol (DAPG), lipopeptides with antifungal activities and/or hydrogen cyanide (HCN) by pseudomonads have been strongly associated with soil suppression (SCHLATTER et al., 2017). Moreover, a variety of compounds such as siderophores, chitinases and the antimicrobial thiopeptide produced by Actinomycetes (mostly Streptomyces) also have a role on suppressiveness to *Fusarium* wilt (CHA et al., 2016) and or competition in potato scab disease (ROSENZWEIG et al., 2012; TOMIHAMA et al., 2016).

In our study, biological properties were at least in part responsible for the suppressive phenotype (<20% baits colonization) to *C. paradoxa*. Seven out of ten soils evaluated significantly increased pathogen growth (baits colonization) after sterilization. Although total fungi, *Bacillus*, *Pseudomonas* and *Trichoderma* were not significantly different between contrasting soils, total

bacteria were higher in suppressive soils. The transferability of suppressive capacity to a conducive soil (>70% baits colonization) endorses the microbial role on suppressiveness. Many works have shown this potential (ELHADY et al., 2018; MENDES et al., 2011). Indeed, pathogen growth in soils with no suppressive microbiota added (control and calcium carbonate treatments) confirms our hypothesis that suppressive phenotype is partially triggered by antagonists.

Target metagenomics analysis of the two contrasting soils, suppressive and non-suppressive, revealed no difference between soils. Representative phyla were Actinobacteria, Proteobacteria, Firmicutes and Acidobacteria. These phyla have been associated to many disease suppressive patterns (LEE et al. 2020; ROSENZWEIG et al., 2012; TOMIHAMA et al. 2016; CHA et al. 2016). Indeed, most part of Actinobacteria have their optimal growth at pH ranging to neutral (BARKA et al. 2015).

On the other hand, among the 25 soil attributes compared in this study, 52% showed a significant difference between contrasting soils in which 84,4% of them corresponded to physicochemical characteristics. Although in our analyses we considered the means of the variables in group of soils (suppressive and non-suppressive), we recognize the limitations of this approach since suppressiveness in each soil may be conferred by different soil properties. Among the 15 chemical factors evaluated, seven were different when suppressive and conducive soils were compared. Soil pH, which was higher in suppressive soils, is involved in the availability of several nutrients and may favor bacterial establishment, which in turn suppresses pathogens (MEYER; SHEW, 1991a; LIU et al., 2020). Additionally, pH may have contributed to the higher levels of calcium, sum of bases, effective cation exchange capacity, and base saturation, all of which are properties linked to the higher fertility of the suppressive as compared to conducive soils. This higher availability of nutrients may have favored establishment of selected groups of microorganisms with antagonistic activity against the pathogen (SCHLATTER et al., 2017). Iron and aluminum, however, showed higher contents in conducive soils. Lower iron contents drive the selection of siderophore-producing microbes that compete for this element and may displace pathogens in the process (PELZER et al., 2011; SCHLATTER et al., 2017). This mechanism has been shown to operate in different suppressive soils (ZACCARDELLI et al., 2013) and may also play a role in soils suppressive to the stem bleeding disease. Aluminum is toxic to plants and microorganisms (KOCHIAN et al., 2015) and may be involved in the lower populations of total cultivable bacteria in conducive soils. Also, all suppressive soils had significantly higher sand

(86.80% vs. 71.40%) and lower clay (7.20% vs. 21.20%) contents, which was also reported to correlate with the suppressiveness to *Fusarium oxysporum* f. sp. *lini* (AMIR AND ALABOUVETTE 1993) and *Rhizoctonia solani* (RODRIGUES et al. 1998).

Calcium can interfere with most of the chemical attributes were significantly different. So we tested the hypothesis that calcium was someway involved in suppressiveness. Lime and gypsum, here simulated by calcium carbonate and calcium sulfate, are widely used in agriculture to balance acidic in surface and depth, respectively, by controlling aluminum saturation. Nevertheless, in our work aluminum was higher in suppressive soils which has been shown as a factor triggering suppressiveness to *Thielaviopsis basicola*, the causal agent of black root rot in tobacco together with pH lower or equal 5 (MEYER; SHEW, 1991a; MEYER; SHEW, 1991b). In contrast, in our work we observed suppression phenotype at higher calcium content and base saturation and pH 7 compared to the conducive soils.

