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CONSERVATIVE LONG TERM CROPPING SYSTEMS MITIGATING DROUGHT STRESSES AND IMPROVING SOIL HEALTH

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Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Agronomia/Fitotecnia, área de concentração em Produção Vegetal, para a obtenção do título de Doutor.

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CONSERVATIVE LONG TERM CROPPING SYSTEMS MITIGATING DROUGHT STRESSES AND IMPROVING SOIL HEALTH

SISTEMAS CONSERVATIVOS DE CULTIVO DE LONGO PRAZO NA MITIGAÇÃO DO ESTRESSE DA SECA E NA MELHORIA DA SAÚDE DO SOLO

Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Agronomia/Fitotecnia, área de concentração em Produção Vegetal, para a obtenção do título de Doutor.

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À Deus, meu refúgio e minha fortaleza.

Aos meus pais, minha base.

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RESUMO

O manejo conservacionista destaca-se pelo sucesso das atividades agrícolas diante de condições climáticas adversas, como a seca. O equilíbrio entre as fracções físicas, químicas e biológicas do solo, centrado na saúde do solo, promove um ambiente resiliente com maior diversidade e abundância das suas funções e processos. Algumas práticas, como as culturas de cobertura, os condicionadores de solo e o plantio direto, podem melhorar a saúde do solo. Este estudo tem como objetivo avaliar práticas sustentáveis de gestão do solo para melhorar a saúde do solo e mitigar os efeitos da escassez de água devido à resiliência ao stress da seca. Em climas tropicais e semiáridos, o manejo conservacionista do solo com o aporte de condicionadores de solo, culturas de cobertura e plantio direto, melhora a capacidade de troca catiônica, favorecendo o aumento da disponibilidade de nutrientes, a manutenção do pH e a ciclagem de nutrientes. Também, a *Urochloa decumbens* favore a porosidade do solo e aumenta a retenção de água. Mudanças no manejo do solo geram diferenças na comunidade microbiana do solo, também afetada por condições de seca. Assim, um manejo com foco na saúde do solo aumenta a resiliência das funções do solo à seca.

Palavras-chave: Sustentabilidade. Resiliência. Estresse ambiental.

ABSTRACT

The conservative management stands out for the success of agricultural activities in the face of adverse climate conditions, such as drought. The balance between physical, chemical, and biological soil fractions, focusing on soil health, promotes a resilient environment with greater diversity and abundance of its functions and processes. Some practices such as cover crops, soil conditioners, and no-till may improve soil health. The objective of this study was to evaluate the effects of sustainable soil management in coffee cropping, aiming to improve soil health and mitigate the effects of water scarcity through resilience to drought. In tropical and semiarid climates, the conservation soil management with the input of soil conditioners, cover crops, and no-tillage, improves the cation exchange capacity, favoring the increase of nutrient availability, maintenance of pH, and nutrient cycling. Also, *Urochloa decumbens* promotes soil porosity and increases water retention. Changes in soil management generate differences in the soil microbial community, also affected by drought conditions So, management focusing on soil health enhances soil functions' resistance to drought.

Keywords: Sustainability. Resilience. Environmental stress.

SUMMARY

CHAPTER 1 BACKGROUND

1 GENERAL INTRODUCTION

Drought is an issue that hits agricultural practices worldwide, intensified due to climate change related to water scarcity, especially in tropical and semi-arid conditions (IPCC, 2021; SÁ JÚNIOR et al., 2011). These regions also have limitations in building soil health because of the struggle of soil organic matter (SOM) accumulation, one of the most important parameters for assessing soil health (BREVIK et al., 2020; KARLEN et al., 1997; LAL, 2020a; LEWIS et al., 2018; NUNES et al., 2021). Water is related to soil health since its availability affects soil biological, physical, and chemical attributes, nutrient uptake, crop development, growth, yield, and health (DAVIS; HUGGINS; REGANOLD, 2023; GHIMIRE et al., 2023; LEHMANN et al., 2020; NUNES et al., 2021; VILELA et al., 2023).

Some conservative agricultural systems can improve soil health. In the global scenario, only 12.5% of the soils are explored under some conservation practices (e.g., crop diversification, no-till, cover crops, soil covering). Although the United States and Brazil have 43.2 and 32.0 million hectares of soil under conservation agriculture, most cultivated areas are under conventional tillage (KASSAM; FRIEDRICH; DERPSCH, 2019). Conventional tillage intensity and bare soils are some of the main issues that impair soil health, mainly due to soil erosion, which causes SOM degradation, reduces water storage, and increases CO_2 emissions (BAVEYE et al., 2020; BORRELLI et al., 2021; CHERUBIN et al., 2021; TIEFENBACHER et al., 2021). In contrast, no-tillage, reduced tillage, diversified crop rotation, organic compost, and manure have carbon (C) sequestration potential up to 500 kg of $C \cdot ha^{-1} \cdot year^{-1}$, which is converted in SOM, capable of retaining up to 20 times its weight in water (STEVENSON, 1994; TIEFENBACHER et al., 2021).

These conservation practices can improve soil health due to the formation of soil aggregates, soil water retention, reduced soil compaction and reduced erosion losses (physical properties); SOM input, cation exchange capacity (CEC), nutrients cycling, pH, soil buffering (chemical properties), and abundance, resilience, and biodiversity of microbiome functions (biological properties) (COSTA et al., 2018; FRANZLUEBBERS et al., 2021; MEDEIROS et al., 2019; VILELA et al., 2023; WULANNINGTYAS et al., 2020).

Also, the circular bioeconomy, conservation, and regenerative agriculture play an important role in soil health and crop sustainability (KASSAM; FRIEDRICH; DERPSCH, 2019; LAL, 2020b; MOREIRA et al., 2023). One of the aims of these practices is to optimize water and inputs in cropping systems (MUSCAT et al., 2021). Due to this management, using on-farm byproducts (e.g., organic compost from farmyard manure), integrated crop rotation, and good agricultural practices, it was possible to recover approximately 60% of all N input and ensure high yields even in the drought season (MOREIRA et al., 2023).

Due to these benefits, the management leading to sustainable agriculture for healthier soils also contributes to greater drought resilience. Therefore, this study aims to assess sustainable soil management practices to improve soil health and mitigate the effects of water scarcity in soil and plants.

2 REVIEW

2.1 Soil health

Soil health refers to the soil dynamic and interactions among chemical, biological, and physical properties and how this affects soil functions, water quality, climate, crop yield, and plant and human health (BREVIK et al., 2020; DAVIS; HUGGINS; REGANOLD, 2023; LEHMANN et al., 2020; MENDES et al., 2021; OLSON et al., 2014). Healthy soil may ensure crop profitability and environmental quality, keeping soil functionality and enhancing plant responses even under environmental stresses (DAVIS; HUGGINS; REGANOLD, 2023; LAL, 2020b; LEHMANN et al., 2020; NAYLOR; COLEMAN-DERR, 2018).

The management focusing on soil health stands out for more productive and sustainable agriculture, since it may improve nutrient cycling, soil suppressiveness, carbon sequestration, plant yield, and drought tolerance, besides long-term environment conservation (COSTA et al., 2018; FRANCO et al., 2020; LAL, 2020a). Some systems can improve soil health such as crop rotation, cover crops, reduced or no-tillage, and organic amendments input (e.g., circular bioeconomy). These agricultural practices may contribute to balanced plant nutrition and integrated disease and pest management, being an alternative to reduce chemical fertilizers and pesticide use (LAL, 2020b).

There are soil health indicators assessed to provide specific information about the soil properties and, when combined with a diverse data set, will allow us to have an overall understanding of the soil health of a site-specific, resulting in a soil health index (LEHMANN et al., 2020; NUNES et al., 2021). The indicators should contribute to enhancing one or all of some ecosystem services, such as plant production, water quality, climate regulation, and human health (LEHMANN et al., 2020).

The indicators used to assess soil health may be SOM content, nutrient availability, CEC, soil pH (soil chemistry), soil aggregation and infiltration, available water capacity (AWC), porosity (soil physics), microbial biomass and activity, enzyme activity, biodiversity due to DNA sequencing (soil biology), besides parameters related to the plants such as yield (LEHMANN et al., 2020; LEITE et al., 2021; NUNES et al., 2021; SINGH et al., 2023). Due to the data diversity, and to help comparison of different indicators, there is the calculation of soil health scores to obtain soil health indices, although there is a struggle to integrate all indicators into a single soil health index (JIAN; DU; STEWART, 2020; LEHMANN et al., 2020).

To apply soil health indices to different sites, the soils must have similar characteristics, address this aim, there is a large range of soil health assessment tools calibrated to different regions (most in the US), soil, and climate conditions. Depending on the indicator, the rating may be high is better, optimal curve, or low is better, meaning that one soil is healthier than another. Some examples of frameworks used for this assessment are CASH, SMAF, USDA-NCRS, principal component regression, and PCA (BREVIK et al., 2020; LEHMANN et al., 2020; NUNES et al., 2021; OBADE; LAL, 2016). In addition, site-specific and climatic constraints, as well as soil taxonomy, must be considered, for instance, a 1% SOM content may be considered adequate under tropical/arid conditions and low when analyzing temperate conditions (LAL, 2020a; LEHMANN et al., 2020).

There is a gap in soil health indicators in tropical Oxisoils and how the management focusing on soil health can contribute to drought resilience, due to adding more water to the system through conservative and circular agriculture principles.

2.2 Tropical and semi-arid soil limitations

Agriculture in tropical and semi-arid regions faces significant challenges due to the unique soil and climate conditions of these areas. Soils in tropical regions are naturally low in fertility, with little organic matter, are high in acidity and toxic aluminum, and may be susceptible to water erosion and nutrient losses (CARDOSO et al., 2012; CARDUCCI et al., 2015; LAL, 2020a; WITHERS et al., 2018). In semi-arid regions, water availability is a critical factor as erratic rainfall patterns and prolonged droughts affect crop productivity and soil erosion increases due to the difficulty of keeping soil covered (DAVIS; HUGGINS; REGANOLD, 2023; KASSAM; FRIEDRICH; DERPSCH, 2019; LEWIS et al., 2018).

So, climate conditions in these regions, especially high temperatures, contribute to the fast SOM decomposition and/or degradation depending on the management adopted. The protection of SOM is related to the constant input of carbon into the production system. This is because the decomposition process depends on the priming effect (PE), in which soil organisms obtain energy from simple materials (litter) and begin the decomposition of the complex and more stable forms of SOM (humic substances -HS) (JENKINSON; FOX; RAYNER, 1985). Thus, the quality and quantity of SOM, and the management of cultivated land can affect the PE, which an increase in easily degradable organic matter is necessary to avoid the degradation of complex and stable fractions (positive PE) (JENKINSON; FOX; RAYNER, 1985; SHAHBAZ et al., 2018). SOM decomposition releases CO_2, so protecting the most stabilized fraction of organic matter (HS) is essential to mitigate these gas emissions from the soil, which also contribute to carbon sequestration (OZLU; ARRIAGA, 2021).

If the input of organic matter in these soils is not constant, the consequences of their depletion can be irreversible. To overcome these challenges and improve soil health, due to sustainable management, carbon inputs must exceed decomposition rates to avoid the loss of most stabilized fractions of SOM. To this end, regenerative agriculture can be applied aiming to regenerate the cropping system and improve crop efficiency even under stress conditions.

2.3 How conservative agriculture plays a role in improving soil health and mitigating environmental stresses

Regarding climate change, there is a scenario of water lessening and increased temperatures (IPCC, 2021), which can impair plants' development and yield since these stresses can also make the plants more susceptible to pathogens (VILELA et al., 2022; VOLTOLINI et al., 2022). So, sustainable management must help plants to be more resilient under these challenges. Some techniques to mitigate the effects of water scarcity in coffee crops (e.g., organic compost and coffee husk - pericarp of coffee fruits after processing) promoted higher soil moisture even without irrigation, with similar responses to the irrigated treatment with bare soil (CASTANHEIRA et al., 2019). It may happen due to SOM input since it can retain up to 20 times its weight in water (STEVENSON, 1994). So, the management leading to improved SOM will contribute to soil health and provide resilience to crops under a stress climate.

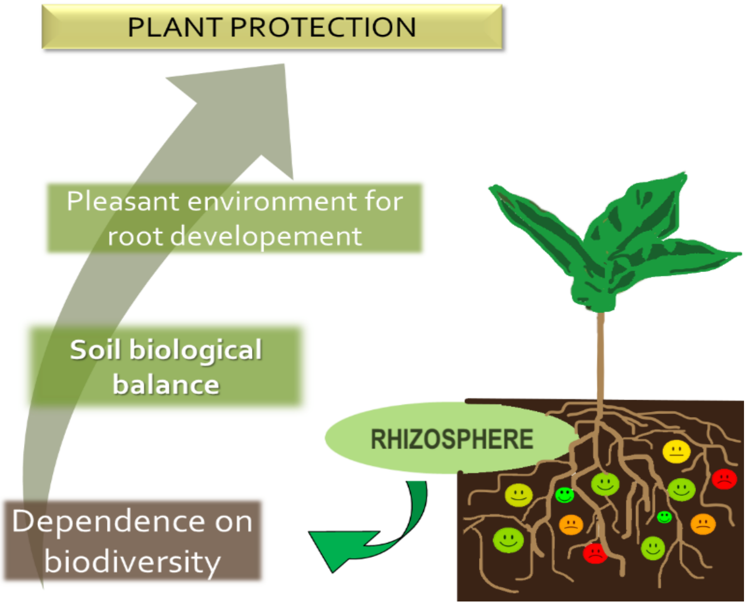
No-tillage is a system that may improve soil health, mainly when it's a long-term activity. In 18 years of this system, there was 50.5% of soil organic carbon input and 2.9% of CEC regarding the area under conventional tillage (WULANNINGTYAS et al., 2020). The use of by-products from the coffee farm post-harvest process (coffee husk) and organic compost of residues from raising chicken (eg., circular agriculture) associated with ecological management of cover crops (*Urochloa decumbens*) may also result in healthier soil. These practices increased by 340% of P availability, contributing to a better environment for coffee plants with an increase in base saturation (70%) and pH (6.5) and a decrease in aluminum saturation (6 to 1.2%) (VOLTOLINI et al., 2020). The sustainable management of coffee plants also results in 53% more grain yield than the conventional system, with only coffee (VOLTOLINI et al., 2022). Green manure of *Arachis pintoi* associated with natural phosphate increases the P uptake of corn (300%), with benefits to biological properties, improving soil microbial biomass carbon (62%) (MEDEIROS et al., 2019).

A healthy soil is capable of ensuring soil functionality, leading to resistance to environmental stress. Conservative soil management with a focus on restoring SOM, improving water content, and, for instance, microbial functions and nutrient availability, may help to achieve healthier soils. So, microbial functionality may be one of the tools used to assess soil health, since healthier soil results in the enhancement of enzyme activities, such as β-1, 4, glucosidase, and phosphatase, involved in C, and P-cycling (DAVIS; HUGGINS; REGANOLD, 2023; SINGH et al., 2023). The presence of some bacterial phyla (Acidobacteria and Firmicutes) in the soil may enhance plants' resilience to stress, indicating high SOM content, which is related to conservation soil management such as no-tillage and cover crops (SINGH et al., 2023).

The calculation of resistance indices helps us to understand how a microbiome's composition and functionality may resist stresses, such as drought (ORWIN; WARDLE, 2004). Even though a high resistance index is not a result of a resistant environment due to the "resistance trap" (DAVIS; HUGGINS; REGANOLD, 2023). If management favors low enzyme activity for instance, and after a disturbance, the characteristic is still low, the resistance index may be higher, only indicating that the disturbance did not negatively affect this trait because it was already bad before.

Land-use intensification and conventional agricultural systems can reduce the complexity and richness of microbial communities, affecting the resistance against environmental stresses (DELGADO-BAQUERIZO et al., 2017; VELOSO et al., 2023; LAU; LENNON, 2011; NAYLOR; COLEMAN-DERR, 2018; PAULA et al., 2014). More complex microbial communities enhance plant performance, with higher shoot biomass, flowering, and chlorophyll content than plants in a low-complexity biological environment (VELOSO et al., 2023; LAU; LENNON, 2011). The soil with a diverse microbiome due to conservation agriculture practices induces suppressiveness against pathogenic organisms (MENDES et al., 2011), providing a healthier environment for plant development (Figure 1). Suppressive soil has about 0% *Rhizoctonia solani* incidence compared to the conducive one (~60%) due to the soil disturbance by heat and reduced rhizosphere biodiversity (MENDES et al., 2011).

Figure 1 - Illustrative scheme of interactions between rhizosphere and microbiome.



Source: Author (2023).

Foliar diseases also may be managed by conservative agricultural systems. In coffee plants, there was a decrease in brown eye spot (BES) incidence in the leaves using coffee husk and organic compost (55 and 75% less than the conventional system) (RESENDE et al., 2022b). There was also a decrease in the fruit disease incidence (76%) using cover crop (*Urochloa decumbens*) regarding the plastic film, characterized by covering the soil without the SOM input (RESENDE et al., 2022b). So, soil moisture, SOM content, and high-efficiency fertilizers result in healthier plants (RESENDE et al., 2022a). Balance among potassium (K), calcium (Ca), and magnesium (Mg), associated with seasons of high-water availability in the coffee leaves, also contribute to the BES incidence decrease (VILELA et al., 2022).

So, soil health is related to plant health, which affects the quality of agricultural products, linked to food security and human health. BES, besides affecting coffee yield, may also affect the quality of beverage (LIMA; POZZA; SANTOS, 2012; POZZA; CARVALHO;

CHALFOUN, 2010). In foliar diseases caused by necrotrophic fungi, sustainable soil management, due to SOM input, may favor species of biological control agents, suppressing the pathogen and resulting in a healthier environment for the plant (LABORDE et al., 2019; RESENDE et al., 2022a; VELOSO et al., 2020). So, the soil microbiome may also affect the quality of coffee beverage, since the complexity of soil microbiome networks is related to the fruit microbiome, resulting in a beverage with complex sensorial characteristics (VELOSO et al., 2020).

3 GENERAL CONCLUSIONS

Long-term conservation soil management with combined sustainable techniques contributes to improving soil health due to the benefits in chemical, physical, and biological properties. Circular agriculture with the return of the by-products to the field and cover crop management contribute to soil conservation with higher plant yield and building soil fertility.

Some biological parameters such as bacterial and fungi richness and diversity should be more studied due to some divergences in the results, in which bacterial communities may resist more management changes. These analyses may be complemented by others more specific such as Taxa abundance and differential abundance test.

There are differences in the microbial community due to management and water availability. The resistance of soil functions to drought disturbance is different between the tropical and the semi-arid soils, with changes also due to soil texture (Halfway and Lamesa). The resistance trap only happened in the semi-arid conditions, there was also observed a dry history, with increasing responses of the enzymes under drought conditions.

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CHAPTER 2 SIX YEARS OF CIRCULAR AGRICULTURE IN COFFEE CROP BUILDING SOIL FERTILITY AND OPTIMIZING WATER

SEIS ANOS DE AGRICULTURA CIRCULAR NO CAFEEIRO CONSTRUINDO A FERTILIDADE DO SOLO E OTIMIZANDO A ÁGUA

RESUMO

Objetivou-se com este trabalho, demonstrar como a agricultura circular aplicada ao cafeeiro por seis anos pode melhorar a matéria orgânica do solo (MOS), construir a fertilidade do solo, e otimizar a água, garantindo altas produtividades. O experimento foi instalado em janeiro/2015 em uma área experimental localizada no município de Lavras, Minas Gerais, Brasil. Os tratamentos consistiram em manejo de cobertura do solo (*Urochloa decumbens*, filme de polietileno e solo exposto), fertilizantes (convencional e de liberação controlada) e condicionadores de solo (casca de café, gesso agrícola, polímero retentor de água, composto orgânico e testemunha). Os teores de MO, nutrientes no solo, potencial hídrico foliar foram analisados durante seis anos, bem como a produtividade e umidade do solo. A partir dessas análises foram calculados o acúmulo total e anual de MOS e nutrientes. Os condicionadores orgânicos (casca de café e composto orgânico) melhoraram o conteúdo de MOS e proporcionaram maior acúmulo total e anual de MOS. O composto orgânico e a casca de café se destacaram entre os demais condicionadores de solo quanto ao acúmulo anual e total de características relacionadas a fertilidade do solo. A cobertura de solo com *Urochloa decumbens* promoveu maior potencial hídrico foliar no cafeeiro. A combinação de condicionadores de solo (composto orgânico, casca de café) com *Urochloa decumbens* e fertilizante de liberação controlada proporcionaram maiores produtividades nos dois biênios analisados. Assim, a *Urochloa decumbens* e os condicionadores orgânicos do solo podem ser utilizados como alternativa para melhorar a MOS e demais nutrientes, com bons resultados a médio prazo em produtividade e manutenção de água na planta.

Palavras-chave: *Coffea arabica* L. Cobertura de solo. Condicionador de solo. Manejo sustentável.

ABSTRACT

This work aims to assess how a long-term circular coffee cropping system may enhance the soil organic matter (SOM), build soil fertility, optimize water, and ensure high yields. The experiment was installed in January/2015 in an experimental area located in Lavras municipality, Minas Gerais State, Brazil. The treatments consisted of soil cover management (*Urochloa decumbens*, plastic film, and exposed soil), fertilizers (conventional and controlled release), and soil conditioners (coffee husk, agricultural gypsum, water-retaining polymer, organic compost, and control). The SOM and nutrients in the soil and leaf water potential were analyzed over six years, as well as productivity and soil moisture. Based on these analyses, the total and annual accumulation of SOM and nutrients were calculated. The organic conditioners (coffee husk and organic compost) improved the SOM content and provided greater total and annual SOM accumulation. Organic compost and coffee husk stood out among the other soil conditioners in terms of the annual and total accumulation of characteristics related to soil fertility. Covering the soil with *Urochloa decumbens* promoted greater leaf water potential in the coffee tree. The combination of soil conditioners (organic compost, coffee husks) with *Urochloa decumbens* and controlled-release fertilizer provided higher yields in the two biennia analyzed. Thus, *Urochloa decumbens* and organic soil conditioners can be used as an alternative to input SOM in tropical soils and build soil fertility by increasing nutrient availability, with medium-term enhancement of yield, soil moisture, and water maintenance in the plant.

Keywords: *Coffea arabica* L. Soil cover. Soil conditioner. Sustainable management.

1 INTRODUCTION

Since coffee cropping began in Brazil in 1727, the growers have been using alternative sources for crop fertilization, such as litter from the native forests and manure. In the following centuries, the organic fertilizers produced from byproducts from the farm and the intensification of mineral fertilizers after the ammonium synthesis could end the extractive coffee cropping system (DAFERT, 1899). So, the long-term establishment of the crop in the same area became possible. At the end of the XIX century, the SOM input to complement mineral fertilization was already the best option for coffee growth and production (DAFERT, 1899; GUELFI, 2017). Unfortunately, this practice loses the space for exclusively chemical fertilization due to the higher amount of organic fertilizer required for the plants and logistic difficulties.

Since 80% of the chemical fertilizers used in Brazilian agriculture are imported from other countries, and most of the P applied is lost by fixation in tropical soils (GLOBALFERT, 2023; WITHERS et al., 2018), there is a need to look back in the past and move forward with old practices (e.g., organic fertilizer) combined with new technologies such as controlled release fertilizers and soil covering. High-efficiency fertilizers (controlled-release) reduce the N losses by volatilization improving this nutrient uptake, also contributing to the reduction of greenhouse gases emissions (GHGs), especially NO_2 (CHAGAS et al., 2019; DOMINGHETTI et al., 2016; LAWRENCIA et al., 2021).

Regarding cover crops, *Urochloa spp.* due to high biomass accumulation and root development, contributes to increased nutrient cycling and water storage, being able to cycle up to 11.5 $kg{\cdot}ha^{-1}$ of P, and to store 40 $m^3.ha^{-1}$ of water in 0-20 cm soil layer. Also, this cover crop increases leaf water potential when compared with bare soil, enhancing the coffee crop efficiency under water deficit (BAPTISTELLA et al., 2020, 2022; CASTANHEIRA et al., 2022; ROCHA et al., 2014; VILELA et al., 2023; VOLTOLINI et al., 2022).

In addition, organic amendments (coffee husk and compost) may increase potassium soil level (K) by up to 70%, soil moisture, and soil organic matter (SOM), resulting in a healthier environment for the plant's development even under unfavorable conditions. The coffee husk is a way to return the K to the field and may complement or replace the chemical fertilizer as a source of this nutrient (CASTANHEIRA et al., 2019, 2022; FERNANDES et al., 2020; VILELA et al., 2023; VOLTOLINI et al., 2020).

So, management that contributes to conservation ag-system (e.g., no-till, cover crops, mulch, integrated crop-livestock-forestry), soil health, and soil carbon sequestration (e.g., biochar, organic amendments, biomass input, increased C stocks) have the potential to enhance the crop's performance in the face of a challenging climate (LAL, 2013, 2020a, 2020b). With a climate changing and challenges with the scarcity of rainfall and fertilizers market crises, some recent research has shown the benefits of conservative soil management and circular agriculture applied to annual crops, aiming to increase SOM input, nutrient and water availability, besides reducing GHGs (LAL, 2013, 2020a, 2020b; MOREIRA et al., 2023).

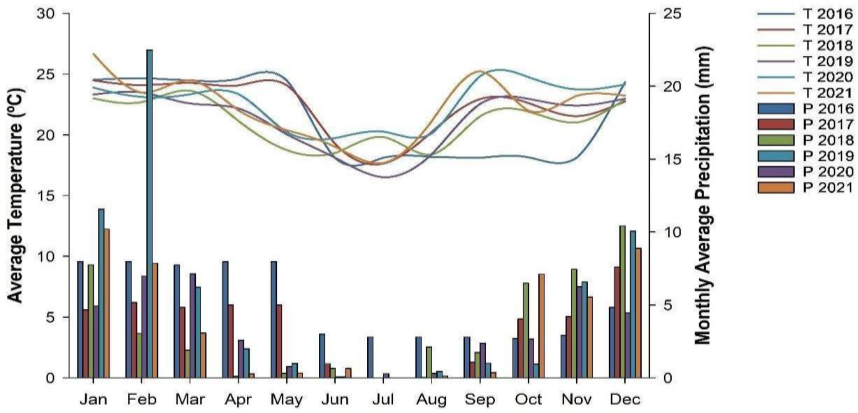
Therefore, circular agriculture contributes to sustainability since this practice aims to optimize water and inputs in cropping systems, which may contribute to the reduction in chemical fertilizers and pesticide use (MUSCAT et al., 2021). Applying circular agriculture, due to the use of byproducts from farm activities (e.g., organic compost from farmyard manure), integrated crop rotation, and good agricultural practices, provides the N recovery by 60% of all input and ensures high yields even in the drought season (MOREIRA et al., 2023).

