UNIVERSIDADE FEDERAL RURAL DO SEMI-ÁRIDO
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
PROGRAMA DE PÓS-GRADUAÇÃO EM FITOTECNIA
DOUTORADO EM FITOTECNIA

TATIANNE RAIANNE COSTA ALVES

MITIGATING ROOT ROT IN MELON MONOCULTURE: INVESTIGATING SOIL MICROBIOME DYNAMICS AND THE EFFICACY OF MICROBIAL AND METABOLITE AMENDMENTS IN DISEASE MANAGEMENT

MOSSORÓ
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Thesis submitted to the Universidade Federal Rural do Semi-Árido, as a requirement for the degree of Doctor of Science in Plant Science.

Research area: Plant pathology

Advisor: Márcia Michelle de Queiroz Ambrósio Prof. Dr.

Co-advisor: Washington Luis da Silva Prof. Dr.

MOSSORÓ

2025

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Dados Internacionais de Catalogação na Publicação (CIP)
Biblioteca Central Orlando Teixeira (BCOT)
Setor de Informação e Referência (SIR)

A474m Alves, Tatianne Raianne Costa.
MITIGATING ROOT ROT IN MELON MONOCULTURE: INVESTIGATING SOIL MICROBIOME DYNAMICS AND THE EFFICACY OF MICROBIAL AND METABOLITE AMENDMENTS IN DISEASE MANAGEMENT / Tatianne Raianne Costa Alves. - 2025.
70 f. : il.

Orientadora: Márcia Michelle de Queiroz Ambrósio.
Coorientador: Washington Luis da Silva.
Tese (Doutorado) - Universidade Federal Rural do Semi-árido, Programa de Pós-graduação em Fitotecnia, 2025.

1. Saúde do solo. 2. Patógenos habitantes do solo. 3. Microorganismos. 4. Cucurbitáceas. I. Ambrósio, Márcia Michelle de Queiroz , orient. II. Silva, Washington Luis da , co-orient. III. Título.

Ficha catalográfica elaborada por sistema gerador autômato em conformidade com AACR2 e os dados fornecidos pelo) autor(a).
Biblioteca Campus Mossoró / Setor de Informação e Referência Bibliotecária: Keina Cristina Santos Sousa e Silva
CRB: 15/120

TATIANNE RAIANNE COSTA ALVES

MONOCULTURE OF MELON CROPS CAUSES CHANGES IN SOIL COMPOSITION AND IN THE ACTIVITIES OF MICROORGANISMS AND THE POTENTIAL OF COMBINED USE OF BACTERIA AND METABOLITES IN REDUCING ROOT ROT OF MELON AND INCREASING ENZYMATIC ACTIVITY OF THE SOIL

Thesis submitted to the Universidade Federal Rural do Semi-Árido, as a requirement for the degree of Doctor of Science in Plant Science. Research area: Plant pathology

Approved on: 22 / 07 / 2025.

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To my parents, Francisca Tacia Costa de Melo
and João Batista Araújo Alves. My grandmother,
Terezinha Araújo Costa de Melo.
And to my brother, Jackson Eduardo Costa Alves.
You are my foundation, my example, and my refuge.
All my effort and dedication are for you!
I DEDICATE!

AKNOWLEDGEMNETS

To God, for the strength, health, courage, faith, and determination He granted me so I could walk this path, complete this stage, and realize this great dream. He is always kind and merciful to us. To Our Lady, for the great blessings constantly bestowed upon my life.

To my parents, Francisca Tacia Costa de Melo and João Batista Araújo Alves, and to my brother Jackson Eduardo Costa Alves, for being my shelter, care, and zeal in the face of adversity. For the encouragement, support, education, and unconditional love you have always given me. I love you infinitely!

To my grandmother Terezinha Araújo Costa de Melo, my second mother and role model. A great supporter of all the achievements I have achieved thus far. I am immensely grateful for all the life lessons you taught me, for the faith you instilled in me, and for everything you have always done for me. I love you immeasurably.

To my advisor, Professor Dr. Márcia Michelle de Queiroz Ambrósio, for whom I have immense admiration, respect, and friendship. I thank you for your guidance, care, patience, constant dedication, and teachings that go far beyond theory. You are a role model for all your students.

To the entire Microbiology and Phytopathology Laboratory team, who were always available to help at every stage of the work. A group of friends who help each other and make workdays easier.

To my friends, for their constant support. Especially Jarlan Lucas, Ana Paula, and Vitória Gomes, for their friendship, support, concern, and constant encouragement. I keep you in my heart!

To Professor Dr. Eulene Francisco and my colleague Éder Francisco, for their invaluable assistance during the analyses.

To Prof. Dr. Washington Luis da Silva, for his co-supervision, funding for soil metagenomic analyses, and collaboration in enriching the research.

To Prof. Dr. Blaire Steven, Jacquelyn LaReau, and Ígor Vinícius, for their partnership and significant contributions to some of the research analyses.

I thank the Examining Board for their corrections and valuable contributions to the improvement of this work.

To the Universidade Federal Rural do Semi-Árido (UFERSA) and the Graduate Program in Plant Science for providing the opportunity to pursue undergraduate, masters,

and doctoral degrees at a top-notch university.

To the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for awarding the scholarship.

To Alltech Crop Science, for their partnership with UFERSA and financial support for part of the research.

To Fazenda Agrícola Famosa and its entire team, for their openness and support during the research.

I thank all the professors at UFERSA for all the knowledge they shared, which contributed to my professional growth.

To everyone who helped me along this path, directly or indirectly.

Thank you very much!

Alves, Tatianne Raianne Costa. **Mitigating root rot in melon monoculture: investigating soil microbiome dynamics and the efficacy of microbial and metabolite amendments in disease management.** 2025. 70f. (Thesis) Doctorate in Agronomy: Phytotechnics – Universidade Federal Rural do Semi-Árido/UFERSA, Mossoró, 2025.

ABSTRACT

The increasing demand for food from limited resources by the human population remains a constant global concern. Brazil is one of the largest food producers in the world and excels in the international trade of certain fruits, such as melons, where it is the second-largest exporter. To meet this demand, melons are cultivated over large areas, often through monoculture practices. This approach can cause significant damage to soil microbiota, decreasing both the quantity and diversity of microorganisms and reducing soil carbon storage. Additionally, monoculture favors the proliferation of phytopathogens that cause serious damage to melons, such as *Fusarium* sp., *Macrophomina* sp., and others. Controlling these pathogens is challenging due to their broad host range and resistance structures, which enable them to survive in the soil for extended periods. Environmental concerns have led to bans on chemical pesticides by importing markets, creating a need for sustainable alternatives to manage these phytopathogens. Therefore, the present study aimed to evaluate microbial activity in clayey and sandy soils subjected to different periods of melon monoculture and to evaluate the effect of alternative products and their combinations on the management of root diseases in melon crops. The first part of the work consisted of two experiments in each soil texture (sandy and clayey) and was carried out at Fazenda Agrícola Famosa. Part of the analyses were conducted at the Universidade Federal Rural do Semi-Árido (UFERSA) in Mossoró, Brazil, and the other part of the analyses was carried out at the Connecticut Agricultural Experiment Station (CAES) in New Haven, United States. The experimental design of the first trial was completely randomized, with four treatments (1 = native area, 2 = 8 years of monoculture, 3 = 15 years of monoculture, and 4 = 20 years of melon monoculture), with six replicates. Soil health indicator enzymes (β-glucosidase, arylsulfatase, and acid phosphatase), microbial biomass carbon, total organic carbon, labile carbohydrate, quantification of total bacteria, sporulating bacteria, and total fungi, and the soil microbiome were evaluated. When the data followed a normal distribution, analysis of variance was performed, and Tukey test was used at a 5% probability level to compare treatment means. When they did not follow a normal distribution, the Kruskal-Wallis test was used, and means were compared using the Bonferroni test. Although melon monoculture is a common practice in melon-producing areas in Brazil and generates high employment, it reduces the activity of microorganisms that are so important for soil health,

hindering the sustainability of this system. The study showed that if producers maintain monoculture for long periods, they need to adopt practices that further promote the activity of these microorganisms, such as increasing soil organic matter, increasing the use and diversity of microorganisms adapted to semiarid conditions, implementing green manure, and reducing practices that disturb the soil. The second part of the study also consisted of two field experiments in areas with a history of diseases caused by soil-dwelling pathogens, using a randomized block experimental design with six treatments and seven replicates. The treatments were previously chosen in a greenhouse experiment, using a completely randomized design with 30 treatments and four replicates. The treatments consisted of beneficial bacteria and fungi, and microbial metabolites. In the field experiments, the treatments were T1 = (*Bacillus subtilis* and *B. licheniformis* + Metabolites 1), T2 = (*B. subtilis*, *Lactobacillus plantarum*, and *Enterococcus faecium* + Metabolites 2), T3 = (Metabolites 1 + Metabolites 2), T4 = (*B. subtilis*, *B. licheniformis* + *L. plantarum* and *E. faecium* + Metabolites 1 + Metabolites 2), T5 = standard management of the farm (*T. asperelum*, *B. subtilis*, Metabolites 1 and Metabolites 2), and T6 = Control. The parameters evaluated were: plant stem diameter, shoot dry mass, disease severity, number of fruits per plant, fruit weight, and ºbrix, and soil enzymes. Analysis of variance was performed, and means were compared using Tukey test at 5% probability. Regarding disease severity, none of the treatments stood out, however, T4 showed promising signs, such as increased soil enzyme activity, a greater number of fruits per plant, and was one of those that provided the greatest reduction in disease severity caused by soilborne pathogens in melons.