This study does not punctuate a single factor triggering suppressiveness. Instead, we highlighted soil microbiota, calcium carbonate and pH as the main drivers to stem bleeding suppression in coconut soils from Sergipe. Although we could not distinguish among them, we could observe a negative correlation between pH and infection when applying calcium carbonate to the soils. Pathogen growth at pH 7 (Supplementary figure S2), achieved as for calcium carbonate as for suppressive microbiota, was diminished tending to zero. Nevertheless, suppressive soils were sandy with higher nutrient contents where total bacteria established at higher populations as compared to the conducive ones. While physical attributes of the soils such as texture cannot be managed, suppressiveness may be induced in the conducive soils through liming and fertilization to act directly or favor the build-up of antagonistic bacteria that would control of the pathogen in the soil, a key stage in *Ceratocystis paradoxa* life cycle. Finally, the level of suppressiveness in our work showed to be an interaction between soil biology, chemistry and physics.

5 Acknowledgements

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7 List of figures and tables

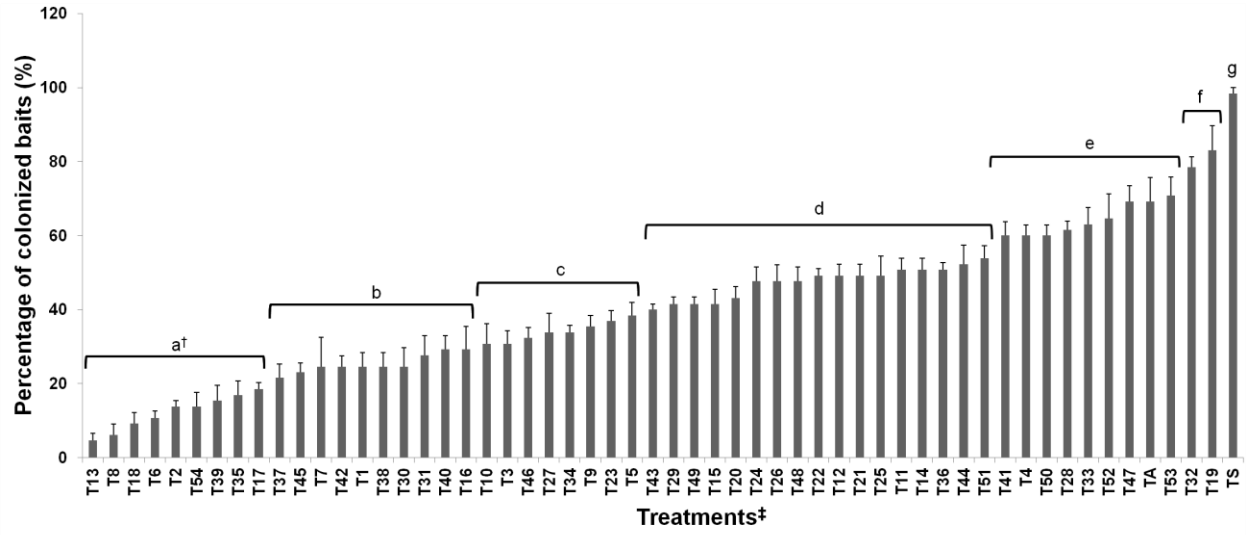


Figure 1 Soil samples classified in suppressive levels as determined by the mean percentage of baits colonized by *Ceratocystis paradoxa*.

† Means followed by the same letter are not significantly different according to Scott Knott's test at 5% probability.

‡ Treatments between T1 - T25 and T26 - T54 were collected in Sergipe and Pará, respectively. Both controls, TA (sand control) and TS (Subsoils) were obtained in Lavras.

Table 1 Percentage of baits colonized by *Ceratocystis paradoxa* in the five suppressive and the five conducive soils after sterilization or in their raw state.