So, integrated practices focusing on recovering and increasing the efficiency of nutrients, building soil fertility, and inputting SOM may enhance the resilience of the crop in the face of drought conditions. Thus, we hypothesize that long-term circular agriculture may enhance the soil organic matter (SOM), build soil fertility, and optimize water, ensuring high coffee yields.

2 MATERIAL AND METHODS

The field experiment was conducted from January 2016 to October 2021. The experimental area was located at latitude 21°13′36.47″ S, longitude 44°57′40.35″ W, and 975 meters altitude, in Minas Gerais State, southeast Brazil. The climate of the region is classified as Cwa with mild summers and dry winters (SÁ JÚNIOR et al., 2011), with average annual temperature and rainfall of 19.4ºC and 1558 mm, respectively (Figure 1).

Figure 1 - Average temperature (T) and monthly average precipitation (P) from 2016 to 2021.



Source: Author (2023).

In January 2016, 'Mundo Novo IAC-379-19' coffee seedling$_s$ were planted with a 3.6-meter spacing between planting rows and 0.75 meters between plants in the row. According to Brazilian Soil Classification System (CURI et al., 2017; SANTOS et al., 2018; SOIL SURVEY STAFF, 2022), the soil of the experimental area was classified as Latossolo Vermelho-Amarelo (CURI et al., 2017), and according to Soil Taxonomy it was classified as Typic Hapludox (SOIL SURVEY STAFF, 2022), with clayey texture (44% clay, 9% silt, and 47% sand).

Before the differentiation of treatments (October 2015), in the experimental area, the soil chemical attributes (for 0-20 cm and 20-40 cm layer, respectively) were: potassium 104 and 48 $mg \cdot dm^{-3}$, phosphorus 4.5 and 1.4 $mg \cdot dm^{-3}$, remaining phosphorus 27.1 and 16.5 $mg \cdot L^{-1}$, calcium 1.5 and 0.5 $cmol_c \cdot dm^{-3}$, magnesium 0.5 and 0.2 $cmol_c \cdot dm^{-3}$, aluminum 0.2 and 0.5 $cmol_c \cdot dm^{-3}$, pH in water 5.0 and 4.6, organic matter 2.1 and 1.3 $dag \cdot kg^{-1}$, potential acidity 3.5 and 4.4 $cmol_c \cdot dm^{-3}$, sum of bases 2.3 and 0.8 $cmol_c \cdot dm^{-3}$, cation exchange capacity 5.7 and 5.1 $cmol_c \cdot dm^{-3}$, base saturation 39.6 and 15.9%, and aluminum saturation of 8.1 and 37.8%. Soil acidity correction and phosphate fertilization were performed according to the results of the soil analysis, following the recommendations of Guimarães et al. (1999).

2.1 Experimental design

The factors under study were arranged in a 3×2×5 factorial scheme of analysis of variance with a randomized block design (30 treatments and three replications) in split-split plots. In the plots were applied three soil covers (plastic film, *Urochloa decumbens*, and bare soil). In the subplots were used two types of fertilizers (conventional and controlled-release fertilizer). Five soil conditioners were distributed among the sub-subplots (coffee husk, agricultural gypsum, water-retaining polymer, organic compost, and control). Each plot had six plants, and the four central ones were used for assessments.

2.1.1 Soil cover

The polyethylene film was a 1.60-m-wide, black-and-white, double-sided. It was set up in the row after planting the seedlings, with the white side exposed to the sun. The polyethylene film was removed in July 2019 due to damage caused by the management of the coffee plots over the years. The *U. decumbens* was grown between coffee rows, which were 1.60 m wide, always being mowed before producing seeds and keeping the biomass under the coffee canopy.

For the bare soil treatment, a 1.00 m band on each side of the planting row was kept free of weeds with weeding and herbicide applications.

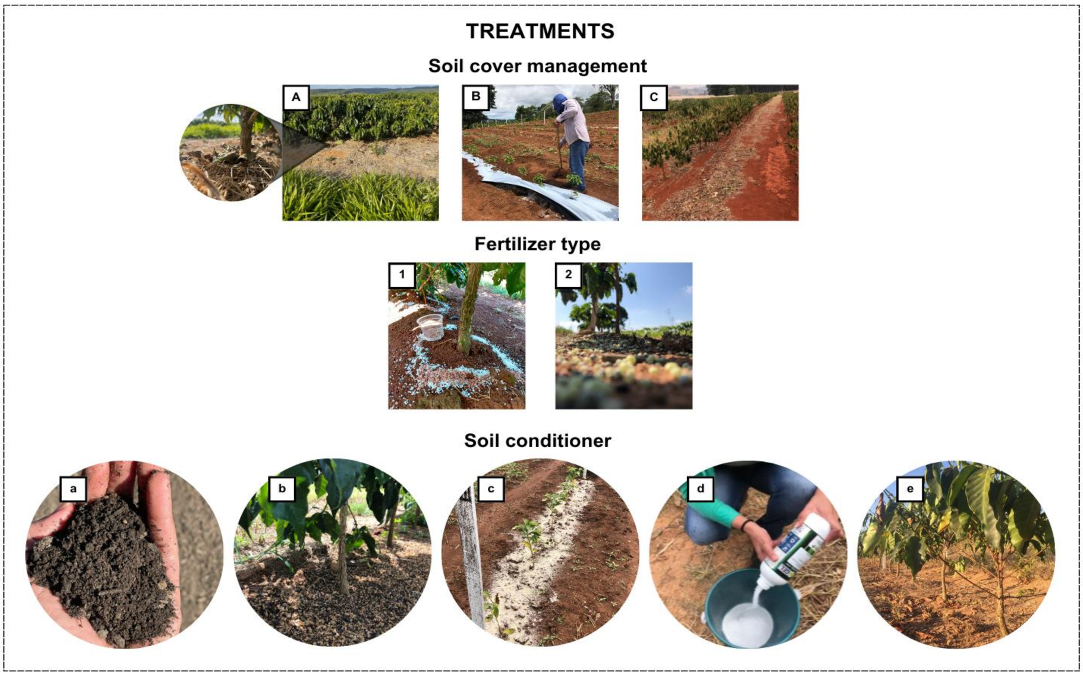
2.1.2 Fertilizer type

Two types of fertilizer were studied to provide nitrogen (N) and potassium (K) to plants. For the conventional fertilizer treatment, the formulation NPK 20-00-20 and conventional urea (45% N) were split-applied. The controlled-release fertilizers were two commercial products coated with elemental sulfur and organic polymers, the first with urea (37% N) and the second with potassium chloride (52% K_2O), used in a single application. The fertilizations were performed according to the results of the soil analysis and the nutritional needs of the coffee plants for post-planting fertilization and first-year-after-planting fertilization (GUIMARÃES et al., 1999).

2.1.3 Soil conditioners

For the coffee husk (pericarp of coffee fruits after processing), agricultural gypsum, and organic compost soil conditioner treatments, the projected canopies were top-dressed with the respective substance immediately after planting (GUIMARÃES et al., 1999). The dose of coffee husk and organic compost was 10 L per plant, uniformly distributed in the respective sub-subplots. The commercial product used to supply organic compost was waste from farms and food industries. Agricultural gypsum was applied in 50-cm bands on each side of the plants at a dose of 300 g m^{-2} based on the results of the analysis of soil from 20 to 40 cm and soil texture (GUIMARÃES et al., 1999). Water-retaining polymer was applied in the planting holes, watered according to the manufacturer's recommendations, and applied at a dose of 1.5 L solution per planting hole, incorporated into the soil. In the years after planting, the same dose was split-applied into two grooves next to each plant within the projection of the coffee canopy (PIEVE et al., 2013).

Figure 2 – Description of treatments with the combination of soil cover management, fertilizer type, and soil conditioners.



Soil cover management with *Urochloa decumbens* (A), polyethylene film (B), and bare soil (C); conventional fertilizer (1) and controlled release fertilizer (2); organic compost (a), coffee husk (b), agricultural gypsum (c), water retaining polymer (d) and control (e).
Source: Author (2023).

2.2 Assessments

2.2.1 Soil fertility

Samples to assess soil fertility were collected from the 0-20 cm layer, once a year, from 2015 to 2021. Samples from 20-40 cm deep layers were collected in 2015 and 2021. In 2015 the sampling was before the treatments' application. The samplings were always collected at the beginning of the rainy season (September). After the collection, the samples were sent for laboratory analysis. The characteristics assessed were soil organic matter (SOM), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), zinc (Zn), cupper (Cu), manganese (Mn), boron (B), iron (Fe), aluminum (Al), cation exchange capacity (CEC), soil pH, sum of basis (SB = $K^+ + Ca^{2+} + Mg^{2+}$), base saturation [V% = ((Ca+Mg+K+H+Al)/CEC) × 100], and aluminum saturation (m%).

The difference between the data from the first (2015) and last (2021) sampling was calculated to assess the total accumulation of nutrients after six years of management. For the annual accumulation assessment, the total accumulation was split per six (total of years assessed).

2.2.2 Gravimetric water content

Soil gravimetric water content (GWC) was assessed by collecting soil samples from the 0–10 cm layer. The samples were collected during the dry season (between July and August), once a year, from 2016 to 2021. A wet mass was obtained immediately after collection and a dry mass after drying at 105 °C until constant weight. The gravimetric soil moisture was calculated as a percentage (Equation 1) (EMBRAPA, 1997):

$$GWC = \frac{WSW - DSW}{DSW} * 100 \quad (1)$$

where:
WSW = wet soil weight (g)
DSW = dry soil weight (g)

The data collection to assess soil moisture was always in the morning right after the leaf water potential assessment. The mean of soil moisture from 2016 to 2021 of all treatments was used in the assessments.

2.2.3 Leaf water potential

The leaf water potential (MPa) was assessed before dawn (3:00 am to 5:00 am), with a Scholander pressure bomb® (model 1000, PMS Instrument Company) with the operation of up to 70 bar. One health leaf was collected per plot, located in the third or fourth pair from the plagiotropic apex. The days for data collection were those without rain.

2.2.4 Crop yield

The harvest of the plants started when about 90% of the fruits were at the ripe stage, between May and July in 2018, 2019, 2020 and 2021. The volume in liters of coffee from each plot was collected. Samples with three liters of coffee were weighed and dried. After hulling, the yield of each plot in kg of coffee at the time of harvest per hectare was calculated based on the weight of the grains. The 2018/2019 and 2020/2021 biennium were calculated by the average of each two-year yield in kg per hectare.

2.3 Statistical analysis

The data obtained were submitted to the assumptions of ANOVA, to verify the normality, homoscedasticity, and independence of the errors by the Shapiro-Wilk, Bartlett, and Durbin-Watson tests, respectively. Data statistical analyses were conducted in R software version 4.2.1 (R DEVELOPMENT CORE TEAM, 2020).

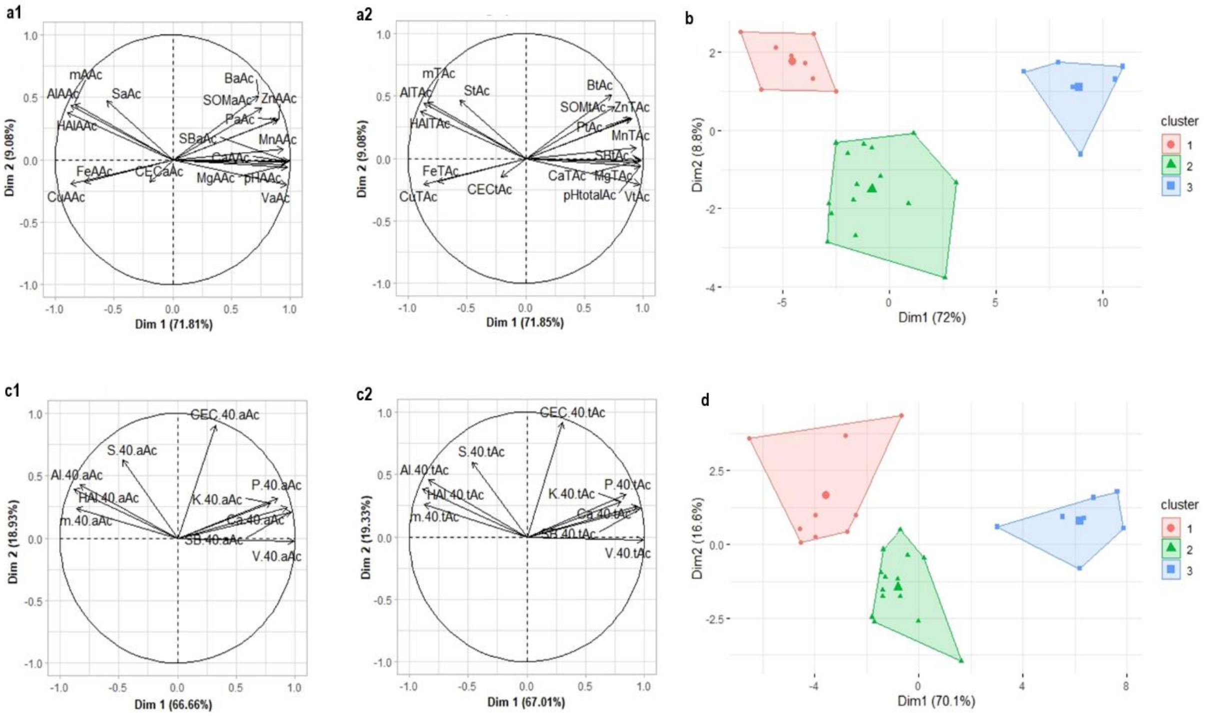
The principal components analysis (PCA) was carried out to assess the clustering of treatments regarding the level of nutrients in the soil, and how such treatments may contribute to soil fertility. The data were clustered based on scores of the first and second components (CRUZ; REGAZZI; CARNEIRO, 2012). The soil chemical characteristics that contributed to at least 80% of the variability explained by the first and second components were selected to be analyzed. The data were submitted to the ANOVA. When significant ($p<0.05$), the means were assessed by the Tukey test.

3 RESULTS AND DISCUSSION

Circular agriculture contributed to build soil fertility in 0-20 cm and 20-40 cm layers during the six years of study, especially increasing the soil organic matter (SOM) due to the organic amendments input (CASTANHEIRA et al., 2019; VOLTOLINI et al., 2020).

In the PCA analysis (Figure 3), there were three clusters regarding the soil amendments, soil cover, and fertilizer-type treatments. In 0-20 and 20-40 cm layers total and annual nutrient accumulation, the same clusters were formed. Cluster #1 had the treatments with the control, agricultural gypsum (G), and water-retaining polymer (P), regardless of soil cover and fertilizer type. The treatments with coffee husk, as a similar characteristic, were in cluster #2, while the ones with organic compost as a common treatment were in cluster #3.

Figure 3 - PCA of soil chemical attributes annual (1) and total (2) accumulation in 0-20cm (a) and 20-40cm (c) deep layers, and clusters of nutrients total and annual accumulation in 0-20cm (b) and 20-40cm (d) deep layers.



Annual (aAc) and total accumulation (tAc) in the 0-20cm layer, annual (40aAc) and total Accumulation (40tAc) in the 20-40cm layer, soil organic matter (SOM), aluminum (Al), potential acidity (H+Al), aluminum saturation (m), sulfur (S), cation exchange capacity (CEC), potassium (K), phosphorus (P), calcium (Ca), magnesium (Mg), sum of basis (SB), base saturation (BS), zinc (Zn), manganese (Mn), cupper (Cu), iron (Fe), boron (B).
Source: Author (2023).

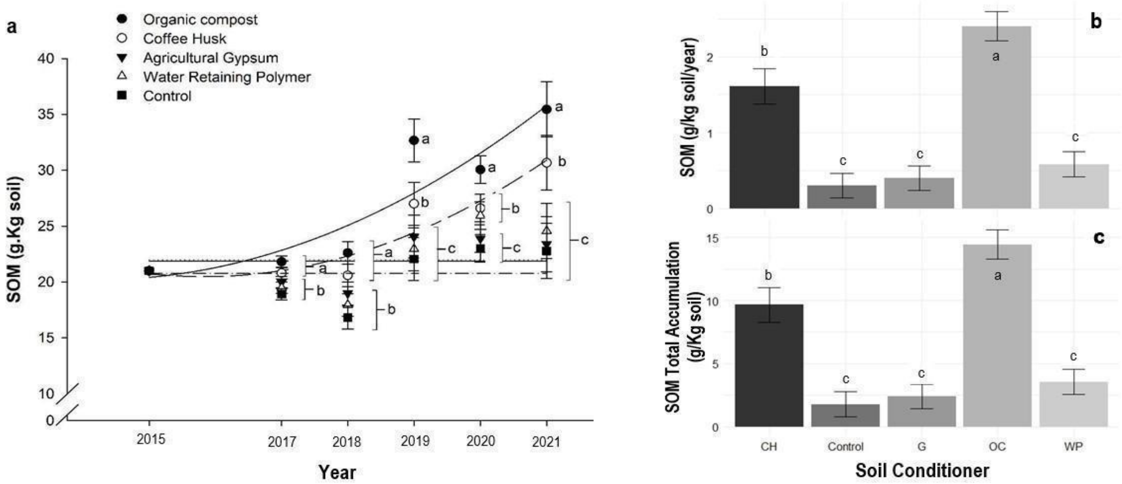
The soil components related to acidity (Al^{3+}, potential acidity (H+Al), and aluminum saturation - m%) and sulfur content (soil bulk 0-20cm) were closer to the first cluster. On the other hand, in the 0-20 cm layer, the CEC, Fe, Cu, Ca, Mg, base saturation, and soil pH were close to the cluster with the treatments that received coffee husk (#2). Also, P, SOM, sum of basis, and other micronutrients (Zn, B, Mn) were close to the third cluster. In soil bulk 20-40cm, cluster #3 was also closer to CEC, Ca, K, P, sum of basis and base saturation, being opposite to the acidity components.

The clusters 2 and 3 are indicative of the contribution of the coffee husk and organic compost to soil fertility, the second one with contributions also in deeper layers (20-40 cm). So, these amendments may contribute to the reduction of chemical fertilization due to the release of nutrients, ensuring balanced plant nutrition (CASTANHEIRA et al., 2019; FERNANDES et al., 2020; VILELA et al., 2023; VOLTOLINI et al., 2020). Also, due to SOM input, these traits may have a buffer effect in the soil, contributing to the maintenance of adequate soil pH and neutralizing Al toxicity (MURPHY, 2015; VILELA et al., 2023; VOLTOLINI et al., 2020).

2.4 Soil organic matter (SOM)

The circular agriculture management, due to the input of organic compost and coffee husk, resulted in the SOM increase during the six years of study (Figure 3).

Figure 4 - Soil organic matter (SOM) content between 2015 (first sampling) and 2021 (last sampling) (a), annual (b), and total (c) accumulation of coffee plants grown under different soil conditioners.



Bars followed by the same letter do not differ by Scott-Knott ($p < 0.05$). Organic compost (OC), coffee husk (CH), agricultural gypsum (G), water retaining polymer (WP), control (C).
Source: Author (2023).

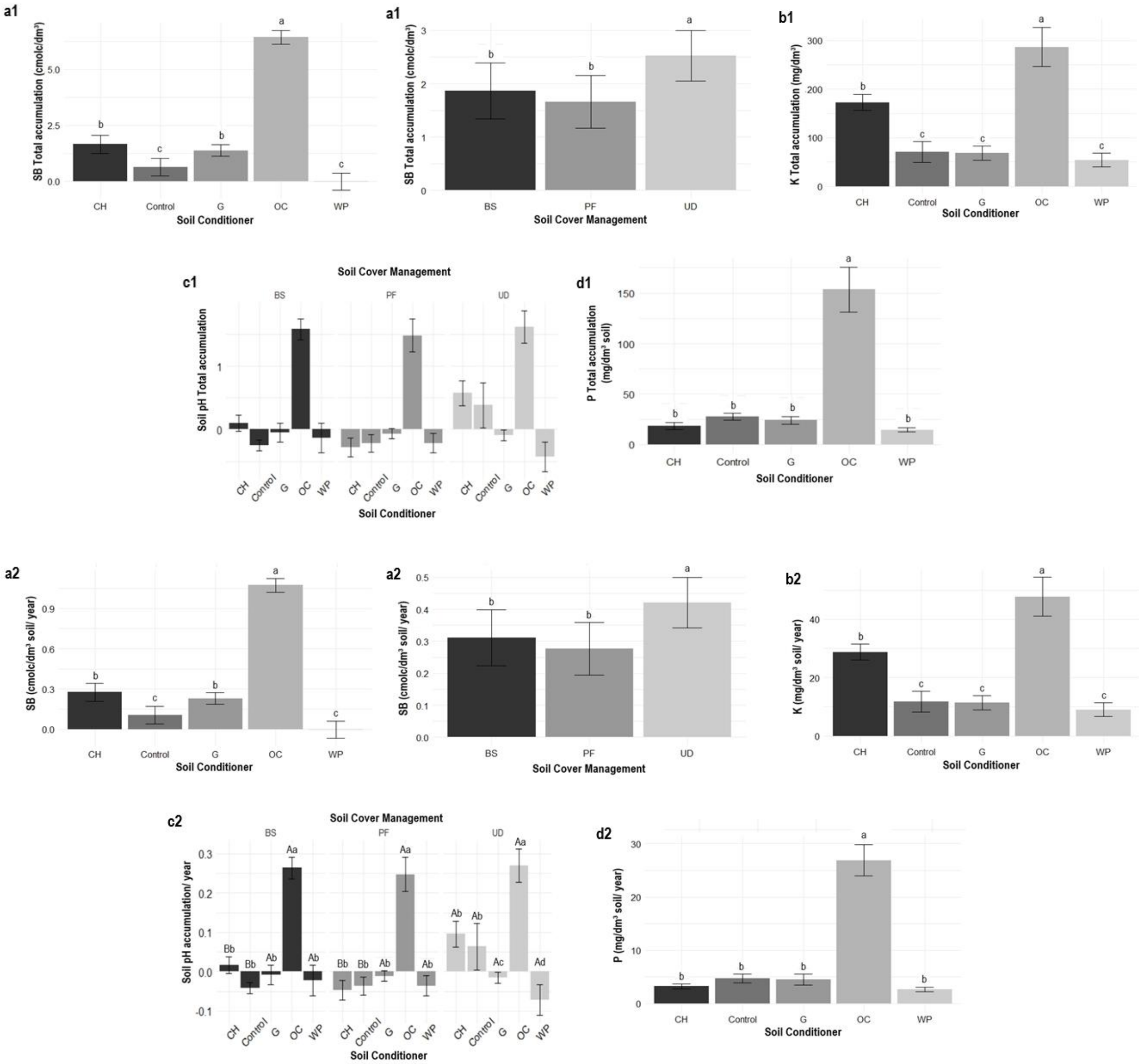
In 2017 and 2018, the coffee husk and organic compost had equally increased SOM regarding the other soil amendments. After these years until the last assessment (2021), the organic compost stood out, followed by the coffee husk. The other three amendments contributed less to the SOM accumulation. Indeed, organic compost and coffee husk addition significantly increased SOM total and annual accumulation (Figure 4 b,c). Due to the organic compost and coffee husk, there were accumulations of 2.4 and 1.6 g·Kg soil^{-1} per year, respectively. The total accumulation of SOM with organic compost (14.4 g·Kg soil^{-1}) and coffee husk (9.7 g·Kg soil^{-1}) were 700 and 439%, respectively, above the total accumulated with the control (1.8 g·Kg soil^{-1}).

SOM is one of the main resources responsible for crop yield and profitability, mainly in tropical agricultural systems (LAL, 2020b). So, since organic conditioners (coffee husk and organic compost) contribute to SOM, the return of these amendments to the field (circular agriculture) results in a more sustainable coffee production system (CASTANHEIRA et al., 2022; MOREIRA et al., 2023; VILELA et al., 2023; VOLTOLINI et al., 2022).

2.5 Soil chemical attributes total and annual accumulation (0-20 and 20-40 cm)

The accumulation of the sum of basis (SB), P, and K in the soil was influenced by the soil conditioners in the 0-20 (Figure 5) and 20-40 cm (Figure 6) layers. Organic compost addition significantly increased SB and soil P levels compared to other amendments. After organic compost, coffee husk and agricultural gypsum also increased SB. Soil cover management with *Urochloa decumbens* improved SB accumulation in the soil. Organic compost and coffee husk input significantly increased soil K levels compared to other soil conditioners. There was an interaction between soil cover management and soil conditioner regarding soil pH annual and total accumulation. In deeper layers (20-40 cm) there was an increase in CEC when using organic compost and the controlled-release fertilizer.

Figure 5 - Sum of bases (Ca+Mg+K) (a), pH (b), phosphorus (c), and potassium (d) total (1) and annual (2) accumulation in the 0-20cm soil depth with coffee plants grown under different soil cover management, soil conditioners, and fertilizer types from 2015 to 2021.