Keywords: Soil health; Soil-borne pathogens; Microorganisms; Cucurbitaceae.

RESUMO

A necessidade crescente de alimentos por parte da população humana a partir de recursos limitados, é sempre uma preocupação mundial. O Brasil é um dos maiores produtores de alimentos do mundo, e se destaca no comércio internacional de algumas frutas, como o melão, sendo o segundo maior exportador. Para conseguir atender a demanda, essa olerícola é cultivada em grandes áreas, e umas das práticas adotadas é o monocultivo. Essa prática pode levar a grandes prejuízos a microbiota do solo, reduzindo a quantidade e diversidade de microrganismos e reduzir o aporte de carbono no solo. Além disso, o monocultivo ajuda a selecionar fitopatógenos que causam grandes prejuízos ao meloeiro, como *Fusarium* sp., *Macrophomina* sp. e outros. O manejo desses patógenos é díficil devido a sua ampla gama de hospedeiros, e por eles também possuirem estruturas de resistência, que permite que eles sobrevivam no solo por longos períodos. Devido a questões ambientais, o uso de defensivos químicos vem sendo banido pelo mercado importador, fazendo-se necessário alternativas sustentáveis para o manejo desses fitopatógenos. Por isso, o presente estudo teve como objetivo avaliar a atividade microbiana em solos argilosos e arenosos submetidos a diferentes períodos de monocultivo do meloeiro; e avaliar o efeito de produtos alternativos e suas associações no manejo de doenças radiculares na cultura do meloeiro. A primeira parte do trabalho constou de dois experimentos em cada textura de solo (arenoso e argiloso), e foi realizado na Fazenda Agrícola Famosa. Parte das análises foram conduzidas na Universidade Federal Rural do Semi-Árido (UFERSA), em Mossoró, Brasil, e a outra parte das análises foram realizadas na The Connecticut Agricultural Experiment Station (CAES), em New Haven, Estados Unidos. O delineamento experimental do primeiro ensaio foi o inteiramente casualizado, com 4 tratamentos (1=Área nativa, 2= 8 anos de monocultivo, 3= 15 anos de monocultivo e 4= 20 anos de monocultivo do meloeiro), com 6 repetições. Avaliou-se as enzimas indicadoras da saúde do solo (β-glicosidase, arilsulfatase, fosfatase ácida), carbono da biomassa microbiana, carbono orgânico total, carbolo lábil, quantificação de bactérias totais, bactérias esporulantes e fungos totais, e o microbioma do solo. Quando os dados seguiram uma distribuição normal foi realizada a análise de variância, e utilizado o teste de Tukey ao nível de 5% de probabilidade para comparação entre as médias dos tratamentos, e quando não seguiram uma distribuição normal, foi utilizado o teste de Kruskal-Wallis, e as médias foram comparadas pelo Teste de Bonferroni. O monocultivo do meloeiro embora seja uma prática comum em áreas de produção de melão no Brasil, e que gera grande empregabilidade, reduz a atividade dos microrganismos tão importantes para a saúde do solo, dificultando a sustentabilidade desse sistema. O trabalho

mostrou que, se os produtores permanecerem com a adoção do monocultivo por longos períodos, é necessário a adoção de práticas que favoreçam ainda mais a atividade desses microrganismos, tais como, aumento da matéria orgânica do solo, aumentar a utilização e a diversidade de microrganismos adaptados a condição semiárida, realizar a adubação verde, e reduzir práticas que perturbam o solo. A segunda parte do trabalho também foi composta por dois experimentos em condição de campo, em áreas com histórico de doenças causadas por patógenos habitantes do solo, com delineamento experimental em blocos casualizados, com seis tratamentos e sete repetições. Os tratamentos foram escolhidos previamente em um experimento realizado em casa de vegetação, em delineamento inteiramente casualizado, com 30 tratamentos e 4 repetições. Os tratamentos foram compostos por batérias e fungos benéficos, e metabolitos microbianos Nos experimentos realizados em campo os tratamentos foram T1 = (*Bacillus subtilis* e *B. licheniformis* + Metabolitos 1), T2 = (*B. subtilis*, *Lactobacillus plantarum* e *Enterococcus faecium* + Metabolitos 2), T3 = (Metabolitos 1 + Metabolitos 2), T4 = (*B. subtilis*, *B. licheniformis* + *L. plantarum* e *E. faecium* + Metabolitos 1 + Metabolitos 2), T5 = manejo padrão da empresa (*T. asperelum*, *B. subtilis*, metabolitos 1 e metabolitos 2) e T6 = Controle. Os parâmetros avaliados foram: diâmetro do caule da planta, massa seca da parte aérea, severidade da doença, número de frutos por planta, peso dos frutos e ºbrix, e enzimas do solo. Foi realizada a análise de variância, e as médias foram comparadas pelo teste de Tukey a 5% de probabilidade. Para a severidade da doença, nenhum dos tratamentos se destacou, no entanto, o tratamento 4 mostrou sinais promissores, como maior atividade das enzimas do solo, um maior número de frutos por planta e foi um dos que proporcionou a maior redução na severidade da doença causada por patógenos do solo no melão.

Palavras-chave: Saúde do solo; Patógenos habitantes do solo; Microorganismos; Cucurbitáceas.

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Figure 1. Stem diameter (A), shoot dry mass (B), and root rot severity (C) in melon plants. Cultivated soils were treated with the mix formulation of alternative products, being T1 = (*B. subtilis* + *B. licheniformis* + metabolites 1), T2 = (*B. subtilis* + *L. plantarum* + *E. faecium* + metabolites 2), T3 = (metabolites 1 + metabolites 2), T4 = (*B. subtilis* + *B. licheniformis* + *L. plantarum* + *E. faecium* + metabolites 1 + metabolites 2), T5 = standard management (*T. asperelum* + *B. subtilis* + metabolites 1 + metabolites 2), and T6 = Control.

Figure 2. Weight (A), number of fruits per plant (B), and °Brix (C) of melon fruits. Cultivated soils were treated with the mix formulation of alternative products. T1 = (*B. subtilis* + *B. licheniformis* + metabolites 1), T2 = (*B. subtilis* + *L. plantarum* + *E. faecium* + metabolites 2), T3 = (metabolites 1 + metabolites 2), T4 = (*B. subtilis* + *B. licheniformis* + *L. plantarum* and *E. faecium* + metabolites 1 + metabolites 2), T5 = standard management (*T. asperelum* + *B. subtilis* + metabolites 1 + metabolites 2), and T6 = Control.

Figure 3. Activity of the enzyme β-glucosidase (A), Arylsulfatase (B), Acid phosphatase (C) of soils. Cultivated soils were treated with mix formulation of alternative products. T1 = (*B. subtilis* + *B. licheniformis* + metabolites 1), T2 = (*B. subtilis* + *L. plantarum* + *E. faecium* + metabolites 2), T3 = (metabolites 1 + metabolites 2), T4 = (*B. subtilis* + *B. licheniformis* + *L. plantarum* and *E. faecium* + metabolites 1 + metabolites 2), T5 = standard management (*T. asperellum* + *B. subtilis* + metabolites 1 and metabolites 2), and T6 = Control.

Supplementary figure 1. Disease severity of naturally infested melon plants and subjected to the application of alternative products and their associations. T1= *Trichoderma asperellum;* T2= *Bacillus subtilis;* T3= *B. subtilis* + *B. Licheniformis;* T4= *Bacillus subtilis* + *Lactobacillus plantarum*+ *Enterococus faecium;* T5= Metabólitos de fermentação 1; T6= Metabólitos de fermentação 2; T7= *T. asperellum* + *B.subtilis;* T8= *T. asperellum* + *B.subtilis* e *B.licheniformis;* T9= *T. asperellum* + B*. subtilis, L. plantarum* e *E. Faecium;* T10= *T. asperellum* + Metabólitos 2; T11= *T. asperellum* + Metabólitos 1; T12= *B.subtilis* + *B. Licheniformis*; T13= *B.subtilis* + *L. plantarum* e *E. Faecium;* T14= *B.subtilis* + Metabólitos 2; T15= *B.subtilis* + Metabólitos 2; T16= *B.subtilis* e *B. licheniformis* + *L. plantarum* e *E. Faecium;* T17= *B.subtilis* e *B. licheniformis* + Metabólitos 2; T18= *B.subtilis* e *B. licheniformis* + Metabólitos 1; T19= *B.subtilis, L. plantarum* e *E. faecium* + Metabólitos 2; T20= *B.subtilis, L. plantarum* e *E. faecium* + Metabólitos 1; T21= Metabólitos 1 + Metabólitos 2; T22= *T. asperellum* +*B.subtilis* + *L. plantarum* e *E. Faecium;* T23= *T. asperellum* + *B.subtilis* + *L. Plantarum* + *E. faecium*+ Metabólitos 2; T24= *T. asperellum* + *B.subtilis* + *L. Plantarum* + *E. faecium*+ Metabólitos 1; T25= *T. asperellum* + *B.subtilis* e *B.*

licheniformis + *L. plantarum* e *E. Faecium;* T26= *T. asperellum* + *B.subtilis* e *B. licheniformis* + *L. plantarum* e *E. faecium* + Metabólitos 2; T27= *T. asperellum* + *B.subtilis* e *B. licheniformis* + *L. plantarum* e *E. faecium* + Metabólitos 1; T28= *B.subtilis* e *B. licheniformis* + *L. plantarum* e *E. faecium*+Metabólitos 1+ Metabólitos 2; T29= *T. asperellum* + *B.subtilis* + Metabólitos 1 + Metabólitos 2; T30= Control.