Treatments	Baits in sterile soil (%)	Baits in raw soil (%)	Difference (%)[†]	*p*-value [a]
T2	38.89	11.11	27.70	0.033
T18	75.00	16.67	58.33	0.004
T8	30.56	22.22	08.34	0.058
T13	22.22	33.33	-11.11	0.031
T6	91.67	38.89	52.78	0.008
T32	80.56	55.56	25.00	0.025
T47	100.00	80.56	19.44	0.006
T19	94.45	83.34	11.11	<0.001
T53	100.00	86.11	13.89	0.002
T52	94.44	100.00	-05.56	0.391
TS [††]	100.00	-	-	-

[†] Reduction or increase in bait colonization when the same soil is tested after sterilization or in its raw state.

[a] Significant at the 0.05 probability level with the t test.

[††] Sterilized control.

Table 2 Population densities (CFU/g of soil) of fungi and bacteria for suppressive (S) and conducive(s) soils.

Treatments	**Total Bacteria**	***Pseudomonas* spp.**	***Pseudomonas* fluorescent**	**B*acillus* spp.**	**Total Fungi**	***Trichoderma* spp.**
T2	6.16×10^5	1.01×10^6	0.00	1.45×10^5	2.35×10^4	9.33×10^3
T18	1.31×10^6	1.93×10^6	0.00	1.16×10^5	2.50×10^4	3.00×10^3
T8	1.01×10^6	1.24×10^6	0.00	1.05×10^5	2.03×10^4	0.00
T13	4.35×10^6	1.75×10^6	6.91×10^4	4.17×10^5	1.66×10^5	1.18×10^4
T6	1.37×10^6	1.25×10^6	0.00	2.46×10^5	3.88×10^4	1.00×10^4
T32	1.59×10^5	1.23×10^6	0.00	2.32×10^5	2.14×10^4	5.00×10^3
T47	2.03×10^5	2.40×10^5	0.00	4.75×10^4	3.26×10^4	0.00
T19	1.77×10^6	1.75×10^6	0.00	1.53×10^5	1.30×10^4	2.00×10^3
T53	1.51×10^6	2.46×10^6	0.00	5.60×10^4	2.23×10^4	2.00×10^3
T52	1.96×10^5	1.10×10^6	0.00	4.55×10^4	1.02×10^4	0.00

Table 3 T test for biological, chemical and physical variables of the 10 selected soils grouped into suppressive and conducive.

Variables	Non-suppressive[†]	Suppressive [††]	*p*-value [a]
Colonized baits (%)	77.44	16.68	<0.00001
Total bacterial (CFU)	5.65	6.14	0.03079
Pseudomonas spp. (CFU)	6.03	6.14	0.6049
Bacillus spp. (CFU)	4.93	5.25	0.08255
Total fungi (CFU)	4.26	4.58	0.2107
Trichoderma spp. (CFU)	2.06	3.10	0.1702
pH (H_2O)	5.02	5.72	0.000216
Available phosphorus (P)	83.20	196.40	0.2064
Potassium (K)	170.50	252.10	0.438
Calcium (Ca)	1.41	3.41	0.003762
Magnesium (Mg)	0.55	1.20	0.09355
Aluminum (Al)	0.44	0.16	0.04465
Sum of bases (SB)	3.65	5.42	0.009972
Effective CEC (t) [c]	2.18	5.12	0.009162
CEC pH 7,0 (T)	2.62	5.28	0.3036
Base saturation (V)	7.60	8.76	0.03115
Aluminum saturation (m)	31.24	57.37	0.08214
Zinc (Zn)	25.60	3.86	0.5318
Iron (Fe)	410.21	19.80	0.01676
Manganese (Mn)	13.79	28.48	0.1479
Copper (Cu)	2.02	16.97	0.2637
Clay (C)	21.20	7.20	0.04
Sand (S)	71.40	86.80	0.028
Silt (Si)	7.20	6.40	0.1778
Particle density	2.55	2.56	0.8065