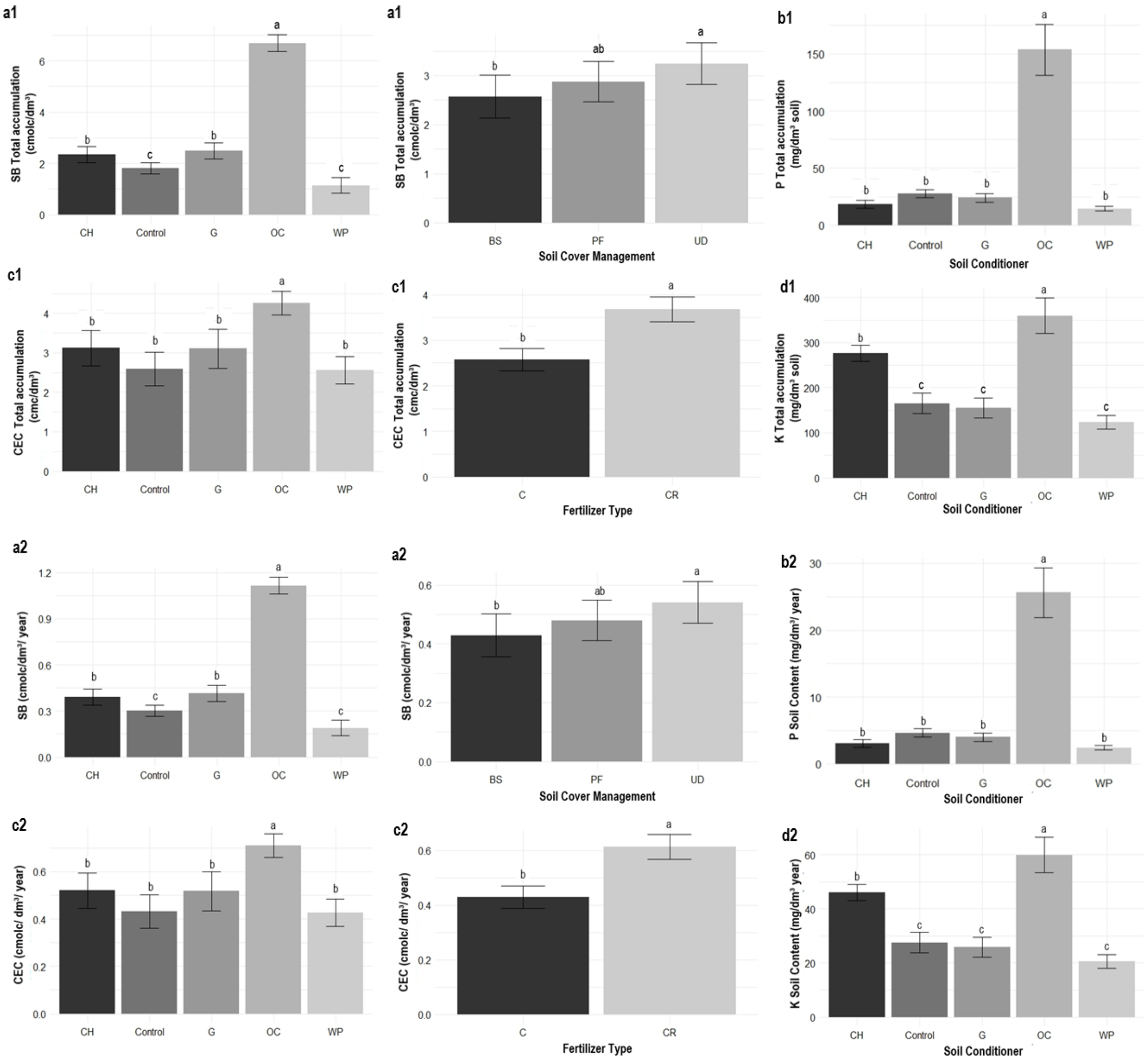


Bars followed by the same letter do not differ by Scott-Knott ($p < 0.05$). Bare soil (BS), polyethylene film (PF), *Urochloa decumbens* (UD), coffee husk (CH), control, agricultural gypsum (G), organic compost (OC), and water-retaining polymer (WP).
Source: Author (2023).

The SB total accumulation (0-20cm) (Figure 5a1) with the organic compost (2.5 mg$\cdot$dm$^{3-1}$) was 32% higher than the control (1.9 mg$\cdot$dm$^{3-1}$). The organic compost input improved the SB by approximately 1 cmo$_c \cdot$dm$^{3}\cdot$year^{-1}, followed by coffee husk and agricultural gypsum (Figure 5a2). The organic compost contributed to the increase by 50 mg$\cdot$dm$^{3-1}$ per year of the K in the soil, with a total accumulation of about 300 mg$\cdot$dm$^{3-1}$. The coffee husk also could contribute to the accumulation of K in the 0-20 cm layer, in lower proportions than the organic compost, but above the other amendments (Figure 5b).

The organic compost combined with all soil cover management increased soil pH (1.5 total and 0.25·year[-1]), followed by the *Urochloa decumbens* + coffee husk (0.5 total and 0.1·year[-1]) (Figure 5c). SOM due to buffer effect may reduce the acidification of the soil over the years (MURPHY, 2015). So, since the organic compost, coffee husk + *U. decumbens* contributes to SOM, these managements may also act as soil buffer (VILELA et al., 2023; VOLTOLINI et al., 2020).

Figure 6 - Sum of bases (Ca+Mg+K) (a), cation exchange capacity (CEC) (b), phosphorus (c), and potassium (d) total (1) and annual (2) accumulation in the 20-40 cm soil depth with coffee plants grown under different soil cover management, soil conditioners, and fertilizer types from 2015 to 2021.



Bars followed by the same letter do not differ by Scott-Knott ($p < 0.05$). Bare soil (BS), polyethylene film(PF), *Urochloa decumbens* (UD), coffee husk (CH), control, agricultural gypsum (G), organic compost (OC), and water-retaining polymer (WP), conventional fertilizer (C), controlled-release fertilizer (CR).
Source: Author (2023).

In the 20-40 cm layer, SB was higher with the organic compost (225% above control) and with *Urochloa decumbens* (27% above bare soil) (Figure 6a). The K content increased by 60 and 45 $mg{\cdot}dm^{3}{\cdot}year^{-1}$ with organic compost and coffee husk, respectively (Figure 6d).

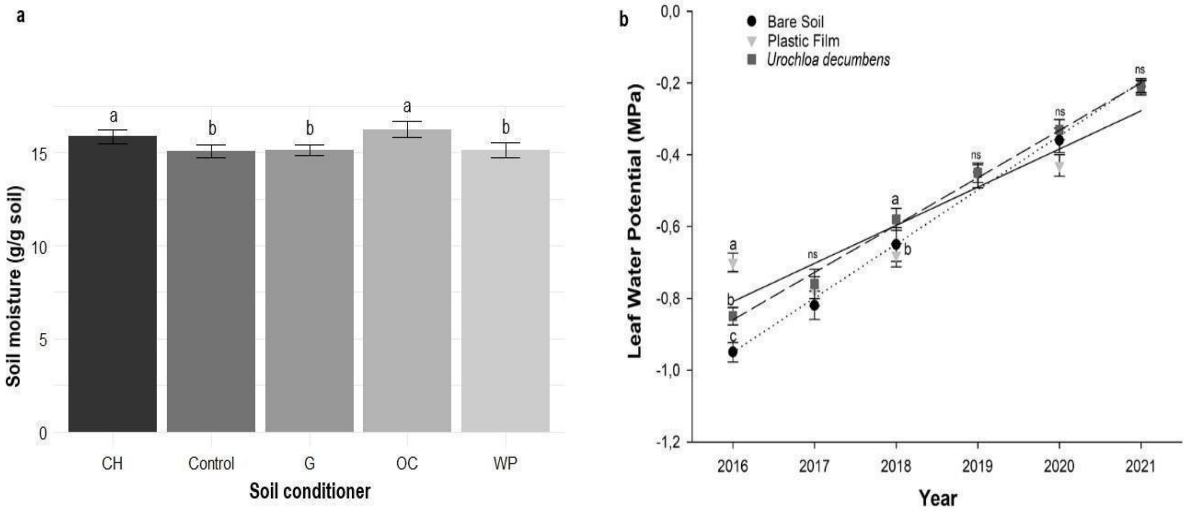
In the first 20 cm soil deep layer (Figure 5), the organic compost input had the potential to increase the P levels by 27 $mg.dm^{-3}$ of soil per year. The total accumulation of P in the soil with the organic compost was about 479% above the control (162 against 28 $mg{\cdot}dm^{-3}$). In deeper layers (20-40cm), the P level also increased by 162 $mg{\cdot}dm^{3}$ after 6 years of organic compost input. The CEC (20-40 cm) increased 4.5 $cmo_{c}{\cdot}dm^{3-1}$ after 6 years of organic compost input, increasing 0.75 $cmol_{c}{\cdot}dm^{3}{\cdot}year^{-1}$, and with controlled release fertilizer the increase was 3.6 $cmo_{c}{\cdot}dm^{3-1}$ (total) and 0.6 $cmol_{c}{\cdot}dm^{3}{\cdot}year^{-1}$. The controlled-release fertilizer also fostered higher CEC, being 52% higher than the conventional fertilizer. The enhanced fertilizer, due to the slow release of nutrients, contributes to increasing soil fertility and plant nutrition (CHAGAS et al., 2019; LAWRENCIA et al., 2021; VILELA et al., 2023).

In tropical soil with naturally poor fertility, most of the CEC comes from SOM, which improves nutrient availability and decreases P fixation (LI et al., 2019; NASCIMENTO et al., 2018; WITHERS et al., 2018). Also, building soil fertility in deeper layers (40 cm) leads to the improvement of crop yield, since there is a better environment for root growth (MOREIRA et al., 2023). Also, organic compost and coffee husk can be used as complementary sources of nutrients since their input improved K, P, SB, CEC, and soil pH (VILELA et al., 2023; VOLTOLINI et al., 2020; ZOCA et al., 2014).

2.6 Soil moisture and Leaf Water Potential

Soil conditioners fostered the mean of six years of soil moisture assessment. The leaf water potential was influenced by the soil cover management in only two of the six years assessed.

Figure 7 - Mean of soil moisture (a) and leaf water potential (b) from 2016 to 2021 of coffee plants grown under different soil coverings, fertilizer types, and soil conditioners in 2021.



Bars followed by the same letter do not differ by Scott-Knott ($p<0.05$). Coffee husk (CH), control, agricultural gypsum (G), organic compost (OC), water-retaining polymer (WP).
Source: Author (2023).

In the mean of six years of circular agriculture practices, the organic compost (15.5%) and coffee husk (15.7%) input resulted in higher soil moisture than the other soil conditioners (Figure 7a). Management that favors soil covering and SOM is more efficient in retaining water and may have the same gravimetric water content as irrigated bare soil (CASTANHEIRA et al., 2019; VILELA et al., 2023).

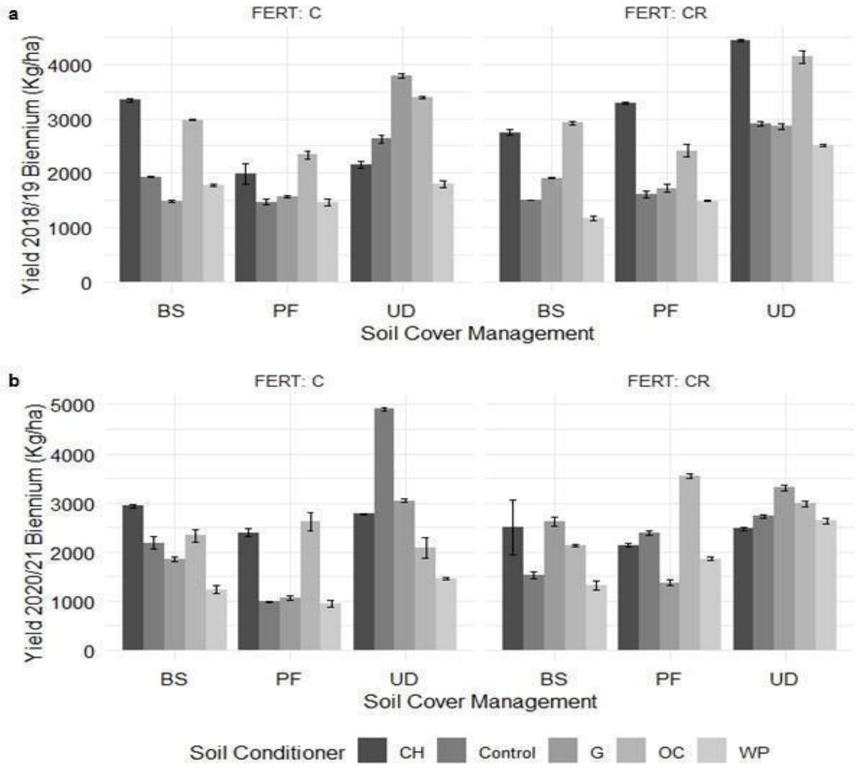
During the six years of assessment, only in 2016 and 2018, there was a significant difference in leaf water potential (Figure 7b). In 2016, a higher value was observed with the soil cover with the plastic film. In the third year of the experiment, the higher leaf water potential was observed in the plots that received the *U. decumbens* as a soil cover management. Also, as the plants were getting older, the leaf water potential was increasing.

Thus, the soil moisture contributes to increase leaf water potential and may be related to the improvement of nutrient uptake efficiency, since this process requires the presence of water and happens by difference of hydric potential (CASTANHEIRA et al., 2022; TAIZ et al., 2017; VILELA et al., 2022). In contrast, the bare soil provided an environment less efficient with lower soil moisture which may result in lower leaf water potential, being harmful to the plant development (CASTANHEIRA et al., 2022; VILELA et al., 2023).

2.7 Yield 2018/2019 and 2020/2021 biennium

The circular coffee cropping system had a significant influence on the coffee yield during the 2018/2019 and 2020/2021 biennium. In the average yield for the two biennia, there was an interaction among soil cover management, fertilizer type, and soil conditioner (Figure 8).

Figure 8 - Yield (Kg·ha^{-1}) of coffee plants grown under different soil coverings, fertilizer types, and soil conditioners in the 2018/19 biennium (a) and 2020/21 biennium (b).



Bare soil (BS), polyethylene film (PF), *Urochloa decumbens* (UD), coffee husk (CH), agricultural gypsum (G), organic compost (OC), water-retaining polymer (WP), conventional fertilizer (C), controlled-release fertilizer (CR).
Source: Author (2023).

The combination of the cover crop (*Urochloa decumbens*) with controlled-release fertilizer and coffee husk or organic compost resulted in a higher yield in the 2018/2019 biennium (Figure 8). Regarding the absolute control (no soil cover + no soil conditioner + conventional fertilizer), the increase in yield with the sustainable practices mentioned was by 53% (3780 kg·ha^{-1}). The association of the polyethylene film with controlled-release fertilizer and coffee husk fostered 66% of the yield, compared with the control (bare soil + no soil conditioner). The same soil cover management with conventional fertilizer and organic compost resulted in a yield of 2700 kg·ha^{-1}, which was 125% above the control plots.

In the 2020/21 biennium (Figure 8), the treatment with *U. decumbens* soil cover management + conventional fertilizer + control had a higher yield (4900 kg·ha^{-1}), followed by the combination of *U. decumbens* + conventional fertilizer + agricultural gypsum (3050 kg·ha^{-1}) or coffee husk (2780 kg·ha^{-1}). The *U. decumbens* + controlled release fertilizer + agricultural gypsum (3310 kg·ha^{-1}) or organic compost (2980 kg·ha^{-1}) had a higher yield than other managements with the same fertilizer (100% above control). The polyethylene film with controlled-release fertilizer and organic compost (3540 kg·ha^{-1}) or coffee husk (2140 kg·ha^{-1}) fostered higher yields than other conditioners, and under the same soil cover, with conventional fertilizer, the organic compost also stood out (2610 kg·ha^{-1}). In bare soil management, the coffee husk resulted in a higher yield in association with the conventional fertilizer (2940 kg·ha^{-1}) and controlled-release fertilizer (2500 kg·ha^{-1}).

The yield oscillation between the two biennia was lower using combined techniques (*U. decumbens* + controlled-release fertilizer + gypsum, organic compost, or coffee husk) than using isolated management (Figure 8). Nutritional balance and the presence of water lead to high yield, so sustainable coffee crop management integrating soil covering and soil conditioners results in higher yield and lower coffee biennially (VOLTOLINI et al., 2022).

U. decumbens, due to the aggressiveness of the root system, contributes to nutrient cycling, and water retention because of the increase of soil porosity, which results in higher coffee yield (BAPTISTELLA et al., 2020, 2022; VOLTOLINI et al., 2022). Since circular agriculture is related to the optimization of water and inputs into the cropping production system, it contributes to improving the nutrient availability and water content, and enhancing plant yield (MOREIRA et al., 2023).

4 CONCLUSION

Circular agriculture due to using organic amendments, especially when combined with *U. decumbens* and controlled release fertilizer enhanced water in the cropping system due to SOM input, resulting in higher coffee yield. These management combinations also contributed to building soil fertility in the 0-20 and 20-40 cm deep layers.

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CHAPTER 3 SOIL HEALTH LEADING TO DROUGHT RESISTANCE OF SOIL FUNCTIONS IN THE COFFEE CROP

SAÚDE DO SOLO NO MANEJO DA RESISTÊNCIA DAS FUNÇÕES DO SOLO À SECA NO CAFEEIRO

RESUMO

O manejo do cafeeiro que conduz a solos mais saudáveis pode aumentar a resistência funções do solo a condições de seca. Objetivou-se com esta pesquisa avaliar o impacto, a longo prazo, do manejo sustentável na saúde do solo do café por meio da avaliação de como a composição e a função dos microbiomas dos solos tropicais brasileiros respondem à seca severa. As parcelas foram selecionadas de um experimento de longo prazo instalado em janeiro de 2016 em uma área experimental localizada no município de Lavras, Estado de Minas Gerais, Brasil. Os tratamentos foram a combinação de casca de café + *Urochloa decumbens*, gesso agrícola + *Urochloa decumbens*, e o tratamento controle com cultivo único de café com solo exposto. Foram avaliados os componentes relacionados à saúde do solo (atributos químicos, físicos e biológicos). O manejo com a casca de café + *U. decumbens* resultou na melhoria dos indicadores químicos, físicos e biológicos da saúde do solo quando comparado ao manejo com solo exposto. Além disso, a combinação de técnicas sustentáveis resultou em maior produtividade do café e qualidade da bebida. A funcionalidade do solo (atividades enzimáticas) foi mais resistente à seca quando manejado com casca de café + *U. decumbens*. As comunidades fúngicas e bacterianas mudaram com o manejo e a disponibilidade de água, com espécies raras sendo favorecidas em condições de seca.

Palavras-chave: *Coffea arabica* L. Plantas de cobertura. Microbioma do solo. Manejo sustentável.

ABSTRACT

The coffee crop management leading to healthier soils may enhance the resistance to drought stresses. The objective of this research was to evaluate the long-term impact of sustainable soil and crop management on soil health through the assessment of how the composition and function of the microbiomes of Brazilian tropical soils respond to severe drought. The plots were selected from a long-term experiment installed in January 2016 in an experimental area located in Lavras municipality, Minas Gerais State, Brazil. The treatments were the combination of coffee husk + *Urochloa decumbens*, agricultural gypsum + *Urochloa decumbens*, and control consisting of single coffee cropping with bare soil. Soil components related to soil health (chemical, biological, and physical attributes) were assessed. The management with the combination of coffee husk + *U. decumbens* resulted in the improvement of chemical, physical, and biological soil health indicators when compared to bare soil management. Also, the combination of sustainable techniques resulted in higher coffee yield and quality of the beverage. Soil functionality (enzyme activities) was more resistant to drought when managed with coffee husk + *U. decumbens*. The fungal and bacterial communities changed with the management and water availability, with rare species being favored under drought conditions.

Keywords: *Coffea arabica* L. Cover crops. Soil microbiome. Sustainable management.

1 INTRODUCTION

Brazil is the largest world producer and exporter of coffee, and Minas Gerais state is responsible for producing 73.6% of *Coffea arabica L.* in the country (CONAB, 2023). Building soil health is a critical aspect to consider since it goes beyond enhancing agricultural systems and ensures soil and food security and human health (LAL, 2013, 2020a). There are different systems to assess soil health, but most of them are calibrated to specific soil conditions in the United States or Brazilian Cerrado, with different crops being cultivated (MENDES et al., 2021; NUNES et al., 2021). So, there is a lack of information regarding soil health indicators for coffee cropping systems in Brazilian tropical soils in Minas Gerais state.

Also, *Coffea arabica* production in the state had two consecutive years of low yield, escaping the pattern of coffee biennially, in which a year with low yield is followed by a year with a high one. So, the 2022 harvest, which should be the highest production, was 0.82% smaller than in 2021 due to adverse climate conditions, mainly drought during coffee blossoming (CONAB, 2023). Soil health is related to the balance among physical, chemical, and biological properties, leading to a resilient and diverse soil environment with plant resistance to biotic and abiotic stresses, especially drought (COSTA et al., 2018; FRANCO et al., 2020; LAL et al., 2020; VILELA et al., 2023).

Management-related practices can improve soil health and optimize water availability, resulting in the sustainability of agriculture systems. Brazil has 32.0 million hectares of soil under conservation agriculture, even though most cultivated soils are under conventional cropping systems (KASSAM; FRIEDRICH; DERPSCH, 2019). Conventional tillage with bare fallow promotes straw removal, destabilization of the soil, and SOM degradation, increasing CO_2 emissions (CHERUBIN et al., 2021; FRANCO et al., 2020; MARTINS et al., 2015; TIEFENBACHER et al., 2021).

Organic amendments input and cover crops intercropped with the coffee crop can contribute to soil health due to the positive effect on the soil-plant system. Coffee husk, one of the by-products of coffee post-harvest, can improve soil moisture, nutrient availability, and soil organic matter (SOM), resulting in a healthier environment for the plant's development even under water deficit (CASTANHEIRA et al., 2019, 2022; RESENDE et al., 2022; VILELA et al., 2023; VOLTOLINI et al., 2022). Agricultural gypsum, as a subsurface soil conditioner, may improve plant rooting in deeper layers due to the increase of nutrient availability and aluminum toxicity neutralization, which make plants more resistant under drought conditions

(CARDUCCI et al., 2015; RAMPIM; DO CARMO LANA; FRANDOLOSO, 2013; SILVA et al., 2015).

Since *Urochloa spp.* can increase soil porosity and SOM, there is an improvement of water retention with more availability to the plants, by adding 40 m^3 ha^{-1} of water in the 0-20 cm soil layer when intercropped with coffee plants (GALDOS et al., 2020; ROCHA et al., 2014). Besides, this system also contributes to increasing P cycling (11.5 $Kg.ha^{-1}$) and enhances the availability of this nutrient to the plants, due to the increase in the phosphatase enzyme activity (BAPTISTELLA et al., 2022; SINGH et al., 2023).

Conservation soil management (eg., no-till, cover crops) contributes to the bacterial community. The presence of Acidobacteria and Firmicutes phyla in the soil is indicative of high SOM content, which increases β-1, 4, glucosidase enzyme activity (SINGH et al., 2023). Such microbiome may be related to the enhance of plants' resistance to stresses.

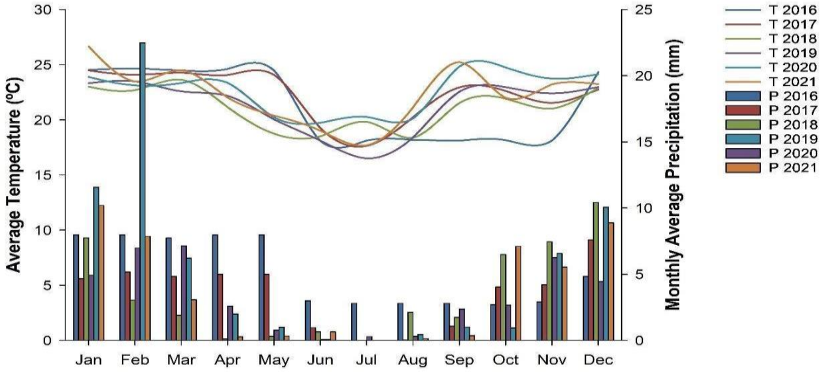
Understanding the interactions between biological, physical, and chemical properties of tropical soil and its effects on plants can contribute to more information regarding soil health in coffee cropping systems. The objective of this research was to evaluate the long-term impact of sustainable soil and crop management on soil health through the assessment of how the composition function of the microbiomes of Brazilian tropical soils respond to severe drought.

2 MATERIAL AND METHODS

2.1 Experimental and management

The study considered a long-term field experiment installed in January 2016 at the Department of Agriculture Federal University of Lavras (UFLA) experimental area near Lavras, Minas Gerais, Brazil (-21.226797 °S, -44.961208 °W, 975 meters above the sea level). The region consists of a tropical climate with an average annual temperature of 19.4°C and an average annual precipitation of 1558 mm. Local rainfall distribution and average temperature during the study period (2016 to 2021) are presented in Figure 1. The soil of the experimental area was classified as Latossolo Vermelho-Amarelo (CURI et al., 2017) according to the Brazilian Soil Classification System (SANTOS et al., 2018) and Typic Hapludox according to Soil Taxonomy (SOIL SURVEY STAFF, 2022) with a clayey texture (Table 1). The grove area was 0.324 ha in size and contained about 1,200 coffee plants. The coffee variety used in the experiment was 'Mundo Novo IAC-379-19' (*Coffea arabica L.*). Distance between trees in the row was 0.75 m and 3.6 m between rows. All trees in the grove were planted at the same time and received the same management with regard the fertilization, and pest control (insecticides, herbicides, and fungicides throughout the year), with products registered for the coffee crop following the guidelines for a sustainable agriculture (REIS; CUNHA, 2010).

Figure 1 - Average temperature (T) and monthly average precipitation (P) from 2016 to 2021.



Source: Author (2023).

Table 1 - Soil's chemical and physical characteristics previous to the experiment installment in 2015.

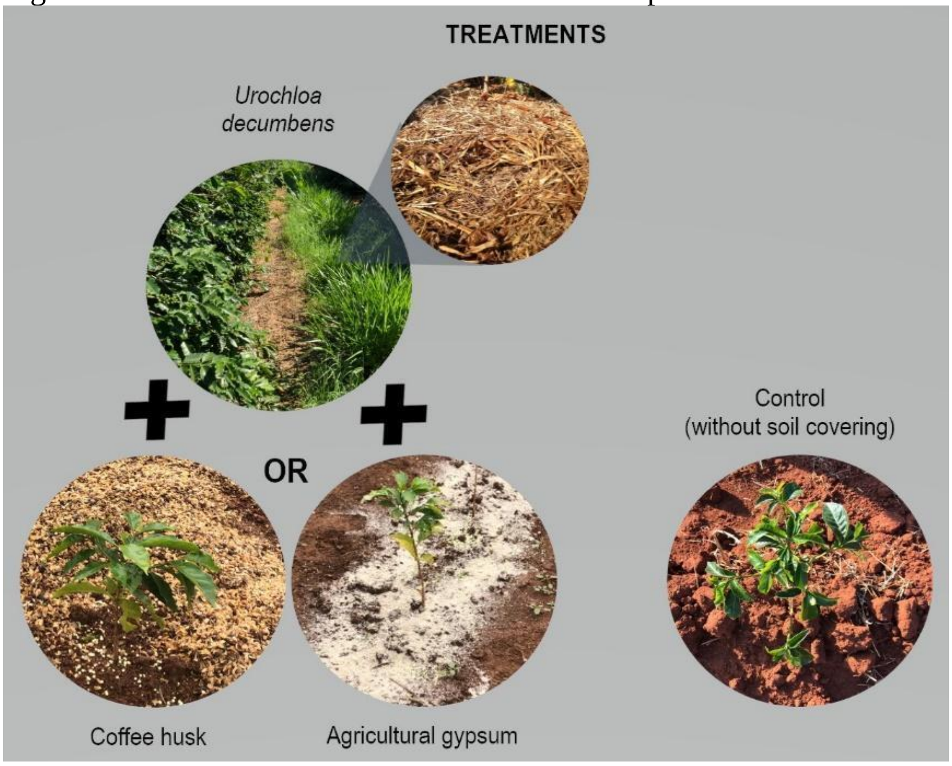
Depth (cm)	pH (H_2O)	P (mg dm^{-3})	K	Ca^{2+}	Mg^{2+}	Al^{3+} (cmol$_c$ dm^{-3})	H + Al	SB	t	CEC
0-20	5.0	4.5	104	1.5	0.5	0.2	3.5	2.3	2.5	5.7
20-40	4.6	1.4	48	0.5	0.2	0.5	4.4	0.8	1.3	5.1
	V (%)	m	SOM (g kg^{-1})	P-Rem (mg L^{-1})	Zn	Fe	Mn (mg dm^{-3})	Cu	B	S
0-20	39.6	8.1	21	27.1	2.9	102.7	22.9	4.1	0.3	35.9
20-40	15.9	37.8	13	16.5	0.7	93.5	10.6	3.2	0.5	60.7
Soil texture classification				Clay		Silt ($kg \cdot kg^{-1}$ soil)			Sand	
Clayey				0.44		0.09			0.47	

Source: Author (2023).