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SUMARY

GENERAL INTRODUCTION

The melon is an herbaceous plant belonging to the Cucurbitaceae family, with an annual cycle, native to Africa and Asia (Filgueira, 2012). It thrives in tropical climates, especially where there is low relative humidity, high temperatures, and high sunlight, factors that favor its cultivation in the Northeast Region of Brazil. Brazil ranks tenth in the world ranking of melon producers, with a production of 862,000 tons in 2023, of which approximately 213,000 tons were destined for export. The Northeast Region is the main producer in the country, accounting for over 90% of national production, with the state of Rio Grande do Norte accounting for over 70% of this total (IBGE, 2025; HF Brasil, 2024). Although it performs well under the climatic conditions of the semiarid Northeast, the performance of the crop can be reduced by several biotic and abiotic factors. Among these, root system diseases caused by soil-borne pathogens (SBPs) stand out. Examples include fungi of the genera *Macrophomina*, *Fusarium*, *Rhizoctonia*, and *Monosporascus* (Medeiros et al., 2015). It is important to emphasize that melon monoculture in these producing regions, combined with some management practices adopted by producers, such as the use of polyethylene mulching, has contributed to the increased incidence and severity of these diseases, causing both quantitative and qualitative losses to crops (Medeiros et al., 2015). These phytopathogens are difficult to control because they have resistance structures that favor the survival of the fungi for several years, even under unfavorable conditions (Andrade et al., 2014). Several species of soil-borne fungi can affect and cause diseases in various economically important crops, such as those belonging to the genera *Fusarium* (which causes vascular wilt) and *Macrophomina* (which causes gray stem rot) (Mihajlović et al., 2017). Some species belonging to the genus *Fusarium* colonize plant vessels, causing xylem obstruction. After root rot, the symptom occurs in the form of wilt, beginning at the ends of branches (Ferreira et al., 2015). This pathogen is a facultative parasite and can survive as chlamydospores in crop residues and soil, favoring high soil moisture and temperatures between 20 and 28 °C (Pavan et al., 2016).

The fungus *M. phaseolina* has soil as its natural habitat, with a wide geographic distribution, and a higher incidence observed in tropical and subtropical regions. Besides its large number of hosts, it can be pathogenic to more than 500 botanical species, according to the literature (Pennerman et al., 2024). This species is characterized by abundant, cottony, dark gray aerial mycelium. Its main source of inoculum is microsclerotia (Nascimento et al., 2016). By attacking plant roots, the pathogen causes dry root rot, charcoal rot, shoot wilting, and

damping off (Gupta et al., 2012). However, this fungus uses sclerotia as its main form of infection and can persist in the soil, in plant debris, or be transmitted through contaminated seeds (Pereira et al., 2012).

Monoculture is one of the practices that helps select these pathogens, as it aims to cultivate a single crop in a given area, aiming to increase production (Bogužas et al., 2022). This practice is used in the cultivation of species destined for export, such as melons, which, combined with other practices such as mulching, promotes an imbalance of beneficial soil microorganisms and the increase in thermotolerant phytopathogens (Bandopadhyay et al., 2020). This practice has a significant environmental impact, causing soil depletion, nutritional imbalance, loss of microbial biodiversity, and pathogen selection (Bogužas et al., 2022).

Although monoculture is an aggressive practice, soil in its equilibrium is a living, dynamic, and biologically active system, where interactions essential for ecosystem functioning occur. It has a complex biological community composed of microorganisms, fauna, and roots, which are fundamental for nutrient cycling, structure formation, and maintenance of soil fertility (Jacobsen & Hjelmso, 2014). Microorganism populations in the soil coexist in an ecological balance that can be significantly influenced by the cultivated species, management adopted, input applications, successive crops, monocultures, and prevailing climatic factors, especially temperature and humidity, which can alter this balance (Jacobsen & Hjelmso, 2014).

Microbial communities are the main sources of enzymes in soil, and these enzymes are essential for the decomposition of carbon and soil organic matter (Moghiminian et al., 2017). The enzymes arylsulfatase, β-glucosidase, and acid phosphatase are associated with the sulfur, carbon, and phosphorus cycles, respectively, and are directly or indirectly related to productive potential and the sustainability of land use (Mendes et al., 2018). Soil enzyme measurements also have great potential as indicators of soil quality and health due to their sensitivity, responding more quickly to changes in management practices than parameters such as soil organic matter (Mendes et al., 2019).

Given the importance of maintaining soil balance through the maintenance and preservation of microbiota, aiming to reduce the impacts of monoculture on the soil, and the need for tools to aid in the management of root diseases, some techniques have been studied and used in practice to increase soil balance and biological activity in different crops, such as the use of biological or alternative products based on microorganisms, metabolites, or their derivatives, which promote soil health, making it a more balanced environment. Beneficial microorganisms promote biological control through antagonistic interactions, which can reduce the occurrence or intensity of diseases (Coêlho et al., 2018). The main mechanisms of

antagonistic action are: antibiosis, competition, parasitism, and predation, and they also act to promote plant growth. Examples of microorganisms that act as biocontrol agents include bacteria of the genus *Bacillus* sp., *Azospirillum* sp., and fungi of the genus *Trichoderma* sp., among others (Coêlho et al. 2018).

Rhizobacteria are among the microorganisms that stand out in biocontrol, as they live and colonize the rhizosphere, promoting plant growth through a symbiotic relationship, favoring the absorption of nutrients, especially through the biological fixation of nitrogen carried out by bacteria of the genus *Rhizobium* sp. and *Azospirillum* sp. (Saharan & Nehra, 2011; Sottero, 2013). Another important genus within rhizobacteria is *Bacillus* sp., due to its antagonistic power, being widely explored, as it expresses great biocontrol potential, acting in the degradation of the cell wall of pathogens that have chitin in their composition (Devkota et al., 2011; Lanna filho et al., 2010). Bacteria of the genus *Bacillus* demonstrated broad-spectrum activity against several pathogens that cause corn diseases and effectively controlled stalk rot, exhibiting a control efficacy of 67.40% (Wang et al., 2024).

Among the fungi that promote beneficial effects on soil/plants, the genus *Trichoderma* sp. stands out. It is a soil-borne fungus, found in both temperate and tropical regions, generally associated with the organic fraction and rhizosphere of plants. It protects plants against pathogens through competition, mycoparasitism, and antibiosis (Lorito et al., 2010; Druzhinina et al., 2011). A study by Gao et al. (2023) showed that *T. viride* significantly reduced the incidence of root rot in soybeans, in addition to promoting plant growth and health. Besides being a key biological disease control agent, it has several other uses, such as producing enzymes for industry, promoting plant development, increasing productivity, and reducing the impacts of salt stress (Meyer et al., 2019). Previous studies analyzing the antagonistic effect of *Trichoderma* sp. on some plant pathogens showed that *T. asperellum* had the potential to antagonize pathogens such as *Sclerotium rolfsii*, *Rhizoctonia solani*, and *Colletotrichum dematium* through the in vitro production of toxic metabolites (Gabardo et al., 2020).

Therefore, we hypothesize that intense and continuous melon cultivation without crop rotation in monoculture regimes alters soil enzyme activity and microbial composition. To test this hypothesis, we evaluated microbial diversity, enzyme activity, and carbon analysis in two soil textures (clay and sandy) subjected to different periods of successive melon monoculture. Given the need for sustainable and efficient tools to manage root diseases that damage melon plants, this study also aimed to verify the effect of products based on bacteria, *Trichoderma*, and metabolites in managing PHS in melon plants.