[†] Arithmetic mean of each variable for conducive and suppressive soils

[a] Significant at the 0.05 probability level

[††] Cationic exchange capacity

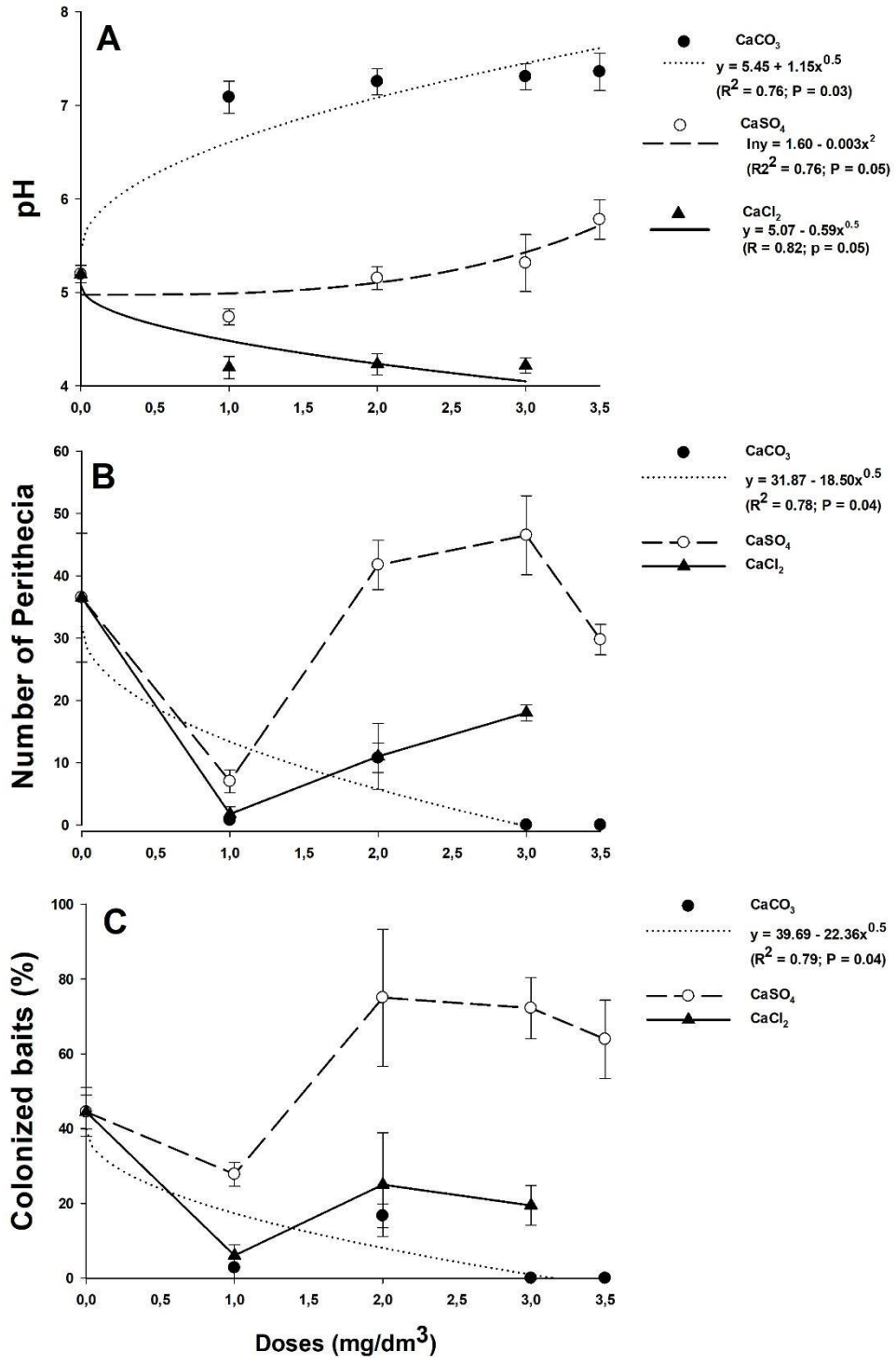


Figure 2 Effect of calcium source and doses on A) soil pH, B) Percentage of baits colonization and C) number of perithecia. Sources were calcium carbonate ($CaCO_3$), sulfate ($CaSO_4$) and chloride ($CaCl_2$) with doses ranging from 0 to 3 and 3.5 mg/dm^3.