2.2 Treatment’s description

The field study considered three treatments comparing different management strategies of the coffee inter-rows: CHUD = coffee husk + *Urochloa decumbens*; GUD = agricultural gypsum + *Urochloa decumbens*; and Control = without soil covering, coffee husk, or gypsum. In the CHUD and GUD treatments, the sowing of the *U. decumbens* was carried out between the coffee rows in December 2015. The grass was planted 1 meter distance from each side of the coffee row, occupying 1.60 m. The ecological management of the grass was conducted by mowing before flowering, with biomass deposition under the coffee canopy. Along with the *U. decumbens*, as soil conditioners, it was used coffee husk (CHUD treatment) or agricultural gypsum (GUD treatment). Both coffee husk and gypsum were applied once every year between 2016 and 2021 (a total of 5 applications) and top-dressed in the coffee rows. Doses were 10 $L.plant^{-1}$ of coffee husk and 300 $g{\cdot}m^{-2}$ of agricultural gypsum, based on the soil chemical analysis from 20 to 40 cm and soil texture (GUIMARÃES et al., 1999). In the control treatment, the soil was kept bare 1.00 m wide on each side from the coffee row with weeding and herbicide applications with products registered to the coffee crop. The herbicides were applied after monitoring and weed identification.

Figure 2 - Scheme of the treatments used in the experiment.



Source: Author (2023).

2.3 Plant and soil sampling and analyses

2.3.1 Crop yield

The fruits from each plot were harvested, and the weight and volume of the harvested coffee were assessed. Samples with 3 L of harvested coffee were weighed and dried. When the samples reached 11.5% moisture, they were weighed before and after being processed. The data obtained was used to calculate the average yield for the 2020/2021 biennium in kg/ha.

2.3.2 Coffee sensorial analysis

The sensorial analysis of coffee was carried out following the Specialty Coffee Association (SCAA, 2013) protocol by three Q-graders (professionally trained coffee tasters at the Coffee Quality Institute). The coffees were assessed according to attributes such as fragrance, sweetness, flavor, acidity, uniformity, clean cup, body, aftertaste, balance, overall, and global score evaluation.

2.3.3 Soil chemical attributes

Soil samples for chemical characterization of the soil were collected at a depth of 20 cm layer at the beginning of the rainy season (September) in 2015 (before the experiment was installed) and in September 2021. Two samples per plot were collected in the projection of the coffee canopy. Soil chemical measurements included: soil organic matter (SOM), potassium (K), phosphorus (P), calcium (Ca), magnesium (Mg), aluminum (Al), pH, base saturation, and aluminum saturation. The SOM and nutrients total accumulation were assessed through the difference between the 2021 and 2015 soil contents. The annual SOM and nutrients accumulation was calculated by dividing the total accumulation per 6 years of experiment.

For SOM measurements, the soil was sieved at 2 mm, air dried, ball milled, and oven dried at 105°C for 2 days. Soil pH was determined in water (1:1 soil/water ratio). P and K were extracted with Mehlich^{-1} solution (H_2SO_4 0.0125 mol L^{-1} and HCl 0.05 mol L^{-1}) at a ratio of 1:10 (v/v) soil/solution and determined by flame emission spectroscopy. Exchangeable Ca and Mg were extracted using a KCl 1 mol L^{-1} (1:10 v/v soil/solution) and determined by atomic absorption spectrophotometry with air–acetylene flame and 5% lanthanum solution to prevent interference. Potential acidity (H+Al) was extracted with Ca (OAc)2 0.5 mol L^{-1} buffered at pH 7. The sum of exchangeable basic cations (SB = Ca^{2+} + Mg^{2+} + K^{+}), cation exchange capacity at pH 7.0 (CEC = SB + H+Al), and base saturation [BS = ((Ca+Mg+K+H+Al)/CEC) × 100] were then estimated.

Table 2 - Soil's chemical characteristics of each plot in 2021.

Soil Management	pH (H_2O)	P (mg dm^{-3})	K	Ca (cmol$_c$ dm^{-3})	Mg	Al	CEC	SOM (g kg^{-1})	BS (%)	m
					0-20 cm depth					
CHUD	5.8	36.0	342.5	4.4	1.2	0.1	8.7	35.0	5.6	4.7
GUD	5.1	20.0	209.5	3.2	0.3	0.4	10.1	27.0	4.1	11.4
Control	4.9	19.7	155.8	1.9	0.3	1.0	8.0	19.2	2.7	22.0
					20-40 cm depth					
CHUD	5.3	35.7	432.7	3.0	0.8	0.1	9.7	-	5.0	2.7
GUD	5.0	24.5	222.3	2.1	0.2	0.4	7.6	-	3.0	12.8
Control	4.9	18.1	170.5	1.4	0.2	0.4	6.6	-	2.1	15.3

Source: Author (2023).

2.3.4 Soil physics attributes

To the gravimetric water content (GWC) assessment, the samples were collected in 2021 with 3 repetitions per treatment, during the dry season (August) from the 0–10 cm layer. The wet soil weight (g) was collected right after sampling and the dry weight (g) was obtained after oven-drying at 105 °C until constant weight (TEIXEIRA et al., 2017).

The collection of undisturbed soil samples was carried out in the 0-10 cm soil depth with a metallic cylinder. We assessed available water capacity (AWC), soil bulk density, total porosity (macro and micropores), and volumetric water content (VWC) (TEIXEIRA et al., 2017).

The stability of the soil aggregates was assessed by sampling the soil clods. The soil samples were sieved so that they were retained on a 4.75 mm sieve. The samples were pre-wetted by capillarity and then vertically shaken in water on a sieve with mesh sizes of 2.0, 1.0, 0.5, 0.25, and 0.105 mm (YODER, 1936).

The soil samples for AWC, soil bulk density, total porosity (macro and micropores), and VWC were collected in 2023 after the third block was eliminated. So, we collected two samples per block.

2.4 Incubation Experiment and Soil microbiome assays

The soil samples for biological assessments were collected from 0-10 cm depth and lyophilized for 48 hours in November 2021. The field-collected soils were used to conduct an incubation experiment to test the coffee microbiome response to severe drought. The soils of each treatment were incubated under two soil moisture conditions: 1) Control conditions, where samples were maintained at approximately 35% water holding capacity (WHC) to sustain a constant low level of microbial activity, and 2) Drought conditions, where samples were initially wetted to 35% WHC and allowed to dry naturally for 15 days before re-wetting. Samples were incubated for 30 days at a 25ºC temperature. The Control treatment represents a continuously watered condition between the high and low levels of rainfall experienced in the field, while the Drought treatment represents a miniaturized bench-scale version of a long period of drying with minimal rewetting of soil during a severe drought season.

Soil microbiome analysis and enzyme assays were conducted before and after the incubation of the soil samples. Microbial nutrient cycling activities were assessed by assaying extracellular enzymes (β-1, 4, glucosidase, leucine amino peptidase and β-1, 4, N-acetylglucosaminidase, and phosphatase) involved in C, N, and P-cycling, using fluorescence-based microplate method (DICK et al., 2018). Total genomic DNA was extracted and purified from soil samples collected at each treatment using commercial extraction kits (DNeasy PowerSoil Kit, Qiagen, Germantown, MD). DNA purity and concentration were quantified using the fluorescence-based assays on a Qubit 2.0 fluorometer (Life Technologies Holdings Pte Ltd, Singapore) and BioTek Synergy HTX microplate reader (BioTek Instruments, Inc., Winooski, VT). DNA from each sample was prepared for Illumina Mi-Seq next-generation metagenomic sequencing at the Texas Tech University (TTU) Center for Biotechnology and Genomics. Bacterial taxonomic groups were detected by amplifying and sequencing the V3 and V4 regions of the 16S rRNA gene that is specific to bacteria and archaea.

2.5 Statistical analysis

The data were submitted to the Shapiro-Wilk test to assess the normality distribution. The data were analyzed by ANOVA, and when significant, the means were compared by the Tukey test ($p<0.05$). The statistical analysis was performed in R software version 4.2.1 (R DEVELOPMENT CORE TEAM, 2020).

The reads were cut to remove adapters and primers using the cut adapt tool (MARTIN, 2011). Denoising was then performed using the DADA2 algorithm (CALLAHAN et al., 2016) available in Qiime2 (BOLYEN et al., 2019) to remove chimeras, and low-quality sequences and determine ASVs (Amplicon Sequence Variants). SILVA 138 (QUAST et al., 2013) and UNITE (NILSSON et al., 2018) were used to assign taxonomy to each bacterial and fungal ASV, respectively. Those classified as mitochondria or chloroplasts were removed from downstream analysis. After all the reads were processed, the final number of 16S sequences per sample ranged from 30670 to 117369 and the ITS ranged from 26969 to 78548. Rarefaction was carried out until all the samples reached an equal sampling depth. Alpha metrics were calculated using the microeco package (LIU et al., 2021). Differential abundance analysis was calculated using the metagenomeSeq package (PAULSON et al., 2013) available on R (R DEVELOPMENT CORE TEAM, 2020).

Microbial abundance, diversity, and function before and after incubation were used to calculate the resistance (RS) of each microbial parameter using the index developed by Orwin and Wardle (2004). Soil resistance to stress is difficult to capture and measure in the field, so imposing drought in the laboratory provides an immediate and controlled comparison of drought-affected and irrigated soils.

3 RESULTS AND DISCUSSION

Sustainable long-term soil management improves soil chemical, physical, and biological attributes, resulting in healthier soil that resists more drought stresses, enhancing coffee yield and quality (VELOSO et al., 2023; VILELA et al., 2023; VOLTOLINI et al., 2022).

3.1 Management effects on chemical soil health and soil organic matter

3.1.1 CEC and soil acidity (pH and Aluminum)

Compared with the Control (bare soil), CHUD increased the CEC by 9% and pH by 18.4% and decreased Al by 90%, while GUD increased CEC by 26.3% and pH by 4.1% and decreased Al by 60% (Table 3). These results indicate that this combined sustainable management contributes to enhancing soil fertility and building a pleasant environment for plant rooting and development (Voltolini et al., 2020; 2022; Vilela et al. 2023). Gypsum, due to being a subsurface soil conditioner, neutralizing Al in deeper layers of the soil, contributes to the rooting of the plants, having a better result when combined with *U. decumbens*, due to the improvement of nutrient cycling (BAPTISTELLA et al., 2020; CARDUCCI et al., 2015; NOGUEIRA et al., 2016). Since tropical soils are naturally with poor fertility, low pH, and high Al toxicity, management that helps to fix these aforementioned issues, contributes to more sustainable coffee farming and may reduce the chemical inputs (CARDUCCI et al., 2015; CASTANHEIRA et al., 2022; JIANG et al., 2015; VOLTOLINI et al., 2020; WITHERS et al., 2018).

Table 3 – Total and annual accumulation of soil CEC and soil acidity in coffee crops under different soil management from 2015 to 2021.

Soil management	Total Accumulation			Annual Accumulation		
	CEC	pH	Al	CEC	pH	Al
Coffee husk + *U. decumbens*	3.0 b	0.8 a	-0.1 c	0.5 b	0.13 a	-0.02 c
Gypsum + *U. decumbens*	4.4 a	0.1 b	0.2 b	0.7 a	0.01 b	0.04 b
Control	2.3 c	-0.1 b	0.8 a	0.4 c	-0.02 b	0.10 a

Means followed by the same letter do not differ by Tukey ($p < 0.05$). CEC: cation exchange capacity; V%: base saturation; K: soil exch. Potassium; P: soil phosphorus content; pH: soil pH in water; Al: soil aluminum content.
Source: Author (2023).

3.1.2 Soil Available Macro-Nutrients (P, K, Ca, Mg)

Changing soil management had a positive impact on nutrient availability. The CHUD management had a higher accumulation of soil P and Mg levels ($p<0.05$), and compared to the bare soil (Control), this management increased the soil P and Mg contents by 83% and 300%, respectively. Soil management that contributes to SOM input, due to the carboxylic and phenolic radicals, may increase the P availability due to the occupation of P fixation sites in the Al and Fe oxides predominants in tropical soils (LI et al., 2019; VOLTOLINI et al., 2020; WITHERS et al., 2018; ZAVASCHI et al., 2020). Also, *U. decumbens* intercropped with coffee trees may improve P cycling, contributing to the supply of this nutrient (BAPTISTELLA et al., 2022).

Regarding potassium, the increase in CHUD was about 119.8% above the Control. The GUD increased by 34.5% the K content when also compared to the Control. Compared to Control, CHUD and GUD increased Ca soil content by 131.6% and 68.4%, respectively. Coffee husk may be a great source of K and supply coffee demands by this nutrient (CASTANHEIRA et al., 2019; FERNANDES et al., 2020; VOLTOLINI et al., 2020). Besides K, coffee husk may also supply other nutrients in lower proportion, such as Ca and Mg (CASTANHEIRA et al., 2019, 2022; VILELA et al., 2023; VOLTOLINI et al., 2020). Gypsum may also be used as a source of Ca to the plants, supplying these nutrients in deeper soil layers (ROCHA et al., 2014).

Table 4 - Total and annual accumulation of soil available macro-nutrients in coffee crops under different soil management from 2015 to 2021.

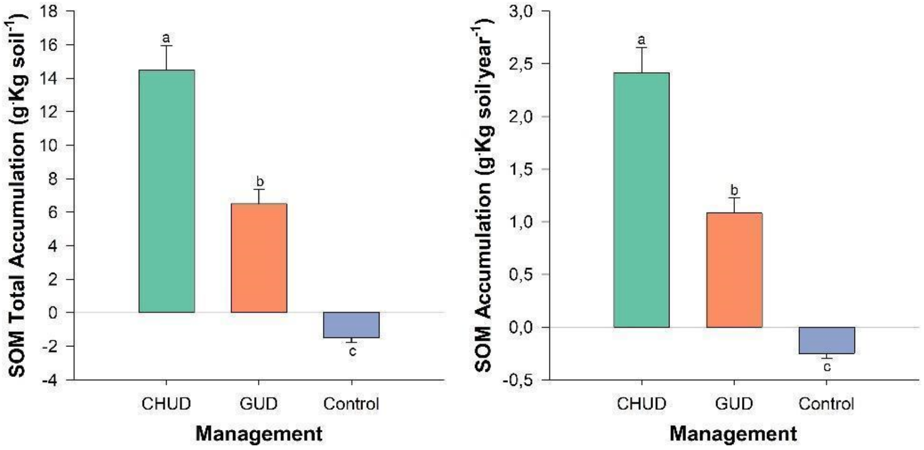
Soil management	Total Accumulation				Annual Accumulation			
	P	K	Ca	Mg	P	K	Ca	Mg
Coffee husk + *U. decumbens*	31.5 a	238.5 a	2.9 a	0.72 a	5.3 a	39.5 a	0.5 a	0.12 a
Gypsum + *U. decumbens*	15.5 b	105.5 b	1.7 a	-0.21 b	2.6 b	16.7 b	0.3 a	-0.04 b
Control	15.2 b	51.8 c	0.4 b	-0.23 b	2.5 b	8.5 b	0.07 b	-0.04 b

Means followed by the same letter do not differ by Tukey ($p < 0.05$). P: soil phosphorus content; K: soil exch. potassium; Ca: soil exch. calcium; Mg: soil exch. magnesium.
Source: Author (2023).

3.1.3 Soil Organic Mater (SOM)

Tropical soils have naturally low SOM content, but they can be managed to increase with positive impacts on soil health and plant yield (LAL, 2020b). The SOM content with CHUD management was 82.3% above the Control, in which there was a negative SOM accumulation, indicative of bare soil contribution to SOM degradation. The SOM decomposition impairs soil health with CO_2 release (LAVALLEE; SOONG; COTRUFO, 2020). The SOM accumulation with GUD was an intermediary, increasing this soil content by 40.6% when compared to the Control. In this treatment, *only the U. decumbens* can input SOM, being a smaller contribution compared with *U. decumbens* + coffee husk, which are two different sources of SOM (BAPTISTELLA et al., 2020; CASTANHEIRA et al., 2022; VILELA et al., 2023; VOLTOLINI et al., 2020).

Figure 3 - Soil organic matter (SOM) total (a) and annual (b) accumulation from 2015 to 2021 in coffee crops under different soil management. Bars followed by the same letter do not differ by Tukey ($p < 0.05$). Coffee husk + *U. decumbens* (CHUD), agricultural gypsum + *U. decumbens* (GUD).



Source: Author (2023).

So, increasing SOM is a solution to the issues that hit tropical soils, since most of its CEC comes from SOM, which also contributes to soil aggregation and porosity, related to the reduction of erosion losses (FRANCO et al., 2020; RIGON; FRANZLUEBBERS; CALONEGO, 2020). The improvement of the physical, chemical, and biological attributes of the soil, due to soil organic matter, also results in the gradual release of nutrients to the plants, improving plant nutrition (DONG et al., 2018; FERNANDES et al., 2020; FRANZLUEBBERS et al., 2021; VOLTOLINI et al., 2020).

3.2 Management impact on physical soil health

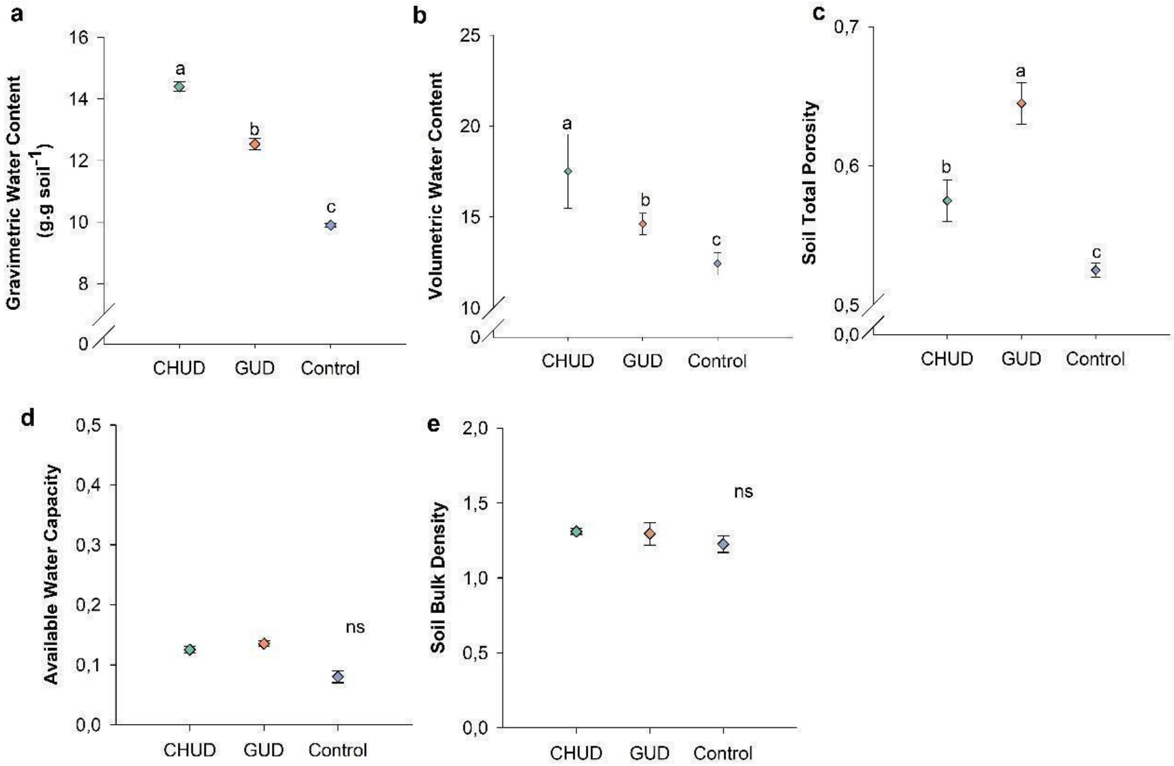
The physical indicators of soil health were affected by sustainable soil management. Practices that favored SOM increase also contribute to the soil gravimetric and volumetric water content increase. CHUD increased by 40% and 35% of the GWC and VWC, respectively, compared to the bare soil. So, SOM can improve the ability of soil to retain water, which explains the contribution of coffee husk due to the SOM input, resulting in higher soil moisture (CASTANHEIRA et al., 2022; VILELA et al., 2023; VOLTOLINI et al., 2020). The combination of *U. decumbens* with organic soil amendments, contributes to increased soil moisture, which the plants more efficient in using water (CASTANHEIRA et al., 2022; VILELA et al., 2023).

Regarding soil total porosity, the GUD management provided the higher porosity (25% above the Control), followed by CHUD, being the control, the treatment with lower soil porosity. *Urochloa* species can improve soil porosity and water retention due to the aggressiveness of the root system and the formation of bio-pores, resulting in greater water retention (FRANCO et al., 2020). Also, since the plant root system may be one of the most important tools to improve soil porosity, the effect of gypsum on the increase of root system, mainly in deeper layers, may explain the higher porosity when these two techniques were combined (ROCHA et al., 2014; SERAFIM et al., 2013; SILVA et al., 2015).

There was no difference in the AWC with the different long-term treatments. The soil bulk density did not change with the different soil management compared to the control, which may be related to the intrinsic characteristics of Oxisols, which due to its resilience may not change such characteristics after long-term different management (SILVA et al., 2021). So, changes in soil management leading to the increase of soil physical quality may not result in differences in soil bulk density and AWC (SILVA et al., 2021). The average soil bulk density (1.27 mg.m-3) is within the values found in Oxisols under cropping systems in Brazilian

Cerrado (SEVERIANO et al., 2011; SILVA et al., 2021), and close to the one found in Oxisols with coffee intercropped with *Urochloa spp* (SILVA et al., 2015).

Figure 4 - Soil gravimetric (a) and volumetric water content (b), total porosity (c), available water capacity (d), and soil bulk density (e) in coffee crops under different soil management. Bars followed by the same letter do not differ by Tukey (p<0.05). Coffee husk + *U. decumbens* (CHUD), agricultural gypsum + *U. decumbens* (GUD).



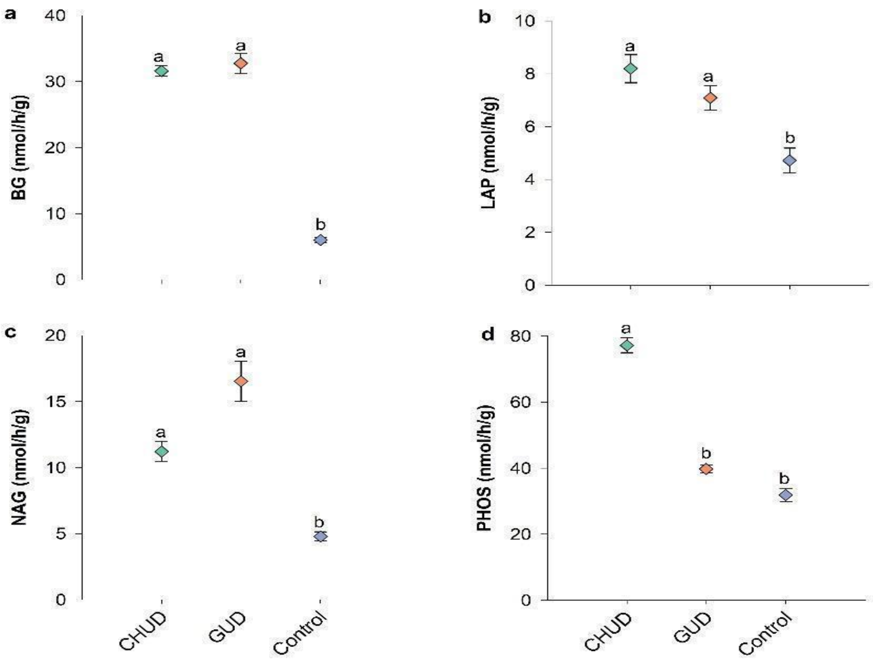
Source: Author (2023).

3.3 Management impact on biological soil health

3.3.1 Soil Enzymes

Integrated sustainable management impacts soil functionality. The *U. decumbens* + coffee husk or agricultural gypsum increases enzyme activities compared to the Control, except the Phosphatase, which the higher activity was observed just in the CHUD management.

Figure 5 – β-1, 4, glucosidase (BG) **(a)**, leucine aminopeptidase (LAP) **(b)**, β-1, 4, N-acetylglucosaminidase (NAG) **(c)**, and phosphatase (PHOS) **(d)** enzyme activities of soils from coffee crop under different soil management. Bars followed by the same letter do not differ by Tukey ($p < 0.05$). Coffee husk + *U. decumbens* (CHUD), agricultural gypsum + *U. decumbens* (GUD).