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ARTICLE I

MELON CULTIVATED IN CONTINUOUS MONOCULTURE REGIMES CAUSES CHANGES IN SOIL COMPOSITION AND MICROBIAL ACTIVITIES

ABSTRACT

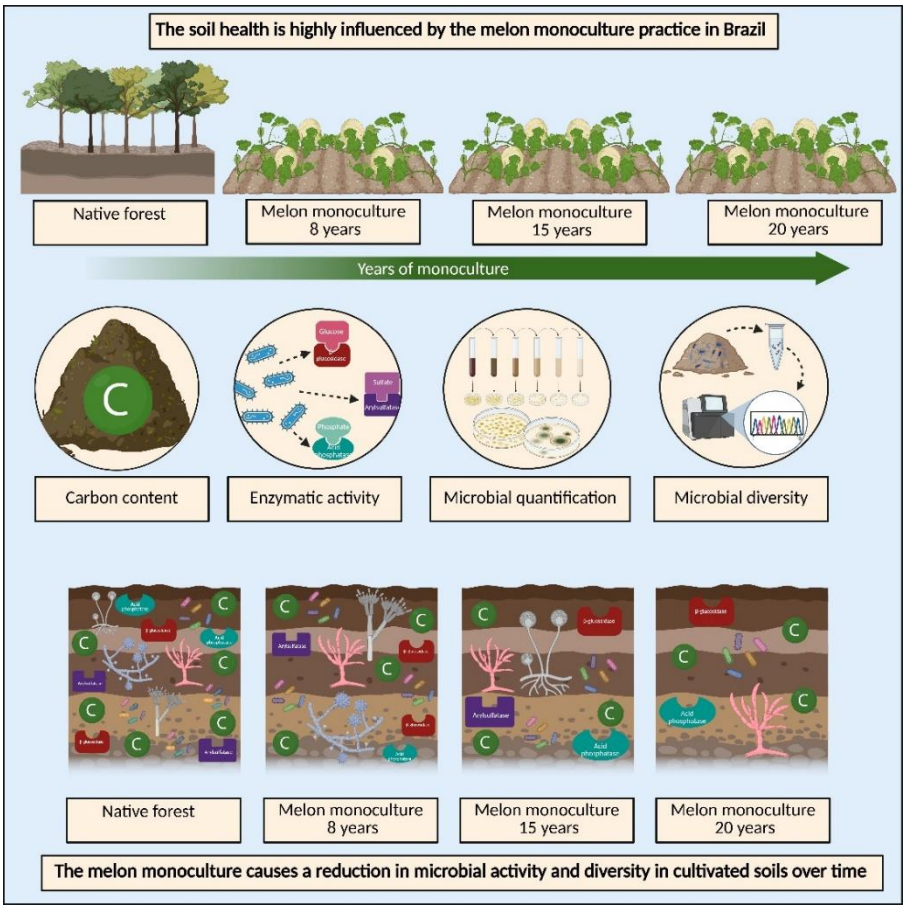
The present study evaluated microbial activity and diversity in clay and sandy soil types subjected to different periods of successive melon monoculture. Soil samples were collected from three farms in the Northeastern semi-arid region of Brazil with 8, 15, and 20 years of melon monoculture. Soil samples were also collected from native areas. To evaluate the biological quality of the soil, the enzymes arylsulfatase, β-glucosidase, and acid phosphatase, total organic carbon, labile carbon, microbial biomass carbon, quantification of total and, sporulating bacteria, and fungi were evaluated. The soil microbiome was also analyzed. The native area showed greater activity of the analyzed enzymes, for both soil textures. In clay soils, there was a reduction in the performance of these enzymes as the years of monoculture increased, with a reduction in β-glucosidase activity of more than 70% compared to native soils. The carbon input of the areas showed a similar behavior, with a decrease of 63.8% in the area with 20 years of cultivation compared to the control. Soil bacterial diversity was significantly lower in melon monoculture sites compared to native soils, as measured by 16S rRNA gene sequencing. Additionally, specific bacterial taxa exhibited altered relative abundances in response to monoculture conditions, with common taxa showing similar shifts across both soil types. Collectively, these data suggest that melon monoculture causes a reduction in carbon content, enzymatic activity, and microbial diversity in cultivated soils over time.

Keywords: Soil health, Microbiome, Organic matter.

HIGHLIGHTS:

- Melon monoculture reduces soil carbon (up to 63.8% in clay soils) and microbial biomass over time.
- Enzyme activity (β-glucosidase, phosphatase, arylsulfatase) declines with prolonged monoculture.
- Microbial diversity decreases, with sandy soils showing faster degradation than clay soils.
- Long-term monoculture increases pathogen-fighting bacteria (e.g., *Bacillus*) and fungi (e.g., *Trichoderma*).
- Soil health indicators suggest sustainable practices (e.g., organic inputs) are needed for melon farming.

GRAPHICAL ABSTRACT:



INTRODUCTION

Brazil is one of the largest food producers in the world and grows various fruits, such as papaya, orange, and mango, which are mainly exported to international markets (MAPA 2023). The Brazilian melon production surpassed 699,000 tons in 2022, produced on 27,457 hectares (IBGE, 2022). The Northeast Region of Brazil is the leading melon producer region, with the states of Rio Grande do Norte and Ceará responsible for more than 90% of all melons produced in the country (IBGE, 2022). This crop has great socioeconomic importance in the region; it generates more than 60,000 direct and indirect jobs, boosting the local economy (Santos et al., 2021).

The successful expansion of melon cultivation in this region is due mainly to its climatic conditions, such as high temperatures (averages between 25 and 35 ºC) and low rainfall (300 to 500mm/year), which favor the production of melon fruits with intensified flavor and high sugar content (Sebrae, 2020; Rocha, 2009). These conditions allow melon to be cultivated all year round, up to three production cycles per year, which facilitates the success of monoculture. In fact, some farms in the region have been producing melon in monoculture for over 20 years now (Figueirêdo et al., 2017). While fostering the production of uniform fruits to attend the international markets, this cultivation practice can have a negative environmental impact, causing soil depreciation, nutritional imbalance of plants, and loss of biodiversity (Long et al., 2018; Zhou et al., 2019; Lu et al., 2020). For instance, studies on rye plantations observed that continuous monoculture carried out for long periods caused a significant reduction in the population of organisms inhabitant in the soil (Bogužas et al., 2022). Furthermore, when evaluating the microbiota in different corn cropping systems, researchers observed that monoculture conducted for 30 and 60 years negatively affected soil microbial diversity, reducing the population of bacterial genera such as *Massilia* sp. and *Haliangium* sp. (Wolińska et al., 2022; Ujvári et al., 2020).

Soil is inhibited by a variety of life forms (*e.g*., bacteria, fungi, protozoa, nematodes, insects, and earthworms), which acts in several processes including nitrogen fixation, biogeochemical cycling, decomposition of organic matter, and degradation of molecules, these processes are essential for promoting soil health and, by extension, fostering agricultural sustainability (Sahu et al., 2019). Moreover, the decomposition and mineralization of organic matter are fundamental for soil protection and to supply nutrients for successor crops (Maluf et al., 2015). However, these processes are also directly influenced by the intrinsic properties of the soil, such as texture and granulometric composition; for example, clay soils have lower decomposition rates compared to sandy soils (Bayer et al., 2011). Nevertheless, the impact of monoculture on the diversity and composition of the soil microbial community is still poorly understood (Xia et al., 2020).

In this study, we hypothesized that intensive and continuous cultivation of melon without crop rotation in monoculture regimes changes the enzymatic activity in the soil and microbial composition. To test this hypothesis, we evaluated microbial diversity, enzymatic activity, and carbon analysis in clay and sandy soil types subjected to different periods of successive melon monoculture.

MATERIAL AND METHODS

1 Sample location and collection

Soil samples were collected from three farms in the Northeast semi-arid region of Brazil. The Apodi and Santa Júlia farms are located in the state of Rio Grande do Norte (RN), with 8 and 15 years of continuous melon monoculture, respectively. The Central farm is located in the state of Ceará (CE) in a 20-year continuous melon monoculture regime. Soil samples were also collected in native areas, free from human interference. In this regard, sandy and clayey soil

samples were collected in preserved areas at the Apodi farm, and these samples were taken as a reference for monoculture periods. Soils, clay and sandy types, were collected from all farms studied, including the native area (Fig 1).

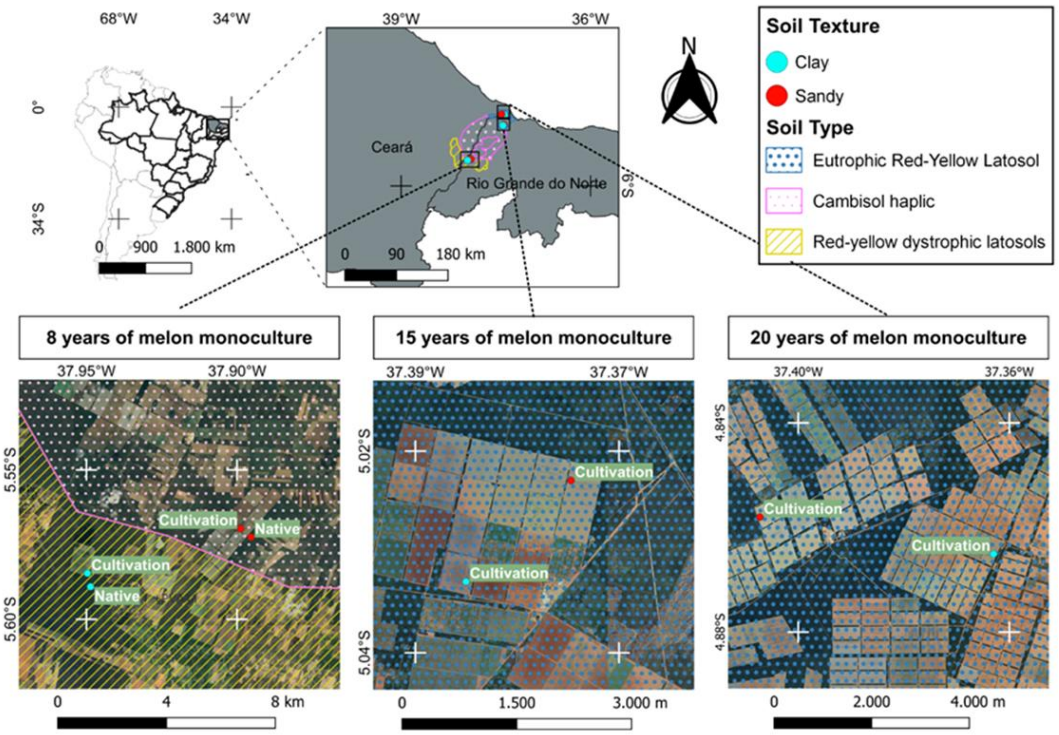


Figure 1. The geographic location of the areas where clay and sandy soils were collected with different periods of melon monoculture (8, 15, and 20 years) and native areas.