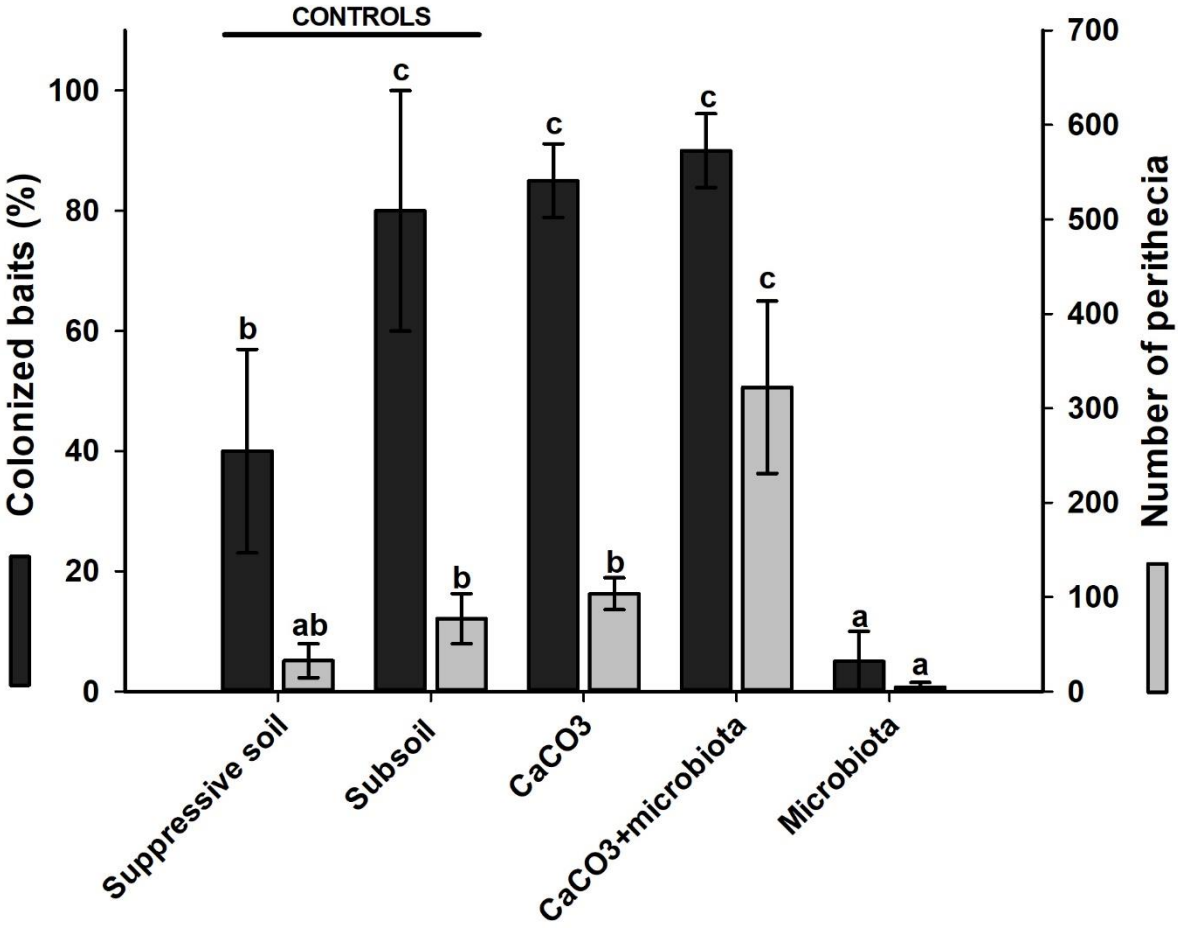


Figure 3 Calcium (1mg/dm^3) and extracted soil microbiota on *Ceratocystis* infection and perithecia number. Original suppressive sample (T2) and subsoil (TS) were used as control. Means were tested with Tukey test $p<0.05$. Standard deviation bars were showed. [a] suppressive soil (T2); [b] microbiota = extracted from soil T2