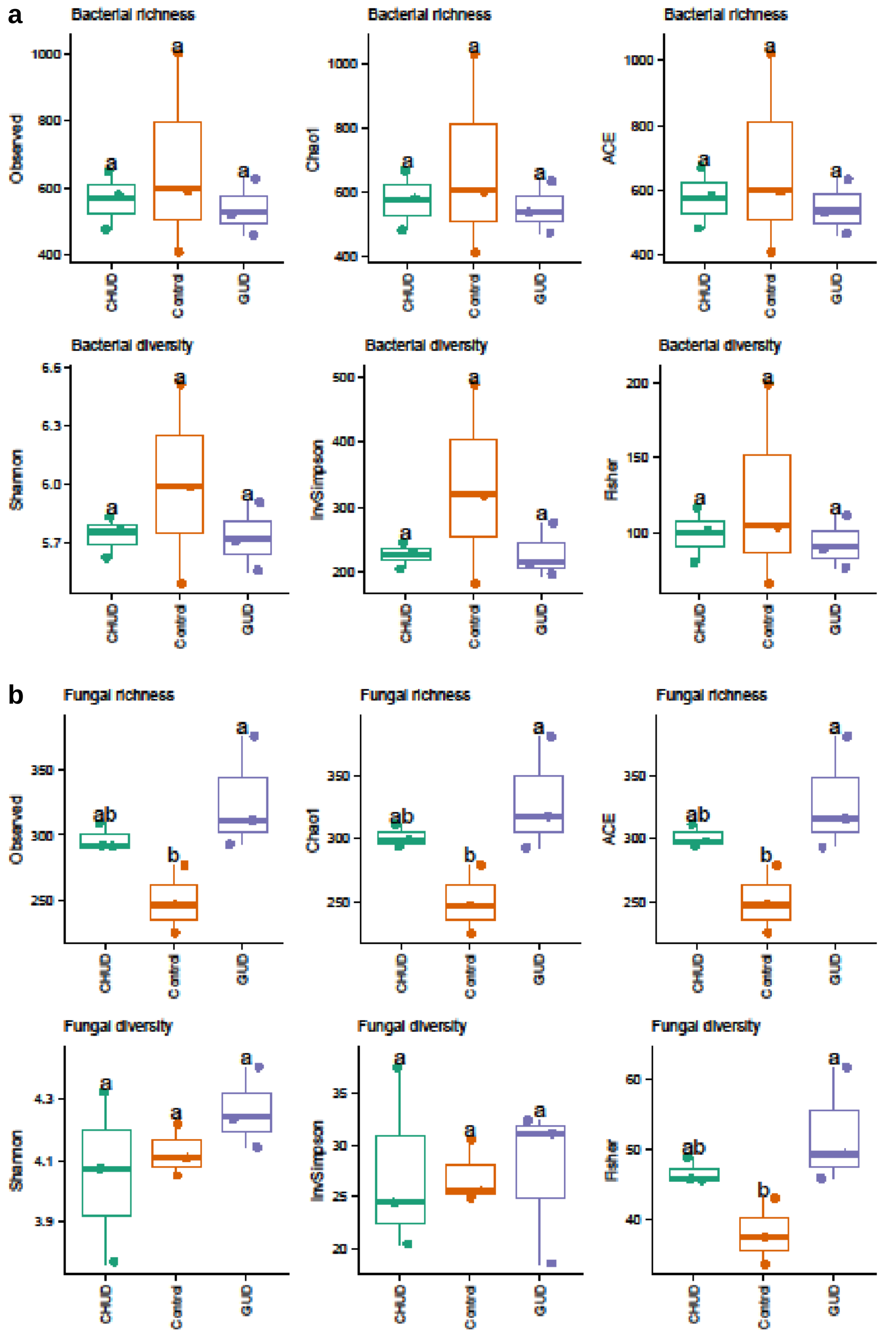
Source: Author (2023).

β-1, 4, glucosidase (BG), leucine aminopeptidase (LAP), and β-1, 4, N-acetylglucosaminidase (NAG) enzyme activities are higher with the combination of *U. decumbens* + coffee husk or agricultural gypsum. Using CHUD and GUD managements, the BG, LAP and NAG activities were 275, 56, and 170% higher than Control. Regarding phosphatase activity, the *U. decumbens* + coffee husk resulted in higher enzyme activity compared to the other treatments (167% above the Control). The SOM input contributes to the increase of enzymes related to carbon cycling, such as the BG (SINGH et al., 2023). The *U. decumbens* as a cover crop intercropped with coffee crops can contribute to the increase of P cycling, which is directly related to the increase of phosphatase activity (BAPTISTELLA et al., 2022). Also, the improvement SOM due to coffee husk may reduce soil P fixation, possibly being related to the higher activity of this enzyme with the combination of *U. decumbens* + coffee husk (SINGH et al., 2023; VOLTOLINI et al., 2020). Techniques that lead to the increase of SOM may increase N cycling in the soil, which might explain the higher activity of enzymes related to N-cycling in treatments that favored the SOM input, due to sustainable management (VELOSO et al., 2023).

3.3.2 Alpha diversity and PERMANOVA test

Soil sustainable management alters the fungal community in the soil, without significant changes in bacterial richness and diversity.

Figure 6 - Alpha metrics of bacterial (a) and fungal (b) communities in the treatments. Treatments with the same letter do not have a difference at a 95 % statistical significance level of the Kruskal-Wallis Rank Sum Test.



Source: Author (2023).

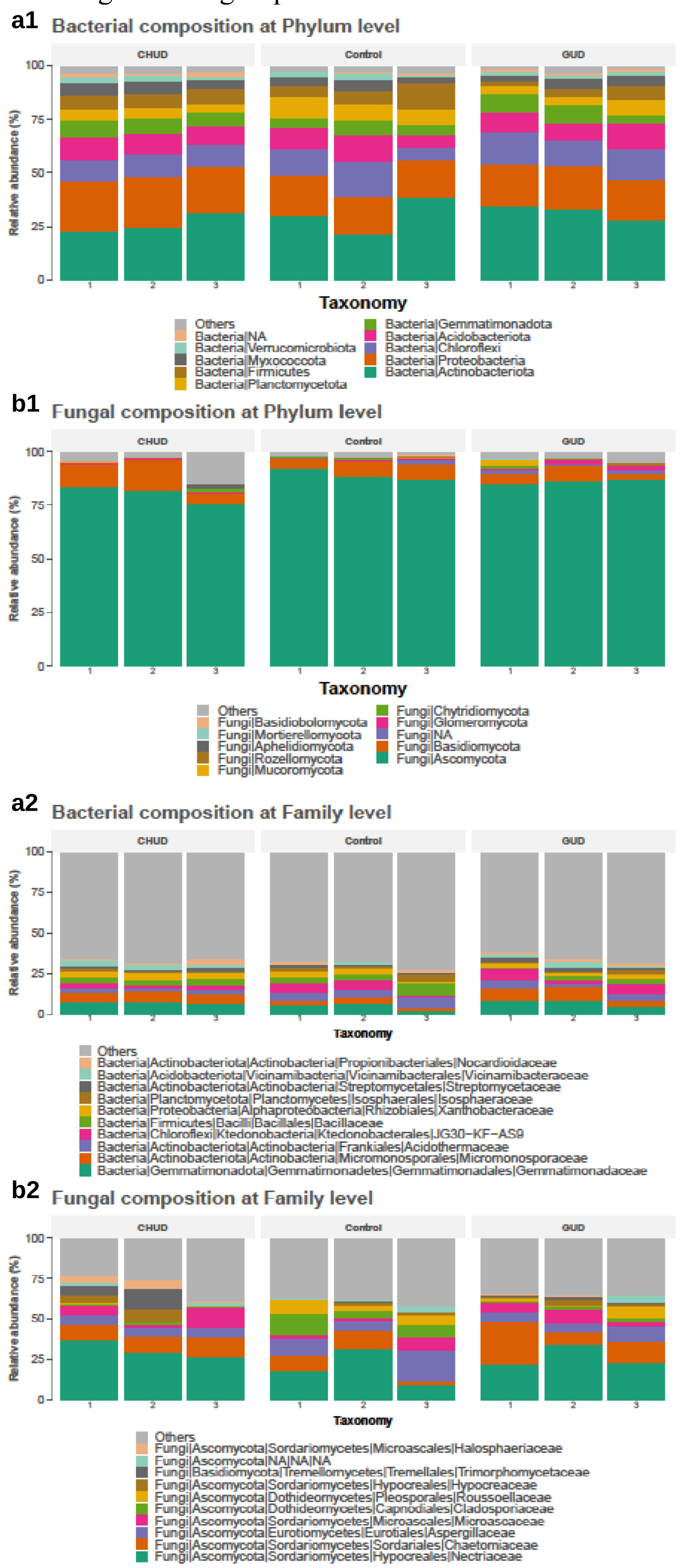
Bacterial richness and diversity did not change with different soil management. The bacterial communities may be less responsive to changes in plant management than fungi communities (LAU; LENNON, 2011). There were differences in fungal richness and diversity (Fisher) regarding soil management, in which the control was lower. Management without using conservation practices (e.g., cover crop, no-till) with disturbed soils, may contribute to the reduction of fungal diversity, lessening soil functionality (VELOSO et al., 2023; WANG et al., 2023).

3.3.3 Taxonomic composition

Bacterial relative abundance (phylum level) did not have major changes with different soil management (Figure 7a1), following the trend observed with alpha metrics assessments. So, bacterial communities may be less sensitive to changes in soil management (LAU; LENNON, 2011). Analyzing bacterial relative abundance at the family level, there were no changes regarding the management, being the 10 more abundant species present in low proportion (less than 30% of the relative abundance) (Figure 7a2). Soils with *Coffea arabica* growing drive to specific soil bacterial communities and may not change with different management, just changing the region and or *Coffea* species (VELOSO et al., 2023). Although there is an abundance of some specific bacterial phyla related to managements with more SOM input, such as Acidobacteria and Firmicutes (SINGH et al., 2023).

Most of the fungi at the Phylum level (Figure 7b1) are Ascomycota, although there were a few Glomeromycota in the GUD management, which are arbuscular mycorrhiza fungi, responsible for the increase in the soil bulk explored by roots and also may be related to the increase of water and P uptake by the plants (VELOSO et al., 2023). In the analysis of the fungal community at family level (Figure 7b2), among the 10 more abundant fungi families, most of those present in the soil under sustainable management are related to biological control and may suppress soil-borne pathogens (KÖHL et al., 1995). There was a presence of fungi from the Hypocreaceae family, related to biological control (e.g., *Trichoderma spp.*), in the CHUD management. Also, the management with gypsum + *U. decumbens* favored the abundance of Chaetomiacea, in which *Chaetomium spp.* is a biological control agent that may suppress pathogens involved in foliar coffee diseases (KÖHL et al., 1995; LABORDE et al., 2019). Thus, this is indicative of healthier soil which is efficient in its functions, resulting in healthier plants in a balanced environment.

Figure 7 - Taxonomic composition of bacterial (a) and fungal (b) communities at Phylum (1) and Family (2) level in each treatment. Only the ten more abundant taxa are shown. The remaining taxa are grouped as ‘others’.

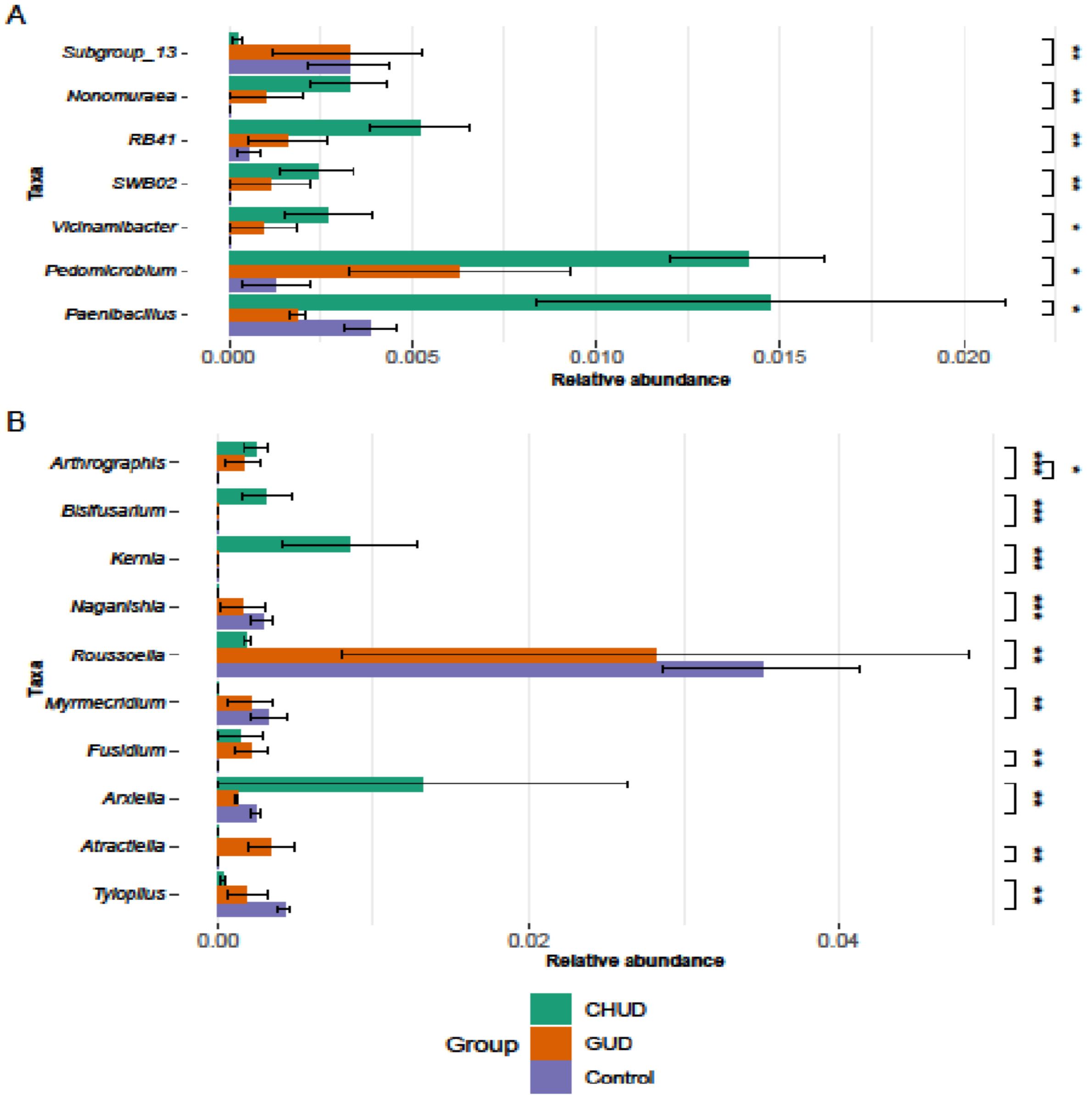


Source: Author (2023).

3.3.4 Differential abundance test

The differential abundance test identifies taxa that tend to be more related to some treatment (PAULSON et al., 2013). Most of the species that are affected by treatments are rare ones (Figure 8). So, *Coffee arabica* growing drives specific soil microbiome communities and networks with changes due to dry and wet environments (VELOSO et al., 2023).

Figure 8 - Bacterial (A) and fungal (B) taxa with significant differential abundance in each treatment (GUD, CHUD, or Control). One (*), two (**), and three (***) represent a statistical difference at the level of 0.05, 0.01, and 0.001, respectively.



Source: Author (2023).

In the bacteria community (Figure 8a), the majority of species are more abundant with CHUD management. Also, CHUD treatment may favor species related to biological control, such as *Paenibacillus spp* (KÖHL et al., 1995). The other species more abundant with CHUD are from Acidobacteria and Firmicutes phyla, which are common in an environment with high SOM and water contents, being related to conservation soil management (SINGH et al, 2023). So, the presence of these bacterial phyla indicates healthier soils and may be related to the enhancement of enzyme activities related to the soil microbial functions, such as β-1, 4, glucosidase, and phosphatase, involved in C, and P-cycling (DAVIS; HUGGINS; REGANOLD, 2023; NUNES et al., 2020; SINGH et al., 2023).

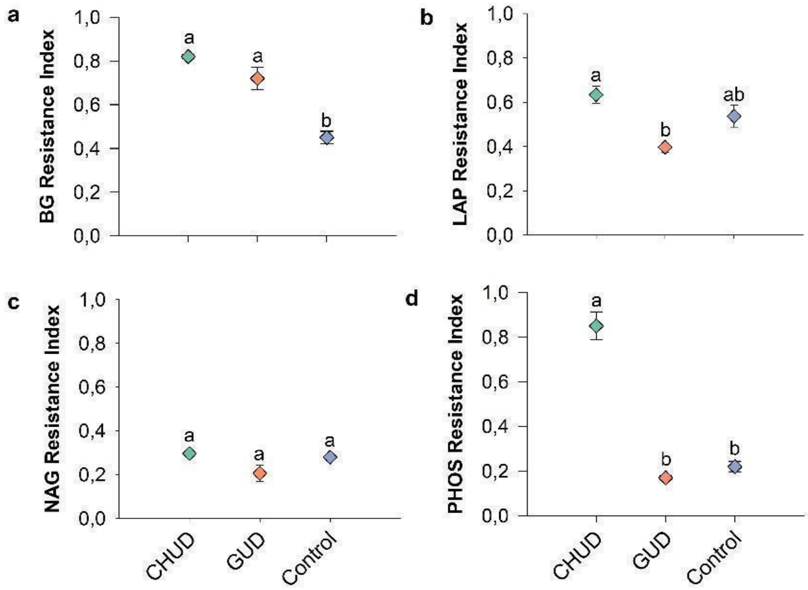
Regarding fungi (Figure 8b), the species with significant differential abundance taxa are also rare ones, being affected by soil management. CHUD management increased *Arthrographis, Bifusarium, Kernia, Fusidium,* and *Arxiella* families. GUD increased *Arthrographis, Fusidium, Atractiella* and *Roussoella.* In the Control, other species were favored, such as *Naganishia, Myrmecridium, Tylopilus,* and also *Roussoella.* There were more species with differences in the relative abundance with the CHUD management.

3.4 Consequences of increased soil health for soil functions and ecosystem services

3.4.1 Resistance of microbial soil functions to drought (Incubation Trial)

There were differences in the resistance index (RS) of enzyme activities (Figure 9). Regarding BG, the management with *U. decumbens* + coffee husk or gypsum favored an environment more resistant to drought when compared with the Control. Only the CHUD provided a higher resistance index of phosphatase and LAP, with no differences regarding the NAG resistance index.

Figure 9 - β-1, 4, glucosidase (BG) **(a)**, leucine amino peptidase (LAP) **(b)**, β-1, 4, N-acetylglucosaminidase (NAG) **(c)**, and phosphatase (PHOS) **(d)** enzyme resistance index of soils from coffee crop under different soil management. Bars followed by the same letter do not differ by Tukey ($p < 0.05$). Coffee husk + *U. decumbens* (CHUD), agricultural gypsum + *U. decumbens* (GUD).



Source: Author (2023).

In an environment without dry memory, there is usually a more humid climate (Figure 1), when the soil is submitted to disturbance, such as drought, the management that favors more soil moisture and SOM, may contribute to higher resistance of soil functions to drought (WANG et al., 2023; VILELA et al., 2023). Also, managements that initially favored higher enzyme activity (Figure 5) were the ones with higher resistance index after the incubation trial (Figure 9), so conservation management, contributes to soil health and resistance against disturbance, such as drought (DELGADO-BAQUERIZO et al., 2017).

3.4.2 Alpha diversity and PERMANOVA test and resistance index (Incubation Trial)

There was no difference in bacterial and fungal alpha metrics resistance indices (Table 5). The absence of differences in the resistance index for richness and diversity means that the parameters did not change with the disturbance. So, the treatments with higher diversity and richness were the same before and after disturbance by drought (DELGADO-BAQUERIZO et al., 2017; ORWIN; WARDLE, 2004). These results may be related to the “resistance trap”, in which an environment may favor a lower diversity and richness that are kept lower after a drought period. So, the index may be higher because the soil remained stable keeping the low parameter after the disturbance, which is not indicative of a good result (DAVIS; HUGGINS; REGANOLD, 2023).

Table 5 - Bacterial and fungi alpha metrics resistance index (RS).

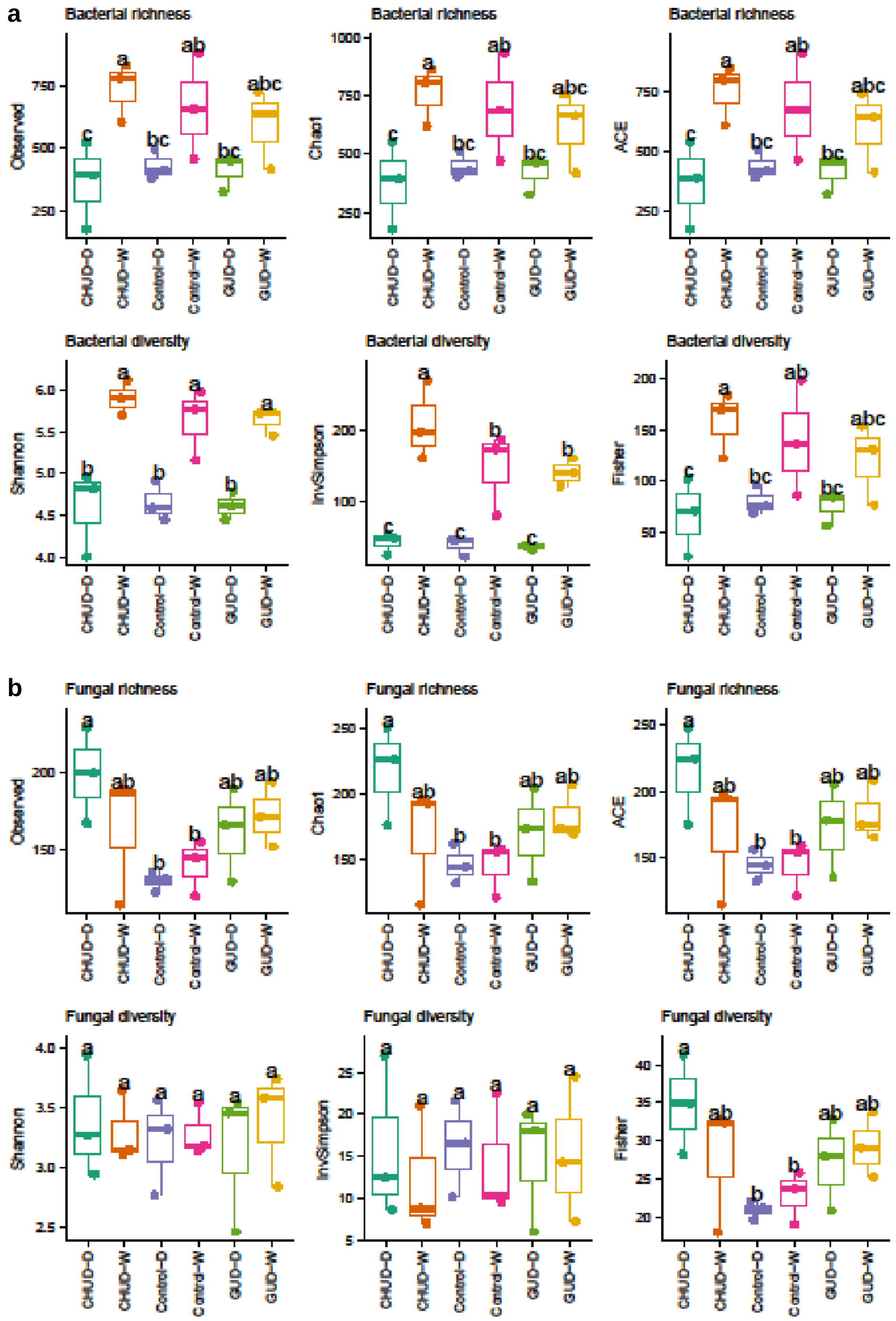
Soil management	Bacterial Richness RS			Bacterial Diversity RS		
	Observed	Chao1	ACE	Shannon	InvSimpson	Fisher
Coffee husk + *U. decumbens*	0.33^{ns}	0.33^{ns}	0.33^{ns}	0.64^{ns}	0.12^{ns}	0.27^{ns}
Gypsum + *U. decumbens*	0.57^{ns}	0.55^{ns}	0.55^{ns}	0.70^{ns}	0.16^{ns}	0.52^{ns}
Control	0.52^{ns}	0.52^{ns}	0.52^{ns}	0.71^{ns}	0.21^{ns}	0.46^{ns}
	Fungal Richness RS			**Fungal Diversity RS**		
	Observed	**Chao1**	**ACE**	**Shannon**	**InvSimpson**	**Fisher**
Coffee husk + *U. decumbens*	0.84^{ns}	0.76^{ns}	0.76^{ns}	0.88^{ns}	0.53^{ns}	0.81^{ns}
Gypsum + *U. decumbens*	0.86^{ns}	0.85^{ns}	0.86^{ns}	0.85^{ns}	0.56^{ns}	0.86^{ns}
Control	0.79^{ns}	0.77^{ns}	0.79^{ns}	0.82^{ns}	0.57^{ns}	0.76^{ns}

^{ns}: non-significant at 95% probability according to Tukey test.
Source: Author (2023).

Nonetheless, changes in the bacterial and fungi richness and diversity can be observed when analyzing the water availability as a second factor under study. So, when comparing the management with different water availability, the drought significantly affected bacterial richness and diversity (Figure 10a). Drought treatment regardless of soil management had less bacterial richness and diversity. So, the presence of water may change bacterial communities when in a complex microbiome (LAU; LENNON, 2011).

Although fungal richness and diversity (Fisher) were affected by soil management regardless of water availability (Figure 10b). The higher fungal richness and diversity (Fisher) were observed with the combinations of *U. decumbens* + coffee husk or agricultural gypsum. Sustainable management and the presence of a complex microbiome drive higher richness and diversity of fungi communities (VELOSO et al., 2023; LAU; LENNON, 2011).

Figure 10 - Alpha metrics of bacterial (a) and fungal (b) communities in the treatments. Treatments with the same letter do not have a difference at a 95 % statistical significance level of the Kruskal Wallis Rank Sum Test.