The treatments were: 1- a native area, 2- an area with 8 years of monoculture, 3- an area with 15 years of monoculture, and 4- an area with 20 years of melon monoculture. Each area analyzed was approximately 4.8 ha in size. Soil collections were carried out with a soil auger in a zigzag movement throughout the area. For this, 15 single samples were collected at a depth of 0-10 cm from the soil line. Subsequently, the single samples were mixed to obtain a composite sample weighing 1kg. Twelve composite samples (replications) were collected per

area in each soil texture. The samples were placed in transparent polyethylene bags and stored in a refrigerator at 5 ± 2 °C for downstream analysis. The experimental design used was completely randomized.

2 Crop management used in the areas analyzed

Each area analyzed presented a type of crop management according to the needs determined by the agronomists who worked in the areas (Table 1). The crop management of the areas within each farm was the same, regardless of the soil texture (sandy or clay), with only the variation in the quantities of products applied.

Table1. Management practices were adopted in areas with different periods of melon cultivation (8, 15, and 20 years) and native areas in soils with sandy and clay textures.

Areas analyzed	Management
Native area	Area completely preserved from human action, populated with forest species native to the caatinga biome, such as: *Piptadenia stipulacea*; *Mimosa tenuiflora*; *Piptadenia moniliformis*; *Caesalpinia ferrea*; *Mimosa caesalpiniifolia* and others.
8 years of monoculture	Management is carried out only with biological products, mainly based on *Trichoderma*, *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus methylotrophyus* and *Bacillus Thuringiensis*. The soil is also routinely enriched with organic compost based on cattle manure, sorghum, monoammonium phosphate (MAP) and bacteria of the Bacillus genus. Furthermore, the active ingredient oxyfluorfen is used in the area.
15 years of monoculture	In this area, biological products based on *B. subtilis*, *B. licheniformis*, *B. methylotrophyus* and *B. thuringiensis* are intensively used, due to the history of high plant mortality due to problems with soil-dwelling pathogens. In addition to biological products, the areas were also flooded, with multiplications based on bacteria of the Bacillus genus, and incorporation of green manures (*Crotalaria juncea* L. and *Pennisetum glaucum* L.) and organic compost based on cattle manure, sorghum and MAP. The active ingredients used in the area were abamectin and oxyfluorfen.
20 years of monoculture	In this area, biological products based on *Trichoderma* spp., *B. subtilis*, *B. licheniformis*, *B. methylotrophyus* and *B. thuringiensis* were used. In addition, organic compounds based on cattle manure, sorghum, MAP and bacteria of the Bacillus genus were also incorporated into the soils. The active ingredients used in the area were abamectin and oxyfluorfen.

3 Soil carbon determination and enzymatic analysis

Soil carbon assessments were carried out at the UFERSA water and soil quality laboratory, for which total organic carbon was determined (Yeomans e Bremner, 1988), labile carbon (Blair et al., 1995; Shang e Tiessen, 1997), and microbial biomass carbon (Islam and Weil, 1998; Brookes et al., 1982). According to Blair et al. (1995), the carbon stock results were used to calculate the carbon management index (BMI). This index refers to the relative measure of changes caused by soil management compared to an original or ideal soil configuration. As the original condition, the native area was used as a reference (BMI=100). The chemical characterization of the samples was also carried out (Table 2), according to EMBRAPA (1999).

Table 2. Chemical analysis of sandy and clay soil types collected in different continuous melon monoculture areas (8, 15, and 20 years old) and native areas.

	Clay soil								
AREA	**Ca cmol/dm³**	**Mg cmol/dm³**	**K cmol/dm³**	**Na cmol/dm³**	**P mg/dm³**	**SO_4 mg/dm³**	**MO g/dm³**	**CTC cmol/dm³**	**pH**
Native area	5.75	1.93	0.62	0.02	7.78	10.84	25.15	11.72	5.8
8 years of monoculture	6.57	1.99	0.87	0.04	88.81	112.96	14.58	11.94	5.5
15 years of monoculture	5.13	1.66	0.51	0.30	115.43	14.78	10.24	10.14	6.1
20 years of monoculture	3.37	0.92	0.18	0.05	118.31	3.12	6.01	6.51	6.1
	Sandy soil								
Native area	4.27	1.31	0.51	0.01	7.78	7.06	12.02	8.46	6.3
8 years of monoculture	2.26	1.27	0.49	0.02	47.09	51.88	5.09	6.43	5.2
15 years of monoculture	3.78	0.98	0.26	0.11	152.38	3.61	6.90	7.27	5.5
20 years of monoculture	6.56	1.00	0.15	0.07	332.23	39.57	5.90	9.81	5.7

Legend: Ca: Calcium; MG: Magnesium; K: Potassium; Na: Sodium; P: Phosphorus in Mehlich 1; SO_4: Sulphur; MO: Organic Matter; CTC pH7.0: Cation exchange capacity; pH: Hydrogen ion potential in water.

Enzymatic analyses were conducted at the Laboratório de Microbiologia e Fitopatologia of the Universidade Federal Rural do Semi-Árido (UFERSA), Brazil. The activities of the arylsulfatase, β-glucosidase, and acid phosphatase were determined (Tabatabai, 1994). This analysis is based on the determination of p-nitrophenol formed after adding specific substrates for each enzyme. To quantify the activities of β-glucosidase, arylsulfatase, and acid phosphatase, the substrates p-nitrophenyl-β- d -glucopyranoside (PNG), p-nitrophenyl sulfate (PNS), and p-nitrophenyl disodium phosphate (P-NPP) were added, respectively. These analyses were carried out directly on air-dried soil samples, and the enzyme activity was expressed in μg p-nitrophenol g^{-1} soil h^{-1}.

4 Quantification of total bacteria, sporulating bacteria, and total fungi present in soil samples

The analyses were carried out at the Microbiology and Phytopathology Laboratory at UFERSA. For the quantification of bacteria, the nutrient agar (NA) culture medium and potato dextrose agar (BDA) were used for the quantification of total fungi. Initially, the samples were serially diluted in 9 mL of water (distilled and sterilized), then plated in the respective culture media, using three plates per dilution. After plating for quantification of total bacteria and fungi, the diluted samples were taken to a water bath at 80 ºC for 20 minutes to analyze sporulating bacteria. After plating the diluted samples, the plates were kept in a BOD-type incubator at 28 ± 2 °C. The colony-forming unit (CFU) count on the plates was carried out when the colonies were wholly formed, and the results were expressed in CFU/g of soil (Borges et al., 2023).

5 Soil microbiome analyses

Soil microbiome analysis was conducted at the Department of Environmental Science

and Forestry, The Connecticut Agricultural Experiment Station (CAES), in New Haven, United States.

5.1 DNA extraction and amplification of 16S rRNA genes

DNA extraction from the soil samples was carried out using the ZymoBiomics DNA Miniprep kit (Zymo Research, Irvine, CA, USA). Initially, 200 mg of soil samples were weighed and transferred to extraction tubes. 750 µL of DNA/RNA Shield™ was added to the soil samples to preserve and stabilize the nucleic acids. Subsequently, DNA was extracted from soil samples using the ZymoBIOMICS™ DNA Miniprep Kit with standard procedures. DNA was quantified in a Nanodrop 2000 (Thermo Fisher Scientific, Waltham, MA), and the quality was verified in 1% agarose gel electrophoresis.

Bacterial 16S rRNA genes were amplified with the primer pair 515F (GTGYCAGCMGCCGCGGTAA) and 806R (GGACTACNVGGGTWTCTAAT) with dual indexing of the Illumina I5 and I7 tags. The DNA samples were amplified with 10 µL Platinum SuperFi II DNA polymerase (Invitrogen), which also included 7.5 µM of mPNA and pPNA peptide nucleic acid (PNA) clamps (mPNA, GGCAAGTGTTCTTCGGA; pPNA, GGCTCAACCCTGGACAG) to block the amplification of host plant mitochondria and plastid rRNA genes, respectively (Lundberg et al., 2013; Steven et al., 2018). PCR conditions consisted of 94 °C for 2 min followed by 30 cycles of 94 °C for 15 s, 60 °C for 15 s, 68 °C for 15 s, and 4 °C for an infinite hold. The resulting amplification products were verified by gel electrophoresis, and cleaning and normalization of individual PCR products were performed with a SequalPrep normalization plate (Invitrogen, Waltham, MA). The normalized PCR amplicons were mixed, and the quantity and quality of the DNA pool were verified using an Agilent TapeStation. The resulting 16S rRNA gene amplicons were sequenced on the Illumina MiSeq 100 system, employing 250 base pair chemistry at the Yale Center for Genome Analysis

(Yale University, New Haven, CT, USA).

5.2 Sequence analysis

Sequencing reads were assembled into contigs and quality screened by using mothur v1.39.5 (Schloss et al., 2009). Sequences that were at least 253 bp in length (contained no ambiguous bases and no homopolymers of more than 8 bp) were used in the analysis. Chimeric sequences were identified by using VSEARCH as implemented in mothur (Rognes et al., 2016), and all potentially chimeric sequences were removed. The 16S rRNA sequences were classified against the SILVA v138 reference database (Quast et al., 2013) using the RDP naive Bayesian classifier as implemented in mothur, and sequences identified as belonging to eukaryotes or that were unclassified were removed. The resulting sets of sequences were assigned to amplicon sequence variants (ASVs), employing a 100% sequence similarity threshold. Negative control samples did show some sequence recovery but were in the range of 32-5291 sequences, or less than 1% of sequences recovered in the sample libraries. The ASV count data was imported into the phyloseq package for further analysis (McMurdie et al., 2013). Potentially contaminating ASVs were removed with the decontam R package using the prevalence model (Davis et al., 2018).