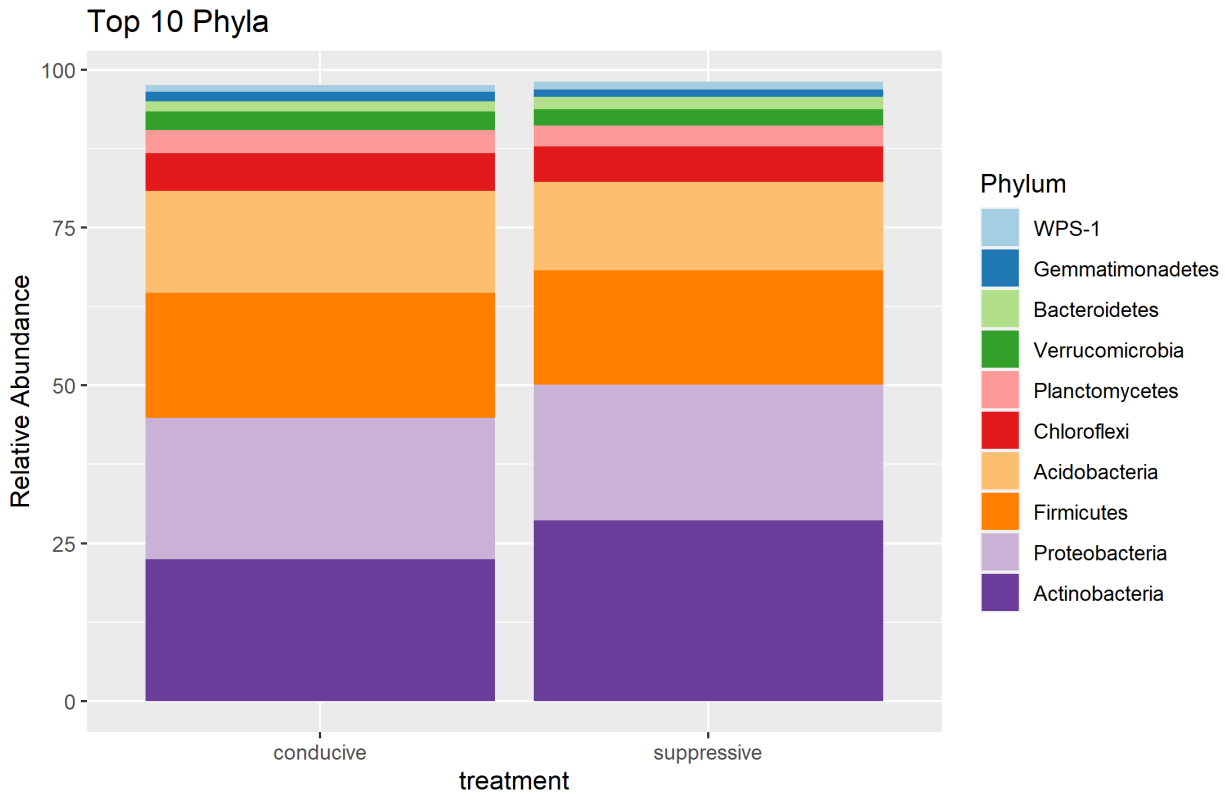