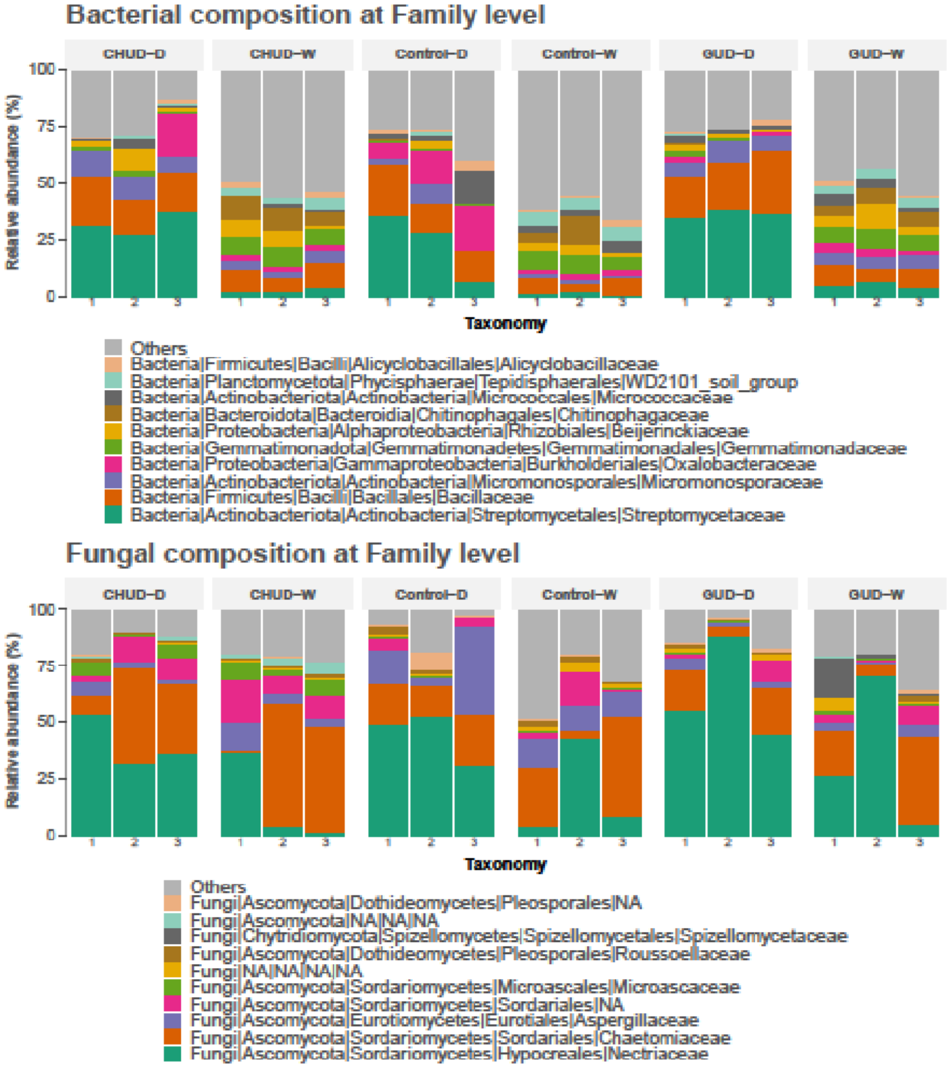
Source: Author (2023).

3.4.3 Taxonomic composition (Incubation Trial)

Regarding soil management, there are more changes in the taxonomic composition of fungal communities than in bacterial ones. When changing water availability, the bacterial communities were affected, with an increase in the relative abundance in drought conditions. There was an increase in other species in the wet (W) treatment, with a decrease in the relative abundance of the 10 more abundant species (Figure 11).

In the fungal communities, besides being affected by drought, the relative abundance also changed among the soil treatments, which is easier to visualize when comparing treatments from the same block (Figure 11).

Figure 11 - Taxonomic composition of bacterial and fungal communities at Family level in each treatment. Only the ten more abundant taxa are shown. The remaining taxa are grouped as ‘others’.



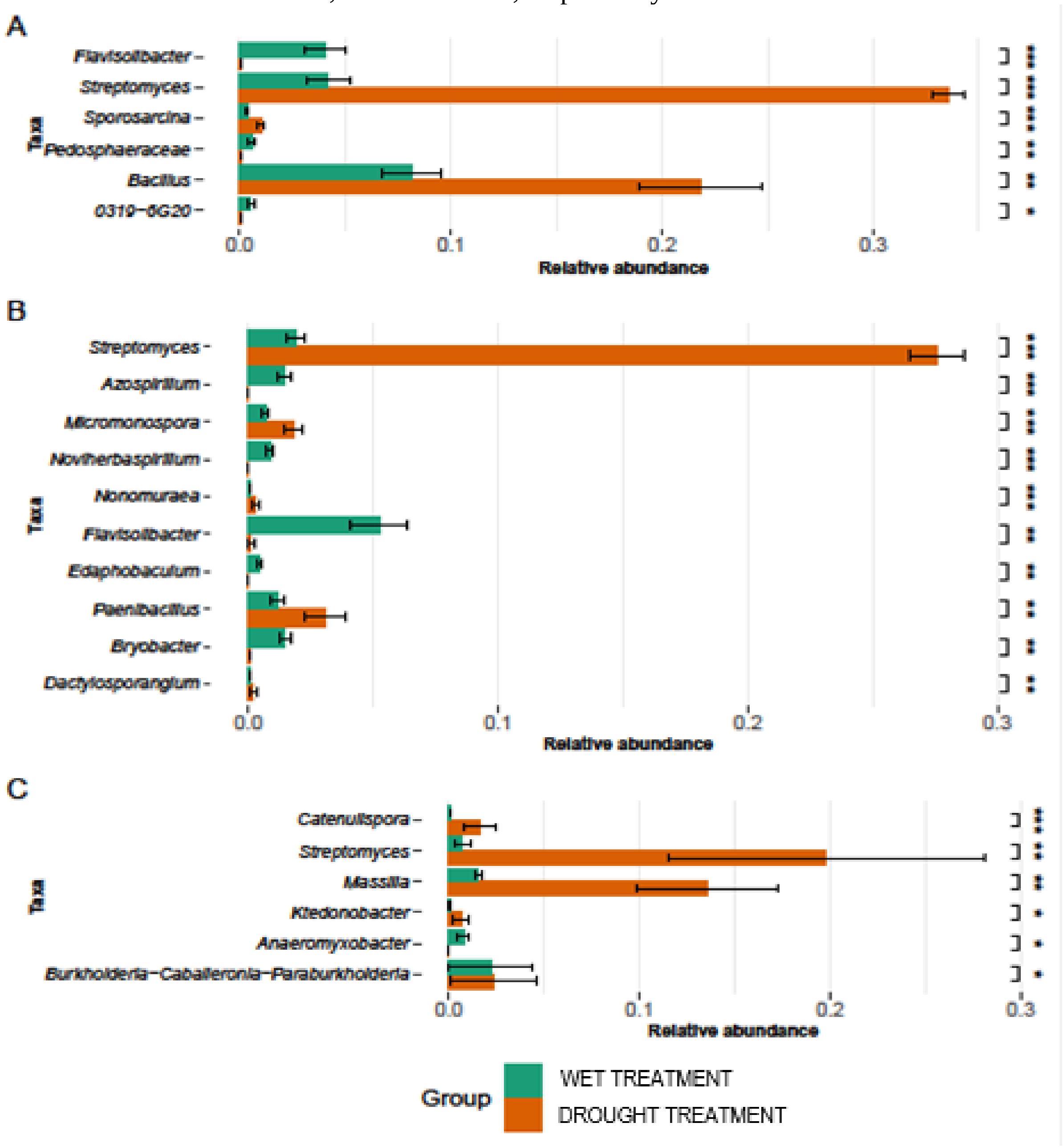
Source: Author (2023).

Dry regions of *Coffea arabica* growing drive to different microbial communities, with less diversity, when compared with humid environments with the same cropping system (VELOSO et al., 2023). Besides, the dry environment drives different communities. The major changes regarding bacteria (Streptomycetaceae and Bacillaceae) and fungi (Nectriaceae), were observed in families with the development of resistance structures so that they can survive in a drought environment (KÖHL et al., 1995). The wetness may result in more competition due to the germination of resistance structure, resulting in different microbial networks, affecting also microbial soil functions (VELOSO et al., 2023; KÖHL et al., 1995; WANG et al., 2023). Even though, Chaetomiaceae was stable in different water availability, not being affected by drought conditions, with no changes in its abundance. Fungi from this family are used in biological control due to their stability in different environments and suppression of foliar disease pathogens (eg., *Cercospora coffeicola*) that may survive in the soil (KÖHL et al., 1995; LABORDE et al., 2019).

3.4.4 Differential abundance test (Incubation Trial)

In the differential abundance test, we can observe that each treatment selects a different microbial community. There were changes in the differential abundance of fungal and bacterial communities regarding soil management and water availability (Figure 12). Rare bacterial taxa were favored by the different soil management and water availability, the more common communities did not change with different soil management and water availability.

Figure 12 - Bacterial taxa with significant differential abundance in each treatment. (A) GUD, (B) CHUD, and (C) Control. One (*), two (**), and three (***) represent a statistical difference at the level of 0.05, 0.01 and 0.001, respectively.



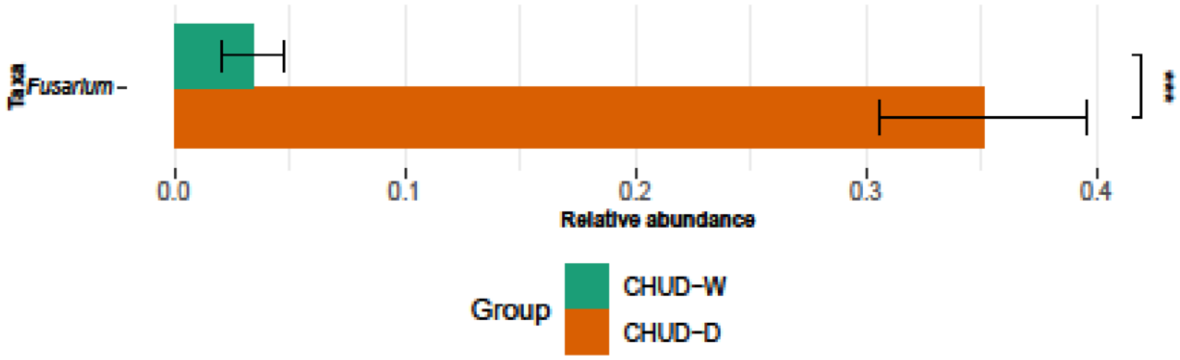
Source: Author (2023).

Streptomyces spp. is present in all management, being the higher abundance in drought conditions (Figure 12). Some bacterial species with resistance structure could survive in a dry environment, being more stable in the face of the disturbance (KÖHL et al., 1995). There are more bacterial species being influenced by drought in the CHUD (Figure 12b) treatment when compared to the others, indicating different bacterial communities regarding different soil management.

For instance, the *Azospirillum spp.* presence in the CHUD treatment may indicate an environment for growth-promoting bacteria, related to the resistance against drought stresses, and is a nitrogen-fixing bacteria (NFB) commonly associated with grass species (FUKAMI; CEREZINI; HUNGRIA, 2018), which may be related to the improvement of enzymes related to N-cycling in the soil (Figure 5b,c). This species is from the Proteobacteria phylum and was found in *Coffea arabica* L. plantations among other NFB (SILVA et al., 2020). In this management, *Paenibacillus*, a biological control agent, was favored by drought conditions, due to a resistance structure (KÖHL et al., 1995).

Regarding fungal taxa, there was a significant differential abundance of taxa only with the CHUD treatment (Figure 13).

Figure 13 - Fungal taxa with significant differential abundance in each treatment. The plot only shows the difference among the 'CHUD' treatments. No statistical difference was found in the other treatments (i.e. 'GUD' and 'Control'). One (*), two (**), and three (***) represent a statistical difference at the level of 0.05, 0.01, and 0.001, respectively.



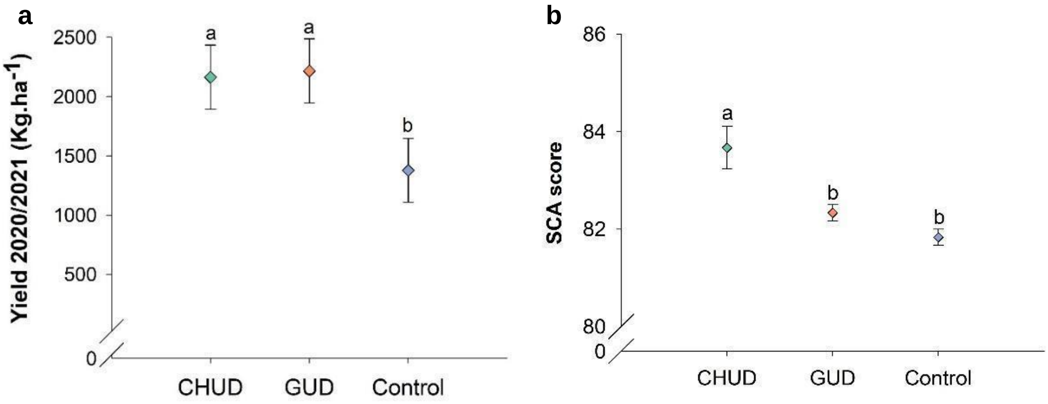
Source: Author (2023).

In the CHUD management, the only species that changed regarding water availability was *Fusarium sp.*, which due to its resistance structure (Chlamydospore) may have an advantage over other species in the soil in drought conditions. Thus, with the wetness, there is the germination of this structure, which makes this fungus less competitive and may provide a better environment for other species to develop and compete due to the moisture. Although there is *Fusarium sp.* in the soil, its presence may not be harmful to the coffee plants, because not all species of this fungus are pathogenic to coffee plants (PFENNING; MARTINS, 2000).

3.4.5 Coffee Yield and quality beverage

Increasing soil health due to sustainable soil management results in the improvement of soil functions and ecosystem services, with positive results in plant yield and quality beverage (Figure 14).

Figure 14 - Yield 2020/2021 biennium (a) and sensorial analysis based on Specialty Coffee Association (SCA) protocol (b) in coffee crops under different soil management. Bars followed by the same letter do not differ by Tukey ($p<0.05$). Coffee husk + *U. decumbens* (CHUD), agricultural gypsum + *U. decumbens* (GUD).



Source: Author (2023).

As a result of long-term management of the soil, and improving soil health (chemical, biological, and physical attributes), there is an enhancement of coffee yield, also ensuring good quality (Figure 14). The soil management with *U. decumbens* + coffee husk or gypsum resulted in a yield about 50% higher than the Control treatment. Combined techniques that favor the organic soil covering, especially *U. decumbens* as a cover crop due to the improvement of soil health indicators (e.g., SOM) contribute to improving coffee yield and reduce biennially (LAL, 2020b; VOLTOLINI et al., 2022).

Regarding quality beverage (Figure 14b), a higher score was observed with the CHUD management. There are a lot of aspects that affect coffee quality, which may be related to the environment, *terroir*, management in the field, and post-harvest techniques. In this study, the changes in the field level with sustainable management (CHUD), may resulted in an environment that favored the improvement of coffee quality, due to better plant nutrition and soil microbiome networks that may favor a fruit microbiome contributed to the quality beverage (VELOSO et al., 2023; MARTINEZ et al., 2014).

4 CONCLUSION

Long-term soil management with the combination of coffee husk and *U. decumbens* intercropped with coffee trees contributes to the improvement of soil health due to the benefits in chemical (CEC, macro and micronutrients, SOM), physical (soil porosity and soil moisture), and biological (enzyme activity, fugal richness, relative abundance of microbial communities) properties. Such results also enhance the resistance of microbial function and community to drought, ensuring high coffee yield and quality.

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CHAPTER 4 SOIL MICROBIOME RESPONSE TO DROUGHT STRESS UNDER CONSERVATION MANAGEMENT IN SEMI-ARID COTTON PRODUCTION SYSTEMS

RESPOSTA DO MICROBIOMA DO SOLO AO STRESS HÍDRICO SOB MANEJO DE CONSERVAÇÃO EM SISTEMAS DE PRODUÇÃO DE ALGODÃO NO SEMIÁRIDO

RESUMO

A seca é um problema que afeta os sistemas de produção em todo o mundo, sobretudo em condições semiáridas. Por isso, nossa hipótese é que o manejo conservacionista do solo em sistemas de cultivo de algodão poderia conduzir à resistência de funções microbianas-chave a secas severas. Os experimentos de campo de longo prazo foram realizados em dois locais de pesquisa agrícola estabelecidos na região das planícies altas do sul (SHP) do Texas, Halfway e Lamesa, da Texas A&M University. Os tratamentos foram três tipos de manejo do solo (plantio direto de algodão com cobertura de centeio - NTCR, plantio direto de algodão com rotação trigo/pousio - NTCW, e plantio convencional com algodão contínuo - CCCT), e dois níveis de irrigação (baixo e alto - 60% ET). As amostras de solo foram coletadas da camada de 0-10 cm de profundidade e na zona associada às raízes. Os solos amostrados no campo foram utilizados para realizar experimento de incubação com finalidade de testar a resposta do microbioma do algodão à seca severa. As análises das enzimas foram realizadas para avaliar a função microbiana por meio da atividade de enzimas extracelulares relacionadas à ciclagem de C, N e P, β-1, 4, glucosidase, leucina amino peptidase e β-1, 4, N-acetylglucosaminidase e fosfatase, respetivamente. As atividades enzimáticas microbianas responderam de forma diferente à seca. As enzimas relacionadas com a ciclagem de N foram mais afetadas pela seca. As enzimas de ciclagem de nutrientes em solos sob manejo conservacionista podem resistir a secas severas, principalmente relacionadas com a reciclagem de C e P.

Palavras-chave: Plantas de cobertura. Plantio direto. Resiliência. Ciclagem de nutrientes.

ABSTRACT

Drought is an issue that has been impairing production systems all over the world, especially in semiarid conditions. So, we hypothesize that conservation soil management in cotton cropping systems could drive resistance of key microbial functions to severe drought. The infield long-term experiments were carried out in two established agricultural research sites in the south high plains (SHP) region of Texas, Halfway and Lamesa, from Texas A&M University. The treatments were three types of soil management (no-till cotton with rye cover - NTCR, no-till cotton with wheat/fallow rotation - NTCW, and conventional till with continuous cotton - CCCT), and two irrigation levels (low and high - 60% ET). The soil samples were collected at 0-10, and 10-20 cm depth, and from the root-associated zone. The field-collected soils were used to conduct an incubation experiment to test cotton microbiome response to severe drought. The enzyme assays were carried out to assess the microbial function through extracellular enzyme activities related to C, N, and P-cycling, β-1, 4, glucosidase, leucine aminopeptidase and β-1, 4, N-acetyl glucosaminidase, and phosphatase, respectively. Microbial enzyme activities responded differently to drought. Enzymes related to N-cycling were more disturbed by drought. Nutrient-cycling enzymes in soils under conservation management could resist severe drought, mainly related to C and P-cycling.

Keywords: Cover crops. No-tillage. Resilience. Nutrient cycling.

1 INTRODUCTION

In a scenario of climate change with a reduction in water availability and temperature increase (IPCC, 2021), the soil can be managed to improve water retention and reduce temperature oscillations (KADER et al., 2017; WULANNINGTYAS et al., 2020). Conservation soil management with the SOM input in the production system, such as cover crops and no-tillage, may result in healthier, resilient, and resistant soil since there is an increase in soil water retention and crop efficiency under drought conditions (DAVIS; HUGGINS; REGANOLD, 2023; NAYLOR; COLEMAN-DERR, 2018; SINGH et al., 2023; WANG et al., 2023). Such management may improve the soil microbiome functions, such as nutrient cycling, and favor an environment that provides plant resistance to stresses, especially drought (DAVIS; HUGGINS; REGANOLD, 2023; SINGH et al., 2023).

Soil microbial functions may be one of the tools used to assess soil health. A healthy soil is capable of ensuring soil functionality, leading to resistance to environmental stress. Some soil management may help to achieve soil health due to the enhancement of enzyme activities related to the soil microbial functions (DAVIS; HUGGINS; REGANOLD, 2023; NUNES et al., 2020; SINGH et al., 2023). Conservation tillage (no-till, reduced tillage) and cover crops increase microbial biomass and activity regarding conventional tillage and bare fallow soil (KIM et al., 2020). The long-term no-tillage system (18 years) was more favorable for the microbial community than the plow area (WULANNINGTYAS et al., 2020). Also, conservation soil management, reducing soil disturbance, contributes to the bacterial community in the soil, resulting in the increase of β-1, 4, glucosidase, responsible for breaking SOM in more stable C, and phosphatase, responsible for making the P-organic available for plant uptake (SINGH et al., 2023).

The soil microbiome presents the possibility of intensifying and optimizing the natural processes that already take place in the environment, contributing to greater autonomy of production systems and minimizing stresses (NAYLOR; COLEMAN-DERR, 2018; PAULA et al., 2014). So, the biological component of the soil can actively participate in plant protection by playing an important role in the promotion of the growth and rooting of species, enabling greater tolerance and resilience of plants against drought (KAVAMURA et al., 2013; NAYLOR; COLEMAN-DERR, 2018; WANG et al., 2023).

The type of management of agricultural systems and soil attributes such as pH, nutrient content, soil texture, soil moisture, and soil temperature can interfere with the function and abundance of microorganisms present in the soil. Besides, the soil microbiome is responsible for nutrient cycling and, mainly, the greater tolerance of plants to stresses, especially drought (NAYLOR; COLEMAN-DERR, 2018; PAULA et al., 2014; SINGH et al., 2023; WANG et al., 2023).

It is necessary to understand the effect of these management techniques on the soil functionality and how it affects the resistance to severe drought. So, we hypothesize that conservation soil management in cotton cropping systems could drive resistance of key microbial functions to severe drought.

2 MATERIAL AND METHODS

2.1 Field experiments

The infield experiments were carried out in two established agricultural research sites in the south high plains (SHP) region of Texas, Halfway and Lamesa. The sites were located at the Texas A&M University Agricultural Complex for Advanced Research and Extension Systems (AG-CARES), where cotton was growing with different management conditions. Long-term research plots from those two sites were selected for this work. The treatments were three types of soil management (no-till cotton with rye cover - NTCR, no-till cotton with wheat/fallow rotation - NTCW, and conventional till with continuous cotton - CCCT), and two irrigation levels (low and high - 60% ET). The soil samples were collected at 0-10 cm depth and from root associated zone. The sampling was conducted in 2021 (Halfway) and 2022 (Lamesa). The soil from Halfway had a clay loam texture and Lamesa had a sandy loam texture.

2.2 Incubation experiment

The field-collected soils were used to conduct an incubation experiment to test cotton microbiome response to severe drought. This study allowed us to assess which conservation management drives the resistance of soil microbiome against severe drought conditions. The soils from each site were incubated under two soil moisture conditions: 1) Control conditions, where samples were maintained at 35% water holding capacity (WHC) to sustain a constant low level of microbial activity, and 2) Drought conditions, where samples were initially wetted to 35% WHC and allowed to dry naturally for 15 days before to re-wetting. Samples were incubated for approximately 30 days with a constant temperature of 25ºC. The proposed Control treatment represented a continuously watered condition between the high and low levels of irrigation experienced in the field. In contrast, the Drought treatment represented a miniaturized bench-scale version of a long period of drying with minimal rewetting of soil during a severe drought season.

2.3 Microbial size, composition, and function assessments

Community size and composition were determined using the Ester-linked fatty-acid methyl esters (EL-FAMES) method (SCHUTTER; DICK, 2000). EL-FAMES were performed in the Lamesa samples after the incubation trial.

Soil microbiome analysis and enzyme assays were conducted after the incubation for Halfway and Lamesa samples. The enzyme assays were carried out to assess the microbial function through extracellular enzyme activities related to C, N, and P-cycling, β-1, 4, glucosidase, leucine amino peptidase and β-1, 4, N-acetylglucosaminidase, and phosphatase, respectively. The fluorescence-based microplate method was used for the assays (DICK et al., 2018).

Microbial function and composition after incubation were used to calculate the resistance (RS) of each microbial parameter (ORWIN; WARDLE, 2004). Soil resistance to stress is difficult to capture and measure in the field, so imposing drought in the laboratory provides an immediate and controlled comparison of drought-affected and irrigated soils. This experiment allowed us to capture which changes occur during drought season as compared to continuously watered soil. Also, to quantify if/how the microbiome, due to long-term conservation management, can resist severe drought conditions.

2.4 Statistical analysis

The data analysis was carried out with the software R version 4.2.1. The data were submitted to the Shapiro-Wilk test and to the ANOVA. When significant the Tukey test was applied to assess the mean of each treatment.

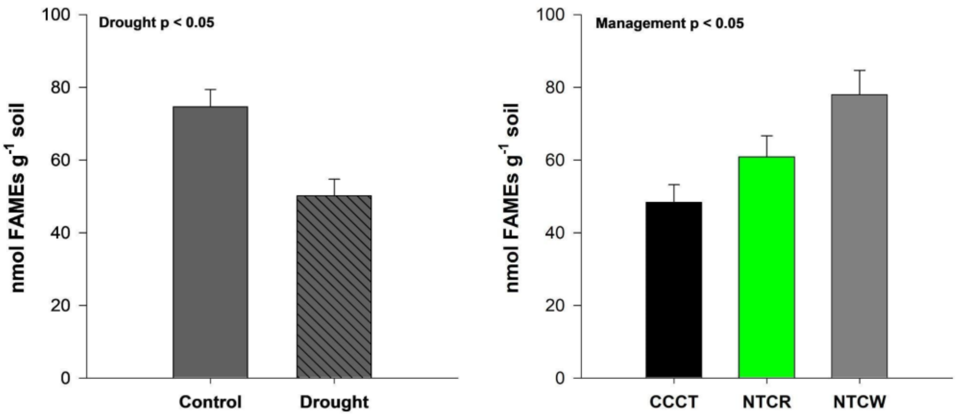
3 RESULTS

3.1 Ester-linked fatty-acid methyl esters (EL-FAMES) - Lamesa, TX site

3.1.1 Microbial community size

Conservation management drives the increase of the microbial community size, which is affected by drought conditions (Figure 1). EL-FAMEs significantly increased due to management but decreased by drought, without interactive effects. The NTCW (80 nmol FAMES.g-1 soil) and NTCR (60 nmol FAMES.g-1 soil) resulted in higher EL-FAMEs, being 67% and 25% above the CCCT respectively. Drought conditions reduced EL-FAMEs by 34% compared to the watered treatment.

Figure 1 - EL-FAMEs of root-associated soil bulk by water availability (a) and soil management (b) with cotton grown under different soil management, and irrigation levels in Lamesa, TX.



Source: Author (2023).