To account for variation in sequencing depth, libraries were randomly rarified without replacement to the size of the smallest sample (18,856 sequences). Alpha diversity metrics were calculated using 100 iterations of subsampling, and the reported values represent the average across these iterations. Similarly, beta diversity statistics were calculated on rarefied datasets with 100 iterations using the average Bray-Curtis distance between samples using the metagMisc R package. The differential abundance of bacterial genera across farm management practices was assessed using the ALDEx2 package in R (Quinn et al., 2019). Prior to analysis, genus-level read counts were transformed using a centered log-ratio (CLR) transformation to

account for the compositional nature of the data. A generalized linear model (GLM) was then used to identify genera exhibiting statistically significant differences in relative abundance among the various farm management practices, using the native soils as a reference level.

6 Statistical analysis

Analysis of variance (ANOVA) was performed on normally distributed data sets, and the Tukey test was used at a 5% probability level to compare treatment means. The Kruskal-Wallis test was used on non-normally distributed data sets, and the means were compared using the Bonferroni Test in R (R Core Team, 2024).

RESULTS

Soil organic carbon, labile carbon and carbon management index

Total organic carbon in areas with clay texture showed a reduction in its averages as the years of monoculture increased. The maximum total organic carbon in the soil (22.4 g kg^{-1}) was observed in the native area. Areas with 8, 15, and 20 years of melon monoculture showed reductions of -33.4, -50.4, and -63.8%, respectively, when compared to the native area. The value of labile carbon reduced as the years of monoculture passed. The native area showed no difference when compared to the area with 8 years of monoculture, but it differed from the other areas. The 20-year-old area showed a reduction of 48.0% in comparison to the native area (Fig 2).

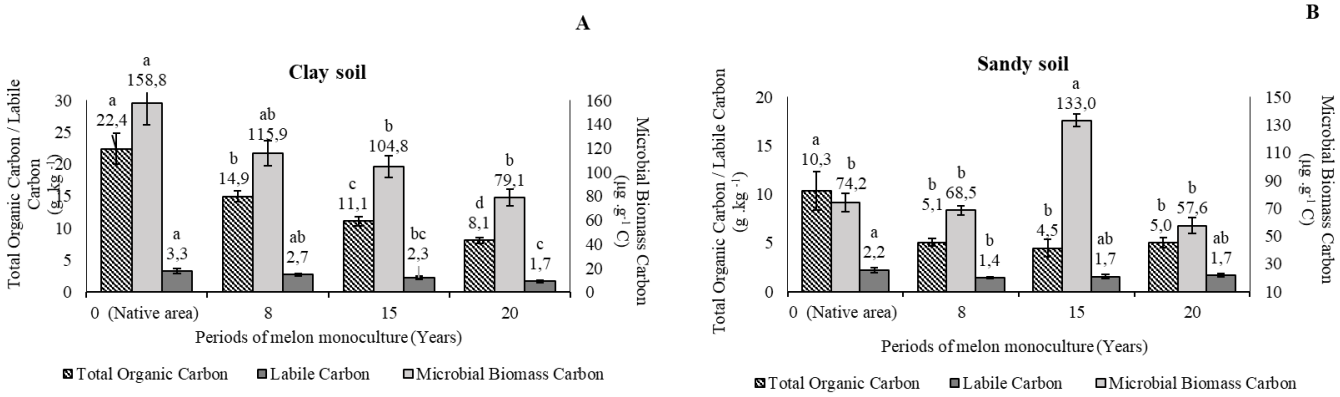


Figure 2. Total organic carbon, labile carbon, and microbial biomass carbon in clay (A) and sandy (B) soils, with different periods of melon monoculture. The means followed by the same lowercase letters within each variable do not differ from each other using the Tukey test at the 5% probability level.

The activity of biomass carbon is reduced as the monoculture usage period increases. This activity in the native was not statistically different from the 8-year monoculture regime; however, it differed statistically from the other monoculture periods. The areas with 15 and 20 years of melon monoculture cultivation showed a 34 and 50% reduction, respectively, in comparison to the native area (Fig 2A).

In areas with a sandy texture, the total carbon input was higher in the native area (10.3 g/kg) than in monoculture areas. Among the cultivated areas, the averages varied between 4.5 g/kg (15 years of monoculture) and 5.1 g/kg (8 years of monoculture), showing no statistical difference between them. In labile carbon, the native area presented averages of 2.2 g/kg, which is similar to the averages presented in areas with 15 and 20 years of melon monoculture, with the area with 8 years of monoculture having lower carbon lability, a contribution of 36.3% lower than the native area (Fig 2B). For microbial biomass carbon in sandy soils, the best results were obtained in the area with 15 years of melon monoculture (133 µg g of C). The native area and the areas with other periods of monoculture did not show any statistical difference between them (Fig 2B).

The carbon management index (BMI) in clay soils reduced significantly as the years of melon monoculture increased. The native area is considered the reference, with 100% of the carbon stock. In contrast, the areas with 8, 15, and 20 years of monoculture showed reductions of approximately 17.6, 32.4, and 49.8% in the BMI when compared to the native area (Fig 3A).

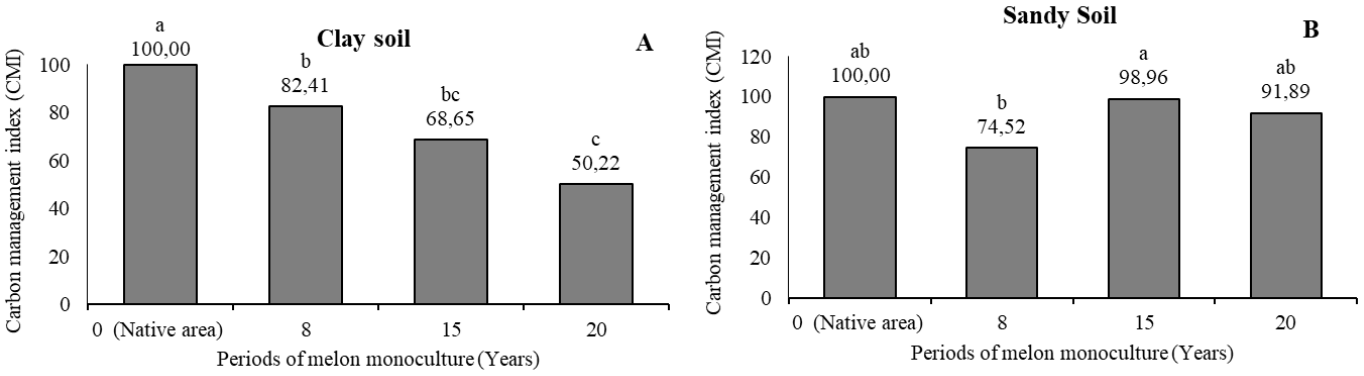


Figure 3. Average carbon management index in clay (A) and sandy (B) soils, with different periods of melon monoculture. Averages followed by the same lowercase letters do not differ from each other using Tukey test at the 5% probability level.

For soils with a sandy texture, areas with 15 and 20 years of melon monoculture showed no statistical difference, with reductions of 1.1 and 8.1%, respectively, when compared to the native area. The area with 8 years of monoculture had a lower BMI, with a reduction of approximately 25% compared to the native area; however, the area with 8 years did not differ from the area with 20 years (Fig 3B).

Soil enzyme activitie

In clay soil, the native area showed higher β-glucosidase enzyme activity, reaching 496 μg p-nitrophenol g^{-1} soil h^{-1}, compared to the other areas (8, 15, and 20 years of monoculture regime) with the same soil texture. The areas cultivated with melon showed a reduction in the

performance of this enzyme as the years of monoculture cultivation increased. For example, in areas with 8 years of cultivation, this enzyme presented a value of 215 µg p-nitrophenol g^{-1} soil h^{-1}, showing a 57% reduction in its activity compared to the native area. After 15 and 20 years of melon monoculture, the average enzymatic activity was similar, with 145 and 144 µg p-nitrophenol g^{-1} soil h^{-1}, with a reduction of 70.7 and 70.9%, respectively, in comparison to the native area (Fig 4A).

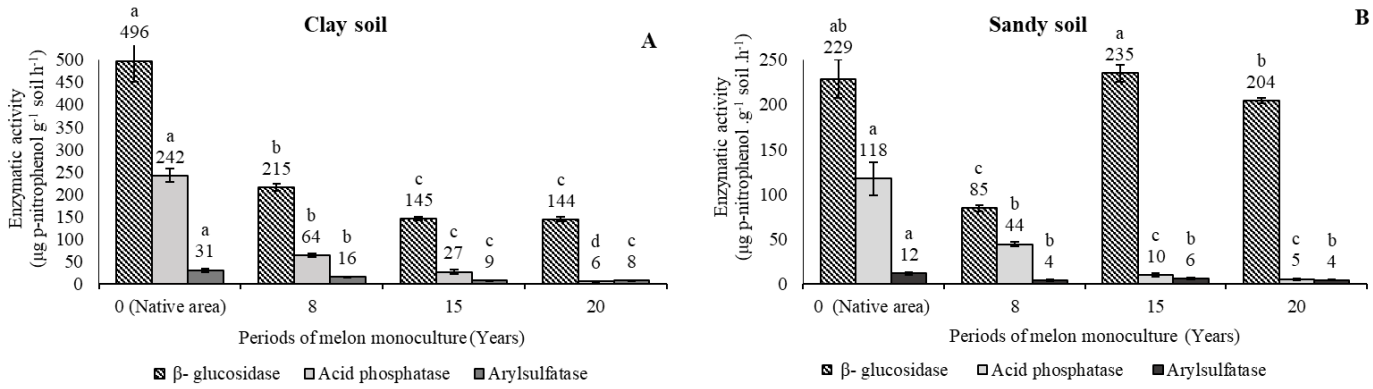


Figure 4. Activity of the enzymes β-glucosidase, acid phosphatase, and arylsulfatase in clayey (A) and sandy (B) soils, with different periods of melon monoculture. The means followed by the same lowercase letters within each variable do not differ from each other using the Tukey test at the 5% probability level.