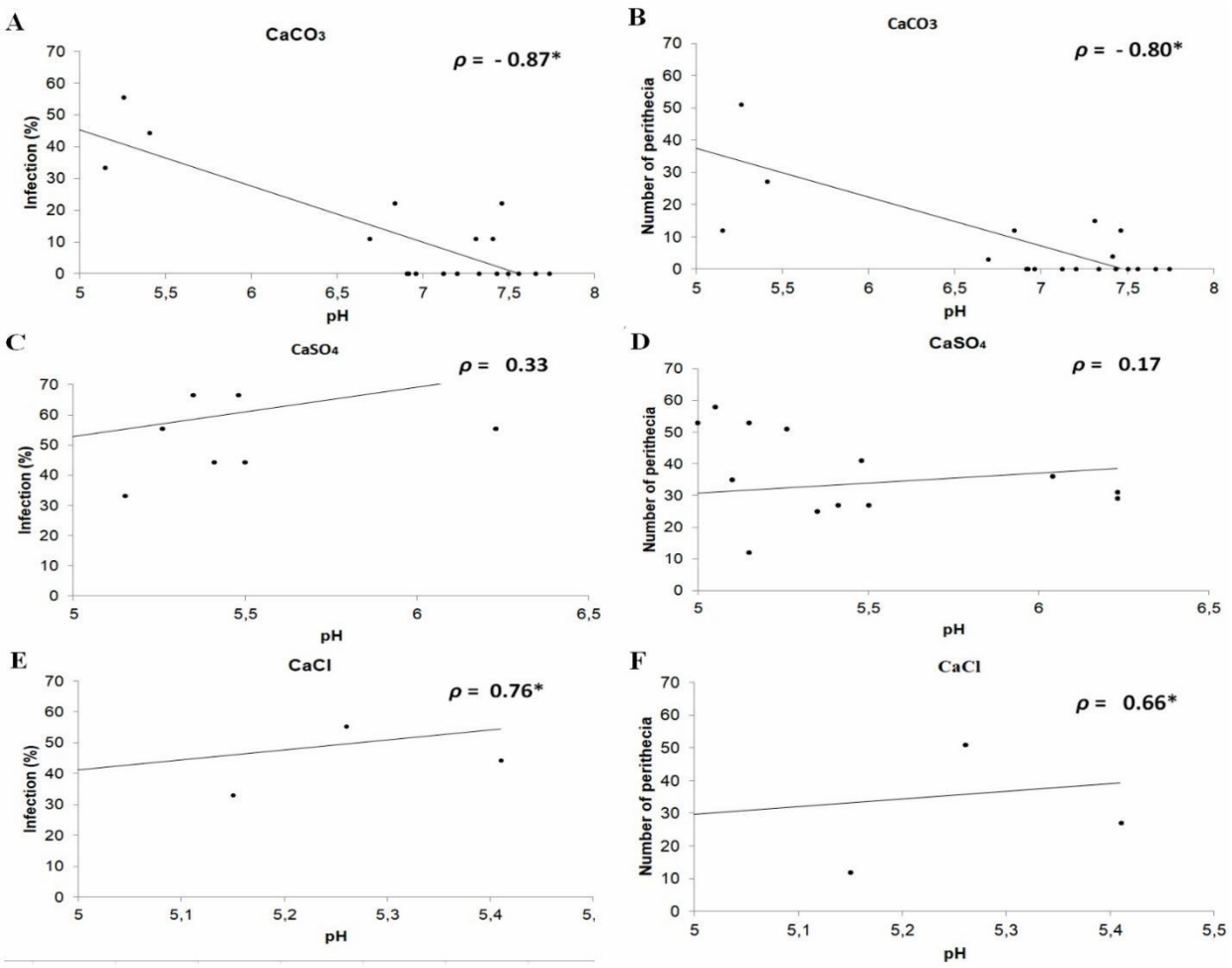