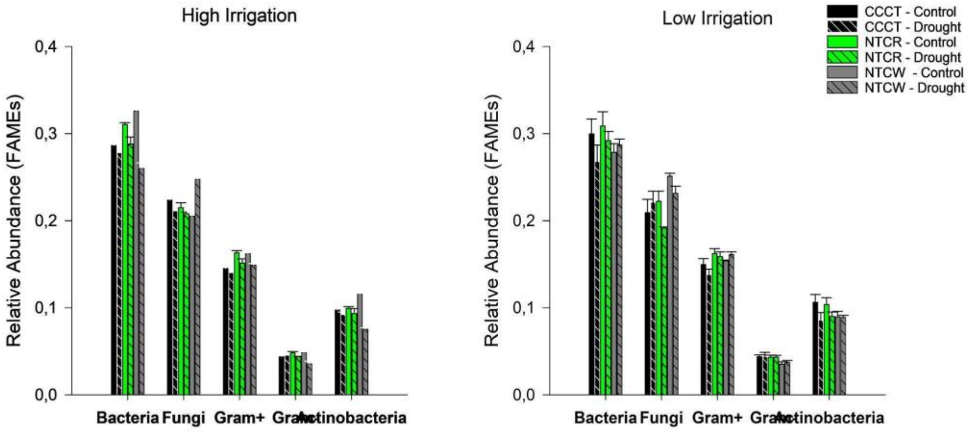
3.1.2 Microbial community composition

The relative abundance of most microbial groups decreased by drought, except fungi in NTCW under high-irrigation field plots. In high irrigation, NTCW, and NTCR + control, bacteria relative abundance was 18% higher than CCCT. The higher bacteria relative abundance was with NTCW and NTCR + Control treatments. In low irrigation plots, NTCR also resulted in higher bacteria relative abundance.

Regarding Gram-positive bacteria, all the conservation managements were (13% average) higher than CCCT, in both high and low irrigation plots. Gram-negative bacteria were affected by management and decreased by drought in the high irrigation plots (Figure 2a), in which NTCR/NTCW + Control contribute to higher relative abundance.

NTCW + Control in high irrigation plots (20% above CCCT) shifted higher Actinobacteria relative abundance, while in low irrigation the CCCT management contributed to the higher relative abundance of this bacteria phyla. In low irrigation, more expressive change was observed with the NTCW + Control treatment, which increased fungi relative abundance by 17% compared to the CCCT.

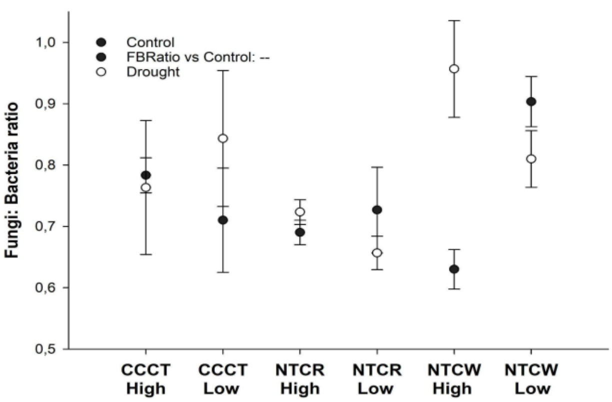
Figure 2 - Relative abundance of root-associated soil bulk with cotton grown under different soil management, and irrigation levels in Lamesa, TX.



Source: Author (2023).

Regarding the Fungi: Bacteria ratio, the higher ratios (~1.0) were observed in the NTCW + high irrigation plots + drought and NTCW + low irrigation + control, being 20% higher than the CCCT treatment.

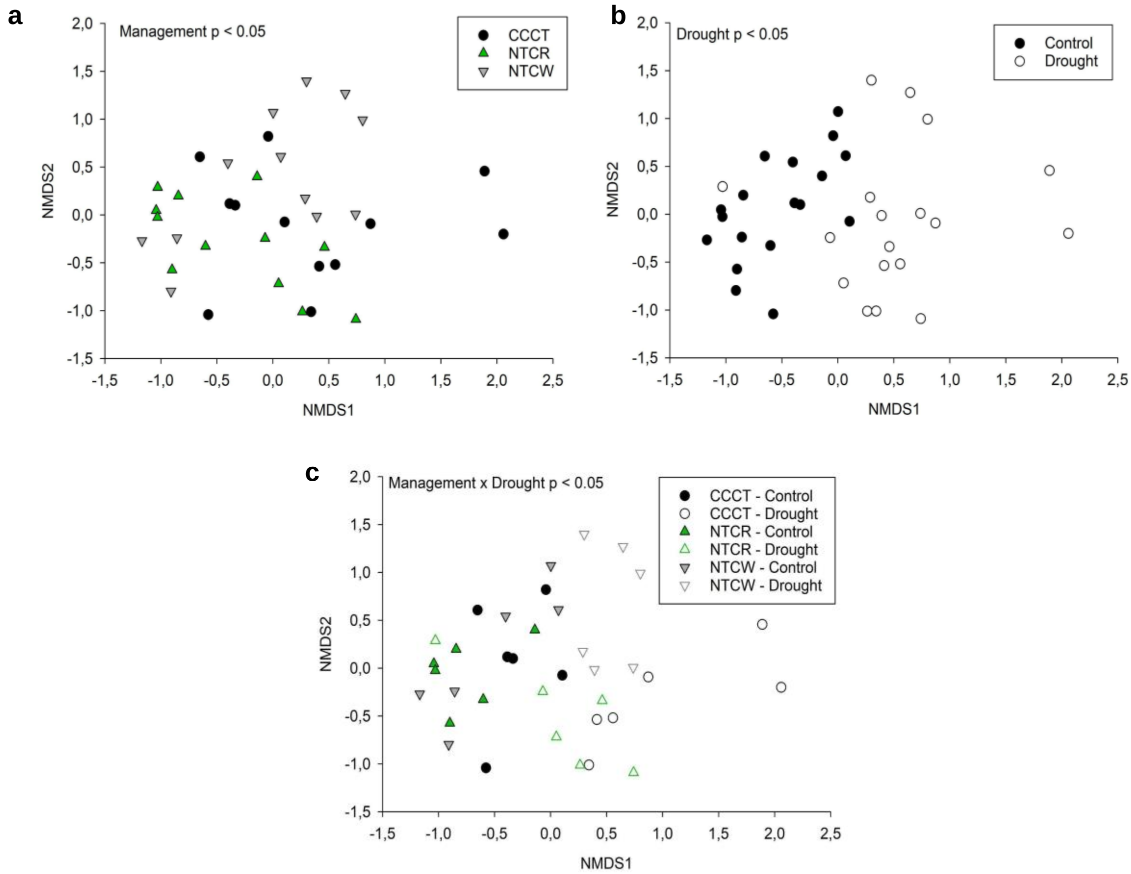
Figure 3 - Fungi: Bacteria ratio of root-associated soil bulk with cotton grown under different soil management, and irrigation levels in Lamesa, TX.



Source: Author (2023).

Microbial community composition was mostly driven by drought conditions. Treatments with drought as a similar characteristic were clustered, with NTCW and NTCR closer to each other.

Figure 4 - Microbial Community Composition of root-associated soil bulk by management (a), water availability (b), and interaction of management with water availability (c) with cotton grown under different soil management, and irrigation levels in Lamesa, TX.



Source: Author (2023).

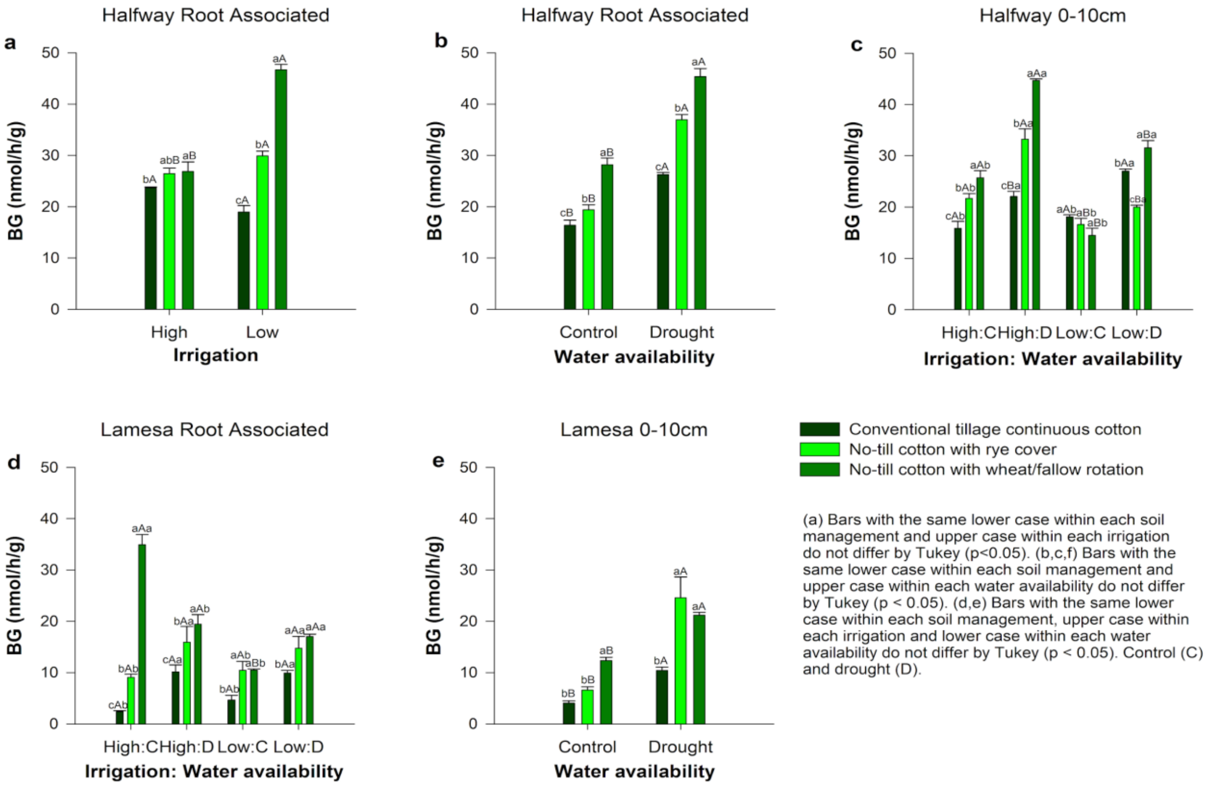
3.2 Microbial function - enzyme activity

Soil conservation management modifies the microbiome functions related to C, N, and P cycling. There was a significant difference between the management of cotton cropping systems, irrigation level, and water availability in the incubation trial for enzyme activities related to C, N, and P cycling.

3.2.1 β-1, 4, glucosidase (BG) enzyme activity

The BG activity was influenced by soil management, irrigation level, and water availability during the incubation trial (Figure 5). Halfway, in the soil bulk root-associated, there were interactions between management and irrigation (Figure 1a) and management and water availability in the incubation trial (Figure 1b). In the same site, with 0-10 cm soil bulk, there was interaction among management, irrigation, and water availability (Figure 1c). In Lamesa, in root-associated samples, there was an interaction between management, irrigation, and water availability (Figure 1d), while in 0-10 cm soil bulk, there was an interaction between management and water availability (Figure 1e).

Figure 5 - β-1, 4, glucosidase (BG) enzyme activity of root-associated (a,b,d), and 0-10 cm (c,e) soil depth with cotton grown under different soil management, irrigation level, and water availability (incubation trial) in Halfway (a,b,c) and Lamesa (d,e) soils.



Source: Author (2023).

In the Halfway site, the soil bulk root-associated in NTCW (50 $nmol \cdot h \cdot g^{-1}$ average) and NTCR (35 $nmol \cdot h \cdot g^{-1}$ average) managements provide the higher BG activity. When there were low irrigation and drought conditions, these conservation managements shifted higher differences compared with the CCCT (25 $nmol \cdot h \cdot g^{-1}$ average) (Figures 1a,b). In 0-10cm soil bulk, the combination of NTCW + high irrigation + drought conditions in the incubation had higher BG activity (by 45 $nmol \cdot h \cdot g^{-1}$), compared with the other management and with this same management under different irrigation and water availability (Figure 1c). The management under both high and low irrigation had a decrease in the enzyme activity when submitted to drought stress, being NTCW about 26% on average higher than CCCT.

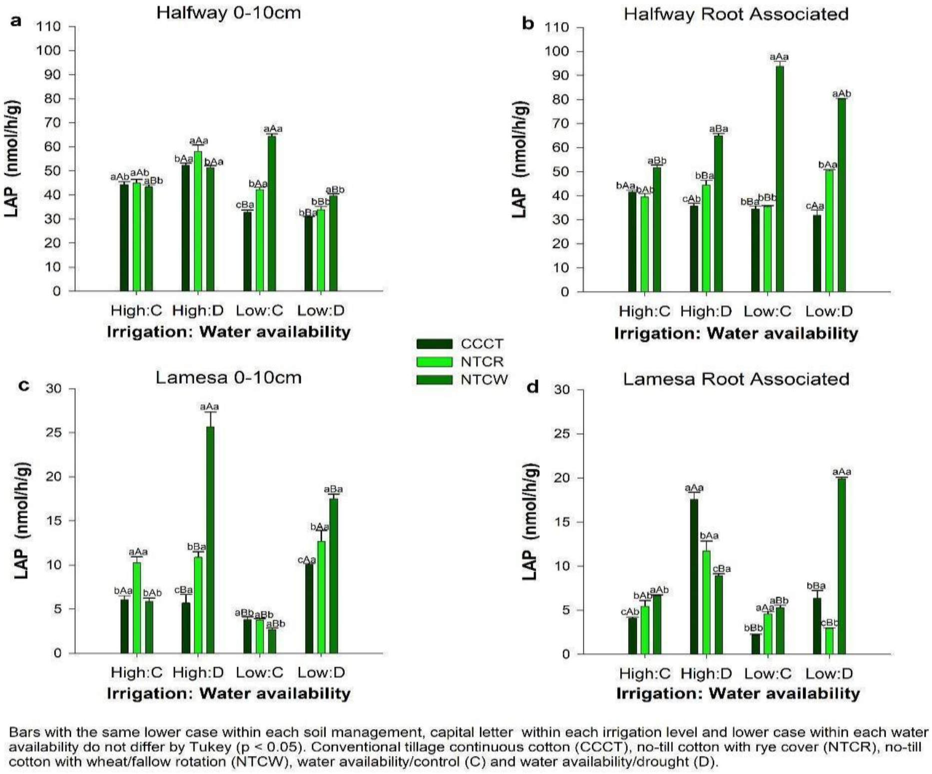
In the Lamesa site, the soil bulk root associated with the higher BG activity was observed in the NTCW with high irrigation and control treatment (35 $nmol \cdot h \cdot g^{-1}$) compared with this same management but under low irrigation or drought conditions. In drought conditions and low irrigation, the NTCW (17 $nmol \cdot h \cdot g^{-1}$ average) and NTCR (15 $nmol \cdot h \cdot g^{-1}$ average) resulted in an activity about 100% higher compared with the CCCT (8 $nmol \cdot h \cdot g^{-1}$ average). In 0-10cm soil bulk, the NTCW and NTCR had higher BG activity under drought conditions, while in control treatment, only NTCW had higher activity of this enzyme. Overall, the conventional management had the lower BG activity.

3.2.2 Leucine amino peptidase (LAP) enzyme activity

LAP activity was also affected by management, irrigation, and water availability (Figure 6). In Halfway 0-10cm soil bulk, LAP activity decreased in low irrigation conditions, except by NTCW management, which was higher when associated with low irrigation and control treatment (Figure 6a). In root-associated soil samples, the higher LAP activity was observed with NTCW management, this activity was even higher when combining NTCW + low irrigation + control (Figure 6b).

In Lamesa 0-10cm soil bulk, the higher LAP activity was in the association of NTCW + high irrigation + drought conditions. NTCW and drought as a common treatment had higher LAP activity. Regarding other managements, there were changes in the enzyme activity only under low irrigation, in which the drought increased LAP activity in all managements (Figure 6c). In root-associated samples, the NTCW with low irrigation and drought conditions had the same LAP activity as the combination CCCT + high irrigation + drought. Except for NTCR + low irrigation, in all the other treatments, the LAP activity increased under drought conditions (Figure 6d).

Figure 6 - Leucine amino peptidase (LAP) enzyme activity of root-associated (b,d), and 0-10 cm (a,c) soil depth with cotton grown under different soil management, irrigation level, and water availability (incubation trial) in Halfway (a,b) and Lamesa (c,d) soils.



Source: Author (2023).

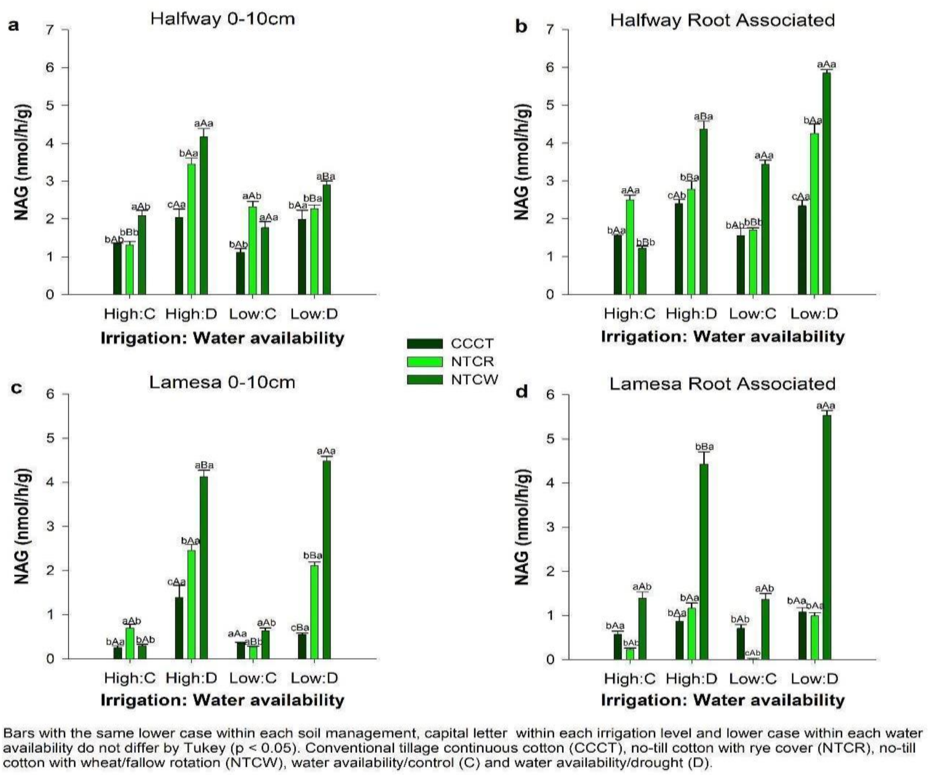
In Halfway 0-10cm soil bulk, the higher activity was with NTCW + low irrigation + control (67 $nmol·h·g^{-1}$), followed by NTCR + high irrigation + drought (60 $nmol·h·g^{-1}$), which were 76% and 13% higher than the conventional management (CCCT), respectively. In root-associated the higher activities were NTCW + low irrigation + control (98 $nmol·h·g^{-1}$) and (84 $nmol·h·g^{-1}$) drought treatments, being 180% and 160% above the CCCT.

In Lamesa 0-10cm soil bulk, the highest LAP activity was 27 $nmol·h·g^{-1}$ (by 350% above CCCT), with NTCW + high irrigation + drought. The other management had changed only under low irrigation, and when submitted to drought conditions, LAP activity increased, being NTCW (18 $nmol·h·g^{-1}$) > NTCR (14 $nmol·h·g^{-1}$) > CCCT (10.5 $nmol·h·g^{-1}$). In the root-associated soil bulk, the combination NTCW + low irrigation + drought had the same LAP activity as CCCT + high irrigation + drought. Although, under the same water availability and irrigation the NTCW (21.5 $nmol·h·g^{-1}$) was 207% higher than CCCT (7 $nmol·h·g^{-1}$). Overall, NTCW associated with lower water availability (low irrigation/drought) had higher LAP activity. Besides, there was a contrast between the maximum LAP activity in the sites, in which Halfway had values close to 100 $nmol·h·g^{-1}$, while Lamesa was below 30 $nmol·h·g^{-1}$.

3.2.3 β-1, 4, N-acetylglucosaminidase (NAG) enzyme activity

NAG activity followed the same pattern observed with the previous enzymes, in which the activity was affected by the interaction among management, irrigation, and water availability (Figure 7). In Halfway 0-10 cm soil bulk, the higher NAG activity was in the NTCW/NTCR + high irrigation + drought conditions (Figure 7a). In root-associated samples, the higher changes were observed with the conservation management (NTCW and NTCR), when submitted to drought (Figure 7b). In Lamesa, the changes in enzyme activities were higher, with a significant increase in the activity under drought conditions, especially in conservation management (Figure 7c,d). Overall, CCCT had lower NAG activity, in both soil bulk samples and sites.

Figure 7 - β-1, 4, N-acetylglucosaminidase (NAG) enzyme activity of root-associated (b,d), and 0-10 cm (a,c) soil depth with cotton grown under different soil management, and irrigation levels in Halfway (a,b) and Lamesa (c,d) soils.



Source: Author (2023).

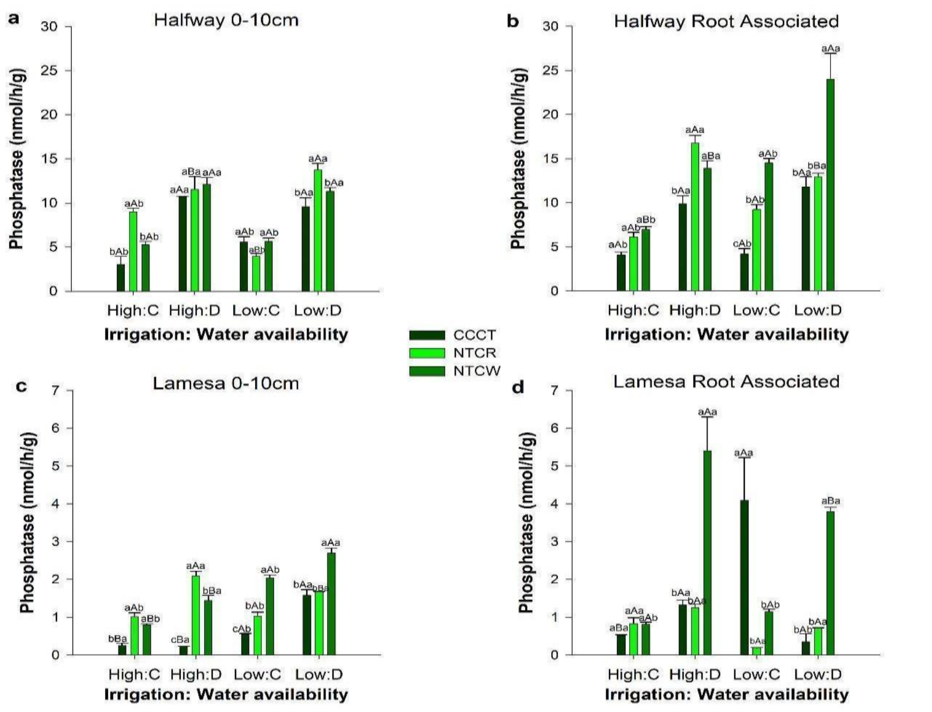
The increase of NAG activity under drought conditions happens mainly in soils under conservation management. In Halfway, in the soil bulk 0-10 cm and root-associated, the NTCW kept the higher NAG activity, especially in drought conditions and low irrigation plots (root-associated samples), increasing on average 50% of the enzyme activity when compared to the

CCCT. In Lamesa, in both samples, the higher NAG activity in NTCW + drought conditions, regardless of irrigation level, was 375% higher than CCCT. The contribution of NTCR to the increase in NAG activity was observed just in the 0-10 cm soil bulk, and when submitted to drought conditions. Compared to the CCCT, this management increased NAG activity by 235%.

3.2.4 Phosphatase enzyme activity

Conservation management had higher phosphatase activity (Figure 8). In Halfway 0-10 cm soil bulk the higher phosphatase activity was in NTCR management (Figure 8a). In root-associated, the higher activity was NTCW + low irrigation + drought (Figure 8b). In Lamesa, higher changes in this enzyme were observed in the root-associated samples, with NTCW + drought treatment, regardless of irrigation (Figure 8d). In 0-10 cm soil bulk, in high irrigation plots, the NTCR resulted in higher phosphatase activity, while under low irrigation, the higher enzyme activity was with NTCW management, regardless of water availability (Figure 8c).

Figure 8 - Phosphatase (PHOS) enzyme activity of root-associated (b,d), and 0-10 cm (a,c) soil depth with cotton grown under different soil management, and irrigation levels in Halfway (a,b) and Lamesa (c,d) soils.



Bars with the same lower case within each soil management, capital letter within each irrigation level and lower case within each water availability do not differ by Tukey ($p < 0.05$). Conventional tillage continuous cotton (CCCT), no-till cotton with rye cover (NTCR), no-till cotton with wheat/fallow rotation (NTCW), water availability/control (C) and water availability/drought (D).

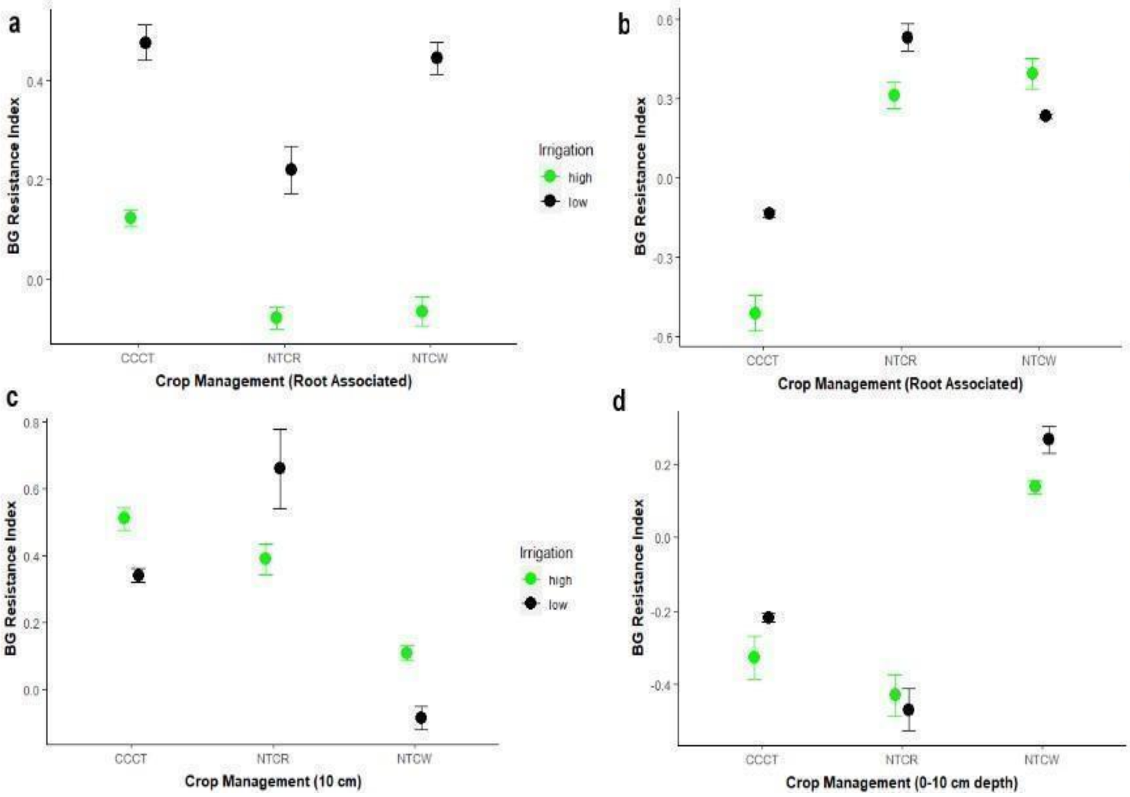
Source: Author (2023).