The activity of the acid phosphatase enzyme was similar to that of β-glucosidase; that is, the native area showed better performance than the other treatments, with an activity of 242 µg p-nitrophenol g^{-1} soil h^{-1}. Over the years of monoculture regime, acid phosphatase activity was reduced in cultivated areas, with 64, 27, and 6 µg p-nitrophenol g^{-1} soil h^{-1}, for areas with 8, 15, and 20 years of cultivation, respectively. When comparing the area with the most extended period of monoculture (20 years) with the native area, a 97.4% reduction in the activity of this enzyme is observed in clay soils (Fig 4A). The native area showed lower activity of the

arylsulfatase enzyme in comparison to the other enzymes evaluated in the present study; however, when compared to the cultivated areas, the native area showed higher performance (31 μg p-nitrophenol g-1 soil h-1 after 8 years of monoculture). Areas aged 8 years of clay soils cultivated in melon monoculture showed a 47.2% reduction in arylsulfatase activity when compared to the native area. Whereas in areas aged 15 and 20 years, the reduction was 70.7 and 70.9%, respectively (Fig 4A).

In sandy soils, the activity of the β-glucosidase enzyme in the native area did not differ statistically from the area with melon monoculture for 15 and 20 years, presenting an activity of 229, 235, and 204 μg p-nitrophenol g^{-1} soil h^{-1}, respectively. However, the area with 20 years of monoculture showed a 10.7% reduction in β-glucosidase activity in relation to the native area. The area with 8 years of melon monoculture was the one with the lowest activity of this enzyme, with 85 μg p-nitrophenol g^{-1} soil h^{-1}, 63% lower than the native area (Fig 4B).

As for the other enzymes, acid phosphatase showed greater activity in the native area (118 μg p-nitrophenol g^{-1} soil h^{-1}) than in cultivated areas. In the area of 8 years of melon monoculture, there was a reduction in the activity of this enzyme of 62.1%, reaching a value of 44 μg p-nitrophenol g^{-1} soil h^{-1}. After 15 and 20 years of monoculture, the loss in acid phosphatase activity was quite pronounced, reaching 91.5% and 95.3%, respectively, in comparison to the native area. The activity of Arylsulfatase in all treatments was lower in relation to the other enzymes analyzed in both types of soil. However, in sandy soil, in a native area, this activity was 12 μg p-nitrophenol g^{-1} soil h^{-1}. For areas cultivated with 8, 15, and 20 years of monoculture, there was no statistical difference, presenting averages of 4, 6, and 4 μg p-nitrophenol g^{-1} soil h^{-1}, with a reduction of 63.9, 50.5 and 62.7%, respectively, in comparison to the native area (Fig 4B).

Total bacteria, sporulating bacteria, and total fungi

For the total culturable bacteria present in clay soils, the area with 20 years of monoculture regime showed no difference from the native area. The areas with 8 and 15 years also showed no difference between them, but they presented lower averages when compared to the native area (Fig 5A). For sporulating bacteria, the area with 20 years of monoculture regime showed higher results, with an average of 1.66 x 10^6 CFU/g of soil. The native area showed similar trends to that of the 15-year-old area, with averages of 4.98 x10^4 and 5.45 x 10^4 CFU/g of soil. As for the total fungi, the 8-year-old area exhibited the highest population of these microorganisms in the soil, followed by the 20-year-old area. The lowest averages were observed in the native areas and with 15 years of melon monoculture regime, which showed no difference (Fig 5A).

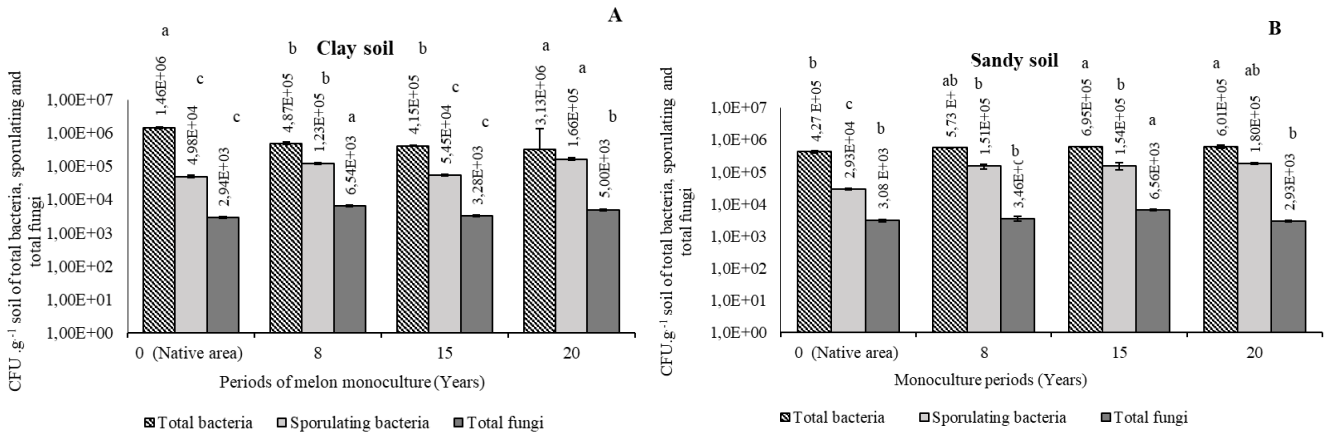


Figure 5. Total bacteria, sporulating bacteria, and total fungi in clay (A) and sandy (B) soils, with different periods of melon monoculture. Averages followed by the same lowercase letters do not differ from each other using Tukey test at the 5% probability level.

In sandy soils, areas with 8 and 20 years of monoculture regime had higher amounts of total bacteria, with averages of approximately 5.73 x 10^5 and 6.0 x 10^5 CFU/g of soil, respectively, followed by the native area, which did not differ from the area with 8 years of

melon cultivation. The area with 20 years of monoculture stood out, considering the sporulating bacteria, followed by 8 and 15 years of monoculture regime and the native area, respectively. The 15-year-old area also had a higher population of total fungi, with 6.56x 10^3 CFU/g of soil. The other areas had no statistical difference between them (Fig 5B).

3.4 Melon monoculture changed the soil microbiome community richness and composition

16S rRNA gene sequencing was employed to characterize bacterial diversity. Through this effort, we recovered 6,731,141 sequences that clustered into 981,806 ASVs. The estimated library coverage ranged from 59.5% to 91.8%, indicating that a substantial portion of the expected diversity was captured with this effort.

Monoculture consistently reduced community richness (observed ASVs and Shannon diversity index), with a soil-type dependent response. Clay soils exhibited reduced diversity only after 15 years, whereas sandy soils showed a significant decrease after 8 years (Fig 6A-B).