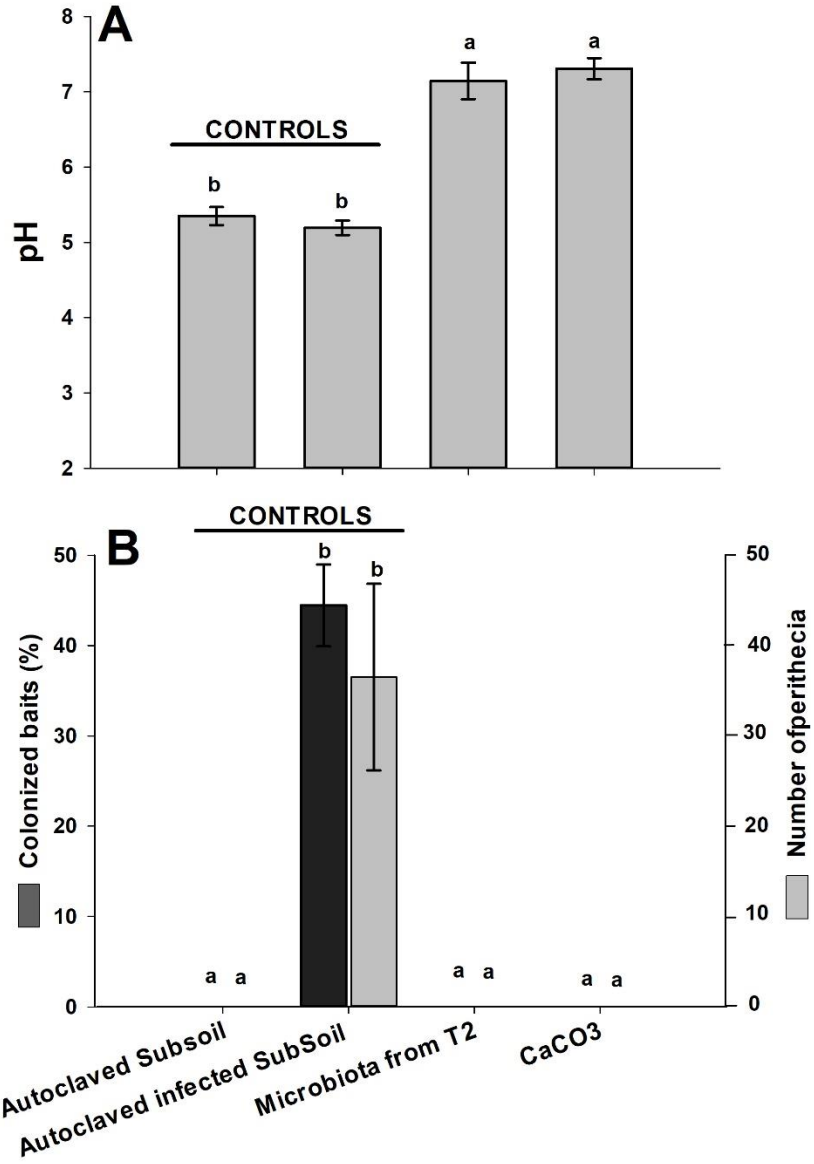
Figure 4 Taxonomic profile and relative abundance at phylum level of contrasting soils, conducive and suppressive, from Sergipe under coconut planting and *Ceratocystis paradoxa*.

8 Appendix

Support material



Support figure S1 Pearson's correlation for calcium sources with pH, baits colonization and number of perithecia.



Support figure S2 Effect of suppressive microbiota, calcium carbonate (1 mg/dm^3) and the resulting pH in the colonization and number of perithecia of *Ceratocystis paradoxa*. Autoclaved subsoil was used as control with and without pathogen inoculation. Means were compared by Tukey test $p<0.05$.

Support table S1. Chemical and physical analyses of sequenced samples, suppressive and non-suppressive

Sample ID	pH	K	Ca	Mg	Al	H+Al	SB	t	T	V	m	O.M.	P-rem	Zn	Fe
		mg/dm^3	cmol$_c$/dm^3							%		dag/Kg	mg/L	mg/dm^3	
Subsoil TS	5.0	28.67	0.13	0.10	0.46	1.86	0.30	0.76	2.16	14.05	60.53	0.53	34.26	0.10	56.54
Suppressive T2	6.9	105.56	2.42	0.71	0.05	0.96	3.40	3.45	4.36	78.00	1.45	0.78	45.68	12.67	27.05
Conducive T19	6.4	78.60	3.10	0.79	0.07	1.29	4.09	4.16		76.05	1.68	1.27	59.74	14.69	26.07