In Halfway 0-10 cm soil bulk, the higher change was in the NTCR + low irrigation + drought (15 nmol.h.g-1). In root-associated samples, the combination NTCW + low irrigation + drought resulted in a phosphatase activity 79% higher than the CCCT in the same water and irrigation conditions. In Lamesa 0-10 soil bulk, the NTCR + high irrigation + drought resulted in about 600% higher phosphatase activity compared to the CCCT, and NTCW + low irrigation + drought resulted in an activity 78% higher than the CCCT. In root-associated, there were even more changes in the phosphatase activity than 0-10 cm in soil bulk. The NTCW + drought, regardless of irrigation levels, increased the phosphatase activity by 375% compared to the CCCT.

3.3 Resistance index (RS) of microbial function

In Halfway (root associated), NTCR with low irrigation had higher RS compared with other management + irrigation combinations. Conservation management had higher BG - RS (Figure 9).

Figure 9 - β-1, 4, glucosidase (BG) resistance index of root-associated (a,b), and 0-10 cm (c,d) soil depth with cotton grown under different soil management, and irrigation level (IRRIG) in Halfway (a,c) and Lamesa (b,d) soils. Cotton with conventional tillage (CCCT), no-till cotton with cover crops (NTCR), and no-till cotton wheat rotation (NTCW).

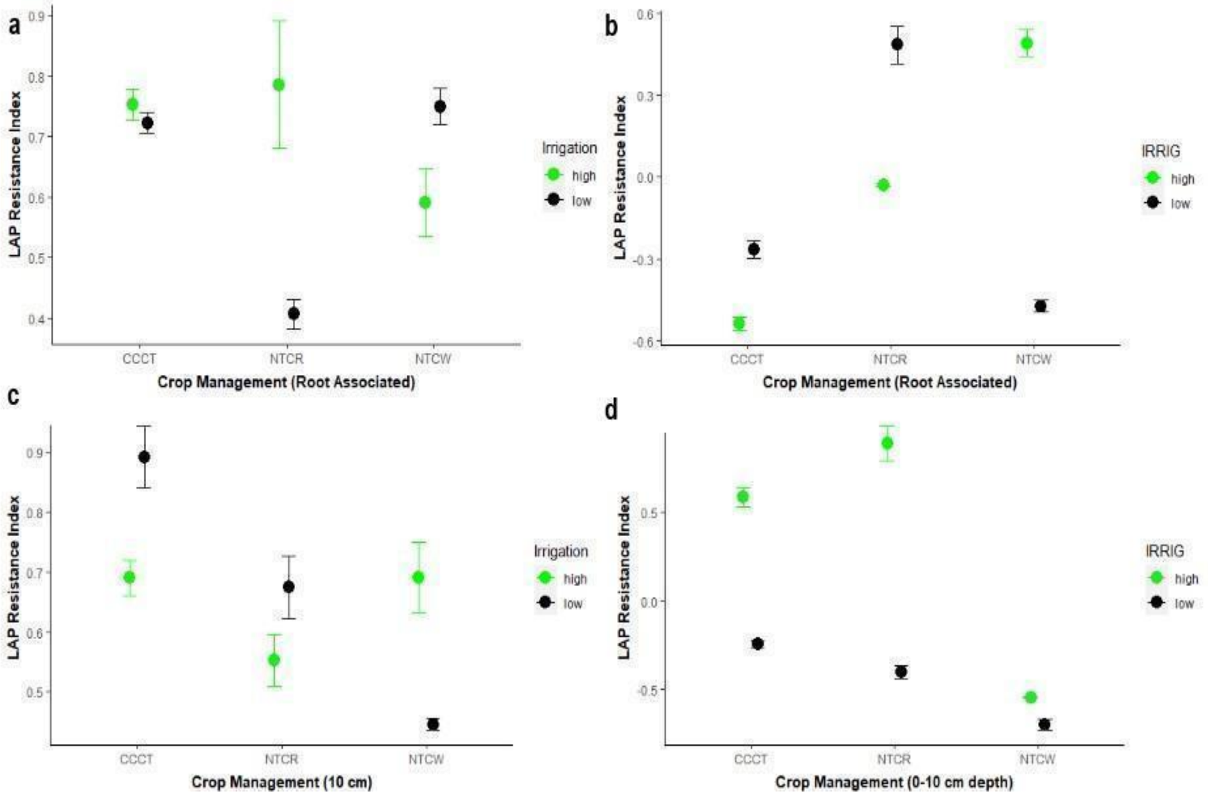


Source: Author (2023).

In Halfway (root-associated), overall low irrigation plots had higher RS than high irrigation. The combination of NTCW/CCCT + low irrigation (0.5) resulted in an increased RS of BG. In 0-10 cm soil bulk, the NTCR + low irrigation (0.7) increased the RS. In Lamesa (root-associated), NTCR + low irrigation (0.5), followed by NTCW + high irrigation (0.4), and under CCCT management, regardless of irrigation, there was a lower RS of BG (-0.25). In 0-10 cm soil samples, NTCW + low/high irrigation increased the BG resistance index, being 100% higher than the CCCT management.

Regarding LAP, conservation management had higher RS (Lamesa). Higher differences between drought and control treatments lead to a decrease in the RS (Figure 10).

Figure 10 - Leucine amino peptidase (LAP) resistance index of root-associated (a,b), and 0-10 cm (c,d) soil depth with cotton grown under different soil management, and irrigation levels (IRRIG) in Halfway (a,c) and Lamesa (b,d) soils. Cotton with conventional tillage (CCCT), no-till cotton with cover crops (NTCR), and no-till cotton wheat rotation (NTCW).



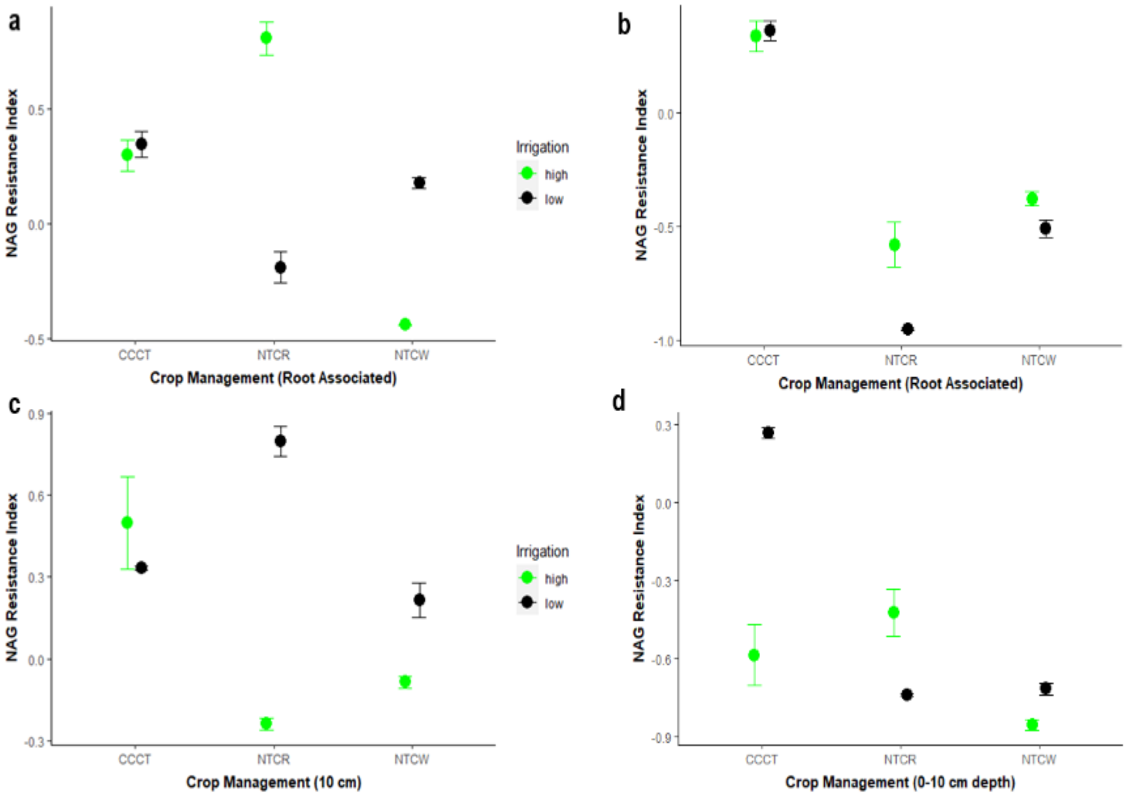
Source: Author (2023).

In Halfway root-associated samples, the NTCW + low irrigation, NTCR + high in irrigation, and CCCT management had the same LAP RS. In 0-10 cm soil bulk, the higher LAP RS was observed in the CCCT + low irrigation management. In Lamesa, the resistance of soil to drought was different from Halfway, mainly in root-associated samples, in which NTCR +

low irrigation and NTCW + high irrigation had a resistance index of 150% higher than CCCT, regardless of irrigation. In 0-10 cm soil bulk, the higher RS was in the NTCR + high irrigation treatment, followed by CCCT + high irrigation.

For NAG activity, the results of the resistance index were different depending on the site and sampling zone (Figure 11). The NTCR management in Halfway had higher NAG activity and less difference between control and drought treatments resulting in higher RS (Figure 11 a,c). Higher RS in CCCT management is related to lower activity under controlled and drought conditions, with a lower difference between the control and disturbed soil (Figure 11 b,d).

Figure 11 - β-1, 4, N-acetylglucosaminidase (NAG) resistance index of root-associated (a,b), and 0-10 cm (c,d) soil depth with cotton grown under different soil management, and irrigation level (IRRIG) in Halfway (a,c) and Lamesa (b,d) soils. Bars followed by the same letter do not differ by Tukey ($p < 0.05$). Cotton with conventional tillage (CCCT), no-till cotton with cover crops (NTCR), and no-till cotton wheat rotation (NTCW).

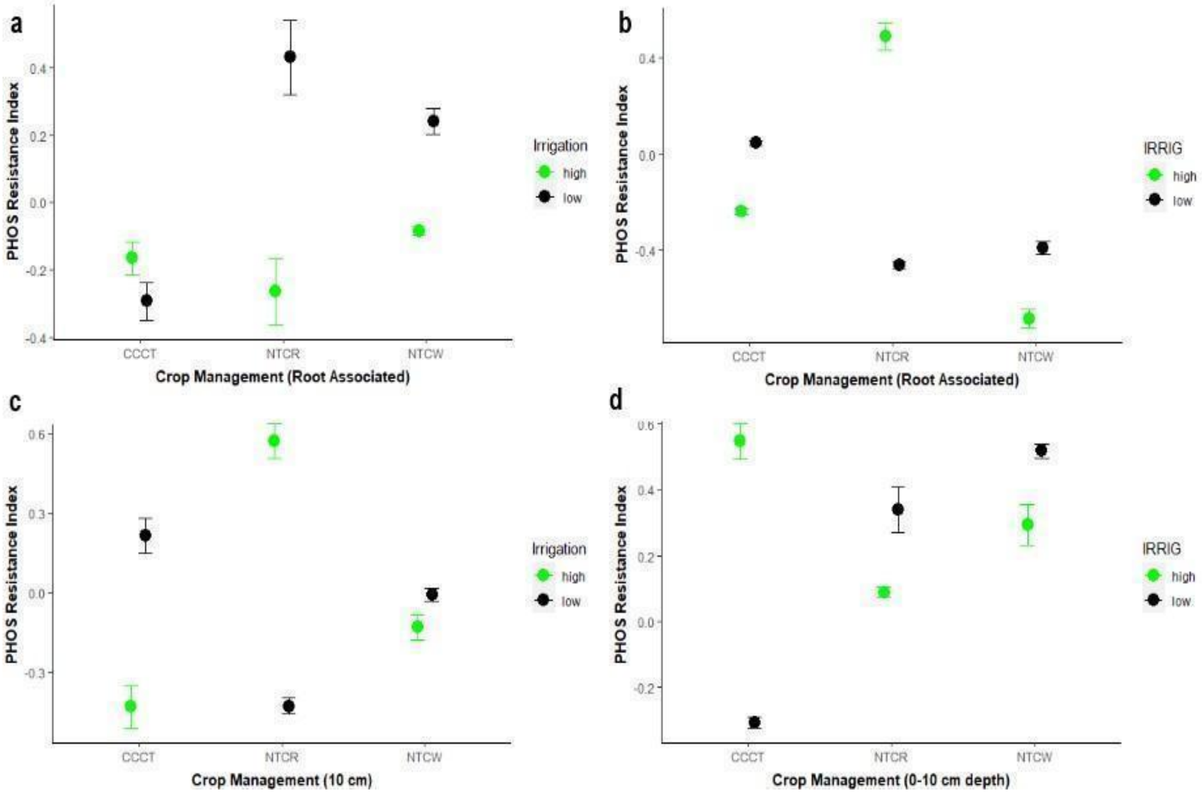


Source: Author (2023).

In the Halfway root associated samples, the higher RS was with NTCR under high irrigation (125% higher than CCCT), and the same management although with low irrigation, had the higher RS in the 0-10 cm depth (142% higher than CCCT). At the Lamesa site, in both sampling zones, the conventional management (CCCT) had higher RS (by 0.35 average), which does not mean a good result since the activity of this enzyme was low under control conditions and was kept low under drought conditions in the incubation trial, being less disturbed.

Regarding phosphatase activity, the conservation managements (NTCW and NTCR) lead to more resistance of this enzyme under drought conditions (Figure 15).

Figure 12 - Phosphatase (PHOS) resistance index of root-associated (a,b), and 0-10 cm (c,d) soil depth with cotton grown under different soil management, and irrigation level (IRRIG) in Halfway (a,c) and Lamesa (b,d) soils. Bars followed by the same letter do not differ by Tukey ($p < 0.05$). Cotton with conventional tillage (CCCT), no-till cotton with cover crops (NTCR), and no-till cotton wheat rotation (NTCW).



Source: Author (2023).

In Halfway root-associated soil, the higher phosphatase RS was observed in the NTCR (0.43), followed by NTCW (0.3), both managements in low irrigation plots, being higher than CCCT with high (-0.18) and low (-0.3) irrigation. In 0-10 cm soil bulk, the higher RS was in the NTCR + high irrigation treatment (0.6), being 300% higher than CCCT with the same irrigation level.

In Lamesa soil bulk root-associated, the NTCR + high irrigation had the higher RS of phosphatase activity (0.45), which decreased when associated with low irrigation. In the 0-10 cm zone, the RS of NTCW + low irrigation (0.55) was the same as CCCT + high irrigation (0.57), followed by NTCR + low irrigation (0.34) and NTCW + high irrigation (0.30). The treatment that less resisted drought disturbance was the CCCT + low irrigation, with the lower phosphatase RS (-0.3).

4 DISCUSSION

4.1 Microbial community size and composition

Microbial community size and composition were affected by drought and management. Since no-tillage and cover crops are alternatives to improve sustainability of agricultural systems due to SOM input and increase in water availability and nutrient cycling, these may help an environment to be more resilient under extreme drought conditions (DAVIS; HUGGINS; REGANOLD, 2023; NAYLOR; COLEMAN-DERR, 2018; SINGH et al., 2023; WANG et al., 2023). So, the EL-FAMEs were higher in wettered conditions and under conservation management (NTCW and NTCR).

Conservation management may improve the abundance of bacteria and fungi, which may decrease under drought conditions (NAYLOR; COLEMAN-DERR, 2018; WANG et al., 2023). Gram-negative bacteria decreased in drought conditions and were higher in conservation management. A stable environment with high SOM may favor an increase of gram-negative abundance, while a disturbance, such as drought, may negatively affect these bacteria (SINGH et al., 2023). In contrast, Gram-positive bacteria may be related to an environment more resistant to stresses, indicating that conservation management contributes to a more resilient and resistant environment (DAVIS; HUGGINS; REGANOLD, 2023; SINGH et al., 2023).

Regarding Actinobacteria phyla, its presence may be related to the systems with less SOM due to continuous soil tillage, and also with systems more adapted to disturbance (SINGH et al., 2023), which may justify the higher relative abundance of these phyla in the conventional tillage management (CCCT). The Fungi: Bacteria ratio was affected by drought and management. The more abundant soil environment tends to have a higher Fungi: Bacteria ratio, although a lower water availability may also contribute to a higher ratio (LAU; LENNON, 2011). So, this justifies the higher Fungi: Bacteria ratio in the conservation management, which had higher microbial relative abundance, and when it was submitted to drought, the ratio remained high.

Microbial composition was most driven by drought, being the conventional management separated from the conservation ones in the graphic dispersion (Figure 4). So, management and drought disturbance shifted changes in the soil microbiome composition due to the disturbance (NAYLOR; COLEMAN-DERR, 2018; SINGH et al., 2023; WANG et al., 2023).

4.2 Soil microbial functionality – nutrient cycling

The soil enzymes related to C, N, and P cycling were affected by management, irrigation level, and drought conditions. Also, different sites, with changes in soil type and environment, may shape different microbial communities, modifying their functionality and responses under disturbances (PAULA et al., 2014; SINGH et al., 2023; VELOSO et al., 2023; WANG et al., 2023).

Conservation management with SOM input, such as cover crop, crop rotation, and no-tillage, may contribute to the improvement of C cycling and consequently increase BG activity (SINGH et al., 2023; WANG et al., 2023). Thus, conservation management (NTCW) shifted higher BG activity, especially when subjected to drought.

LAP and NAG activities, related to N-cycling, were also higher in the conservation management (NTCW and NTCR) and increasiling affected by drought. Conservation management may increase the nitrogen-fixing bacteria (NFB) diversity, the N of microbial biomass, and the soil organic N, which may be related to the improvement of N-cycling and results in a higher LAP and NAG activity (KIM et al., 2020; SINGH et al., 2023; VELOSO et al., 2023; WANG et al., 2023).

Regarding phosphatase activity, the increase of this enzyme may be related to conservation management (e.g., no-tillage), which favors the presence of arbuscular mycorrhizal fungi (AMF), leading to the increase of enzymes related to P-cycling (SINGH et al., 2023; VELOSO et al., 2023).

The water availability also interferes with enzyme activities, so soils with historic drought periods may develop a “dry memory” and may be less disturbed by drought conditions (WANG et al., 2023). So, agricultural systems in semi-arid conditions such as this may shift different communities that even with conservation management have this dry memory, resulting in high activity under drought conditions.

4.3 Resistance of soil functions to drought

Conservation management drives more resistance of soil functions to disturbance. The calculation of some resistance index (RS) helps to understand how a microbiome's functionality may resist stresses (ORWIN; WARDLE, 2004).

Overall, the RS of enzyme activities were higher under conservation management (NTCW and NTCR), with a low influence on the irrigation levels, depending on the site and sampling zone. Negative RS values in the conventional management (CCCT) were indicative of low resistance to drought disturbance, so the activity of the enzyme was very affected by drought, with a high reduction of the activity when submitted to this disturbance (DELGADO-BAQUERIZO et al., 2017; ORWIN; WARDLE, 2004).

Otherwise, some conditions resulted in higher RS in conventional management (CCCT), especially enzymes related to N-cycling. If an environment favors low enzyme activity, and after a disturbance, such as drought, this activity remains low, the resistance index may be high. However, this result did not indicate that the soil resisted the stress, it only means that an attribute that was already bad because of the environmental conditions remained the same, which is called a "resistance trap" that may happen in these circumstances (DAVIS; HUGGINS; REGANOLD, 2023).

5 CONCLUSION

The differences in resistance of key microbial functions to severe drought observed between the studied locations may be related to soil texture.

Microbial enzyme activities responded differently to drought. Enzymes related to N-cycling were more disturbed by drought. Also, nutrient-cycling enzymes in soils under conservation management could resist severe drought, mainly related to C and P-cycling.

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APPENDIX A – CHEMICAL ANALYSIS OF THE ORGANIC COMPOST USED IN THE EXPERIMENTS

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CPF/CNPJ	085.644.526-69	**Cultura/Safra**	
Empreendimento	-	**Entrada - Saída**	26/02/2021
Técnico/Solicitante	MARINA SCALIONI VILELA	**Pacotes**	01-FOBL-BASICO , 09-ADICIONAL (Ca) , 10-ADICIONAL (Mg) , 11-ADICIONAL (S) , 12-ADICIONAL (B) , 14-ADICIONAL (Cu) , 15-ADICIONAL (Fe) , 16-ADICIONAL (Mn) , 19-ADICIONAL (Zn)

Resultados da análise de Fertilizantes Orgânicos

Código de Barras	Descrição amostra	pH	C.T.C	Umidade	Biureto	ANÁLISE GRANULOMÉTRICA			ANÁLISES QUÍMICAS						
						Passante ABNT 10 (P1)	Passante ABNT 20 (P2)	Passante ABNT 50 (P3)	M.O.T	C.O.T	R.M.T	R.M.I	R.M.S	P_2O_5	K_2O
			mmolc/kg	%											
FOBL21-161	COMPOSTO ORGANICO	8,26	NS	8,30	NS	51,47	27,11	7,95	43,40	25,12	NS	NS	NS	3,42	3,62

MACRONUTRIENTES						MICRONUTRIENTES									OUTROS ELEMENTOS QUÍMICOS							
N	P	K	Ca	Mg	S	B	Cl	Cu	Fe	Mn	Mo	Ni	Zn	Al	Cd	Cr	F	Na	Pb	Se	Si	SiO_2
%																						
2,35	1,49	3,00	10,39	0,62	0,53	0,01	NS	0,01	0,50	0,05	NS	NS	0,05	NS	NS	NS	NS	NS	NS	NS	NS	NS

pH = potencial hidrogênio; Umidade = Umidade; ABNT 10 (P1) = Peneira 2mm; ABNT 20 (P2) = Peneira 0,84mm;
ABNT 50 (P3) = Peneira 0,3mm; Biureto = Biureto; M.O.T = Matéria orgânica total; C.O.T = Carbono orgânico total;
R.M.T = Resíduo mineral total; R.M.I = Resíduo mineral insolúvel; R.M.S = Resíduo mineral solúvel; C.T.C = Capacidade de troca de cátions;
N = Nitrogênio total; P_2O_5 = Pentóxido de Fósforo; K_2O = Óxido de Potássio; Ca = Cálcio;
Mg = Magnésio; S = Enxofre; B = Boro; Cl = Cloro;
Cu = Cobre; Fe = Ferro; Mn = Manganês; Mo = Molibdênio;
Ni = Níquel; Zn = Zinco; Al = Alumínio Cd = Cádmio;
Cr = Cromo; F = Flúor; Na = Sódio; Pb = Chumbo;
Se = Selênio; Si = Silício; SiO_2 = Óxido de Silício; NS = Não solicitado;

Informações relevantes:
1 - Não assumimos responsabilidade pelas interpretações dos resultados analíticos.
2 - Recomendações de calagem e adubação sempre devem ser feitas mediantes consulta de um Engenheiro Agrônomo.
3 - O presente laudo não possui finalidades jurídicas.

APPENDIX B – CHEMICAL ANALYSIS OF THE COFFEE HUSK USED IN THE EXPERIMENTS

Laboratório de Análises Agropecuárias - Ltda.
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Cliente: ALISSON ANDRE VICENTE CAMPOS - 65963 || 085.644.526-69
Empreendimento:
Técnico responsável: MARINA SCALIONI VILELA
Pacotes: QUÍMICO MINERAIS
Descrição: CASCA DE CAFE

Data de entrada: 26/02/2021
Data de saída: 02/03/2021

Resultados de Alimentos - Análises Químicas

Informações da Amostra				
Código de Barras				1100000087
			60d	4a
Umidade	%	6,72	-	-
Matéria Seca	%	93,28	-	-
Carboidratos			**60d**	**4a**
FDA	%MS	NS	NS	NS
FDN	%MS	NS	NS	NS
FDNmo	%MS	NS	NS	NS
Lignina	%MS	NS	NS	NS
Amido	%MS	NS	NS	NS
Proteínas			**60d**	**4a**
Proteína Bruta	%MS	NS	NS	NS
PIDA	%MS	NS	NS	NS
PIDN	%MS	NS	NS	NS
NNP	%MS	NS	NS	NS

Gordura			60d	4a
Extrato Etéreo	%MS	NS	NS	NS
Minerais			**60d**	**4a**
Cinzas	%MS	NS	NS	NS
Cálcio	%MS	0,28	-	-
Fósforo	%MS	0,09	-	-
Magnésio	%MS	0,08	-	-
Potássio	%MS	2,96	-	-
Sódio	%MS	0,00	-	-
Enxofre	%MS	0,11	-	-
Cloreto	%MS	NS	NS	NS
Alumínio	ppm	870,49	-	-
Boro	ppm	21,65	-	-
Cobre	ppm	14,54	-	-
Ferro	ppm	144,59	-	-
Manganês	ppm	27,69	-	-
Zinco	ppm	5,89	-	-
DCAD	meq/100gr	NS	NS	NS

Penn State			60d	4a
TMP	mm	NS	NS	NS
Desvio Padrão	mm	NS	NS	NS
KPS			**60d**	**4a**
Amido	%	NS	NS	NS
KPS		NS	NS	NS
FDNfe			**60d**	**4a**
MS	%	NS	NS	NS
FDN	%	NS	NS	NS
Tam. Partículas de Grãos			**60d**	**4a**
TMP	mm	NS	NS	NS
Desvio Padrão	mm	NS	NS	NS

Informações relevantes:
1 - Não assumimos responsabilidade pelas interpretações dos resultados analíticos.
2 - Recomendações de calagem e adubação sempre devem ser feitas mediantes consulta de um Engenheiro Agrônomo.
3 - O presente laudo não possui finalidades jurídicas.
4 - Todas amostras analisadas serão descartadas após noventa dias.

Amostra nº 1100000087
Análises Químicas. Página 1 de 2