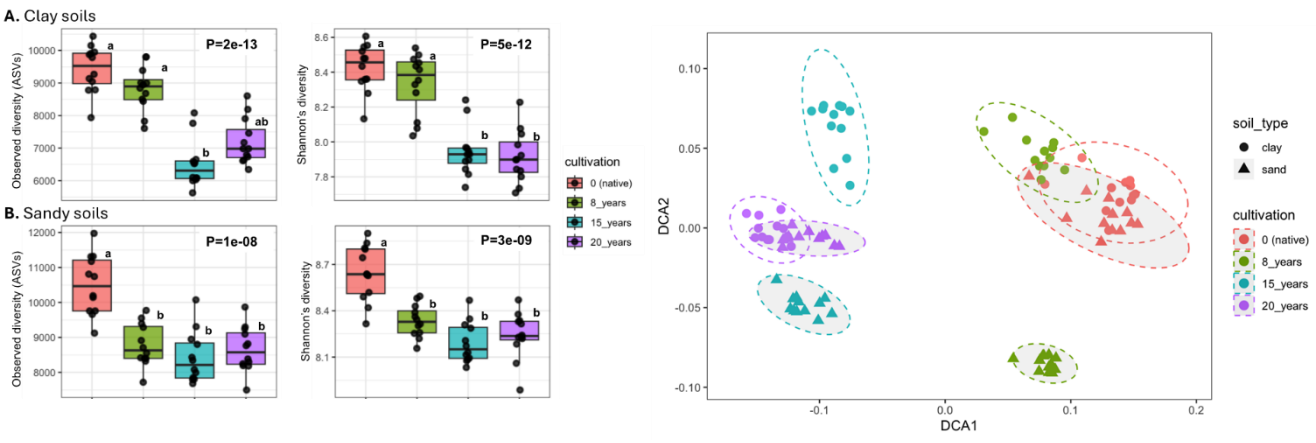
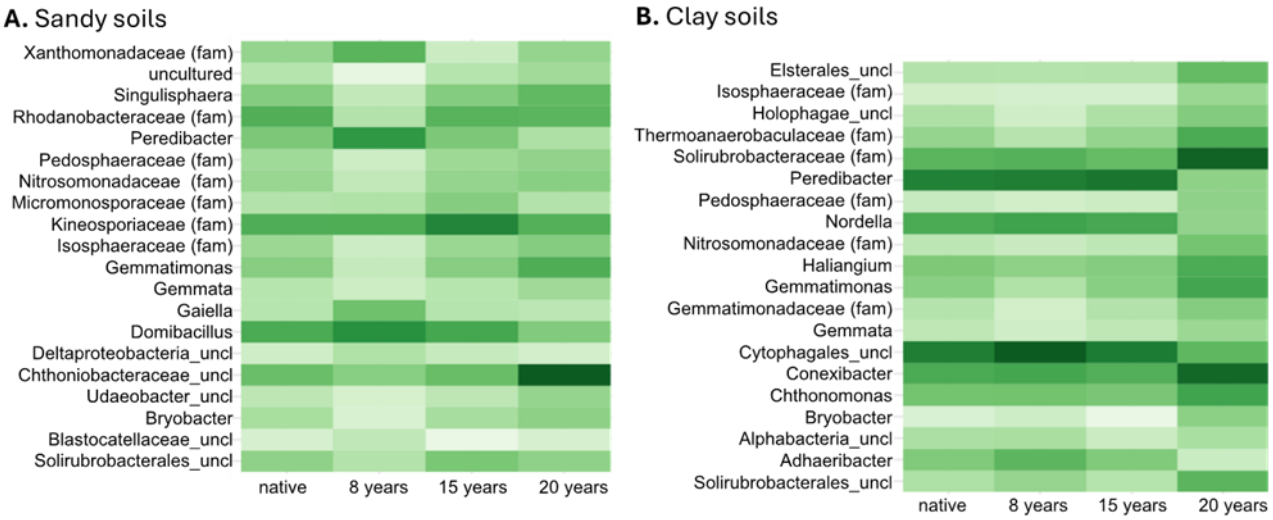


Figure 6. Bacterial diversity. (A) Alpha diversity associated with soil type. P-values are from a Student t-test. (B) Alpha diversity associated with location. Letters indicate significant differences ($p < 0.05$) based on a one-way ANOVA with a post hoc Tukey-Cramer test. (C)

Detrended Correspondence Analysis (DCA) ordination of samples based on Bray-Curtis distances.

A similar pattern was observed in beta diversity. Detrended correspondence analysis (DCA) revealed that 15 and 20-year monoculture sites clustered distinctly from native samples, as well as 8-year monoculture sites (Fig 6C). Consistent with alpha diversity, clay soils remained more similar to native soils than sandy soils. These findings suggest that adaptations to monoculture occur more rapidly in sandy soils.

16S rRNA genes were classified to the genus level to assess the influence of farm management on microbial community composition, focusing on identifying differentially abundant genera that distinguished native soils from monoculture soils. Monoculture induced significant changes in the abundance of 267 genera in sandy soils and 222 genera in clay soils. A substantial overlap of 144 (54%) genera was observed in the taxa identified as responsive to monoculture in both soil types. Fig 7 displays the 20 genera exhibiting the largest average effect



sizes of differential abundance for each soil type. Notably, 9 of these genera were common to both soil types, indicating there was also a conservation of highly responsive taxa. These shared genera, such as *Bryobacter* (Acidobacteriota), *Gemmata* (Planctomycetota), and *Peredibacter* (Pseudomonadota), highlight the taxonomic breadth of microbial responses to melon monoculture (Fig 7).

Figure 7. Differentially abundant genera due to farm management and monoculture conditions. (A) Heatmap depicting the relative abundance of bacterial genera across different farm management practices. Each column represents a sampling location, and each row represents a bacterial genus exhibiting significant differential abundance with the native set as a reference. The color scale indicates relative abundance, and values are row-scaled (z-scored), where 0 represents the mean relative abundance for each ASV across all samples, and 1 and -1 represent one standard deviation above and below the mean, respectively. Columns (samples) were clustered using hierarchical clustering with complete linkage based on Euclidean distances calculated on the row-scaled data to group samples with similar microbial communities. (B) This figure displays the 10 genera exhibiting the largest positive (enriched) and negative (depleted) average effect sizes in abundance when comparing managed soils to native soil samples (reference). Each point represents a genus-level taxonomic bin that was identified as significantly different. For bins that could not be classified to a named genus, the deepest identifiable taxonomic level is indicated (fam=Family, ord=Order, and cla=Class).

DISCUSSION

Melon was chosen for this study because it is a crop that presents a high disturbance cultivation system due to the agricultural practices used throughout the crop cycle. In the Northeast Region of Brazil, melon cultivation is conducted in up to three cycles per year with intense practices, such as soil preparation, application of fertilizers and chemical pesticides, use of plastic covering (mulching), and other cultural practices. Strong pieces of evidence show that these practices contribute significantly to the reduction of carbon in the soil, leading to degradation and loss of nutrients, impacting the general health of the soil (Norris E Congreves, 2018). Soil health is an essential factor for maintaining and/or increasing agricultural productivity. Any changes to the chemical, physical, biochemical, and biological indicators of soil health may interfere with crop production and yield (Wang et al., 2012). Such changes can

be induced by crop management, such as monoculture, or simply when native areas are transformed into agricultural cultivation areas (Quesada et al., 2009).

To the best of our knowledge, this is the first study designed to evaluate the biological qualities of soils cultivated with melon in a continuous monoculture regime. However, some studies have highlighted the problems in the activities of soil microorganisms caused by the replacement of native fields to monoculture areas, such as studies with pasture, sugarcane, and grapevine (Sarto et al., 2020; Liu et al., 2021; Tayyab et al., 2021). Furthermore, Saviozzi et al. (2001) showed that areas cultivated with corn for over 40 years reduced enzymatic activity compared to the native area. A literature review by Peng et al. (2021) highlighted that replacing native forests with crops reduces soil enzymatic activity. The authors hypothesized that this is probably due to the lower carbon input in cultivated areas, as a large part of the biomass is reduced with monoculture.

For most of the soil quality parameters evaluated in this study, the soils of native areas were superior compared to others. This was evidenced by the activities of the enzymes β-glucosidase, acid phosphatase, and arylsulfatase, in which a reduction in the activities of these enzymes was observed as the melon was grown in monoculture over time in both types of soil. These enzymes are indicators of the activity of soil microorganisms, for example, β-glucosidase acts in the C cycle, making glucose available, which is a source of energy for microorganisms (Tabatabai, 1994; Acosta-Martínez et al., 2008). Phosphatase activity in the soil indicates the action of microorganisms in the phosphorus mineralization process, releasing phosphate into the soil in a form that can be absorbed by plants (Moreira and Siqueira, 2006). Thus, when greater activity of these enzymes is observed in the soil, greater activity of the microorganisms is confirmed, as reported by Saviozzi et al. (2001). These authors observed when evaluating several soil enzymes, including β-glucosidase and phosphatase, that as these increased, there was also an increase in other indicators of microbial activity, such as biomass respiration and

microbial biomass carbon.

The sulfur mineralization process is mediated by the enzyme arylsulfatase, which is the main enzyme participating in the cycle. It acts through the hydrolysis of sulfate ester bonds, releasing sulfate ions in free form. Part of the released sulfate will be available in the soil solution and absorbed by plant roots (Tabatabai, 1994). In the present study, the highest activity of this enzyme was observed in the native area, for both soil textures; however, the cultivated areas showed low arylsulfatase activity, indicating lower sulfur availability in the soil.

In addition to enzymatic activity, it is essential to evaluate the dynamics of soil organic carbon, such as total organic carbon and primarily microbial biomass carbon (Santos et al., 2022), as this element is directly related to the activity of microorganisms. In soil, nutrient cycling favors the development of plants as well as plant health. Herein, we found that greater amounts of carbon were observed in native forest areas, both in the form of total organic carbon, labile carbon, and microbial biomass than in monoculture melon fields. With the change in land use from native areas to melon monoculture over a period of up to 20 years, there is a drastic reduction in carbon input in all forms studied.

Other researchers also observed a reduction in carbon in the soil when they replaced native areas with crops. Sarto et al. (2020) discovered that areas cultivated with corn and marandú grass (*Urochloa brizantha* (Hochst. Ex A. Rico) had lower total organic carbon and microbial biomass content when compared to the native area, corroborating our results. Strong pieces of evidence show a reduction in soil organic carbon in areas cultivated with pasture compared to the native area (Dalal et al., 2021), this trend gets exacerbated with the adoption of monoculture (Saviose et al., 2001). This practice severely results in the loss of biodiversity, affecting the structure and fertility of the soil, as the same plant is cultivated repeatedly, without crop rotation, leading to soil compaction, increased erosion, and reduced water retention capacity; in the long term, these changes compromise agricultural productivity and the

sustainability of the local ecosystem (Belete and Yadete, 2023). We found that melon monoculture over long periods reduced soil carbon content in all forms studied. This is a bad sign since organic carbon serves as a source of nutrients for soil microorganisms, benefiting biological activity (Maluf et al., 2015). Furthermore, soil carbon storage acts as an effective climate change mitigation mechanism by sequestering carbon in the form of organic matter; soils help prevent the release of carbon dioxide (CO2) into the atmosphere, contributing to the reduction of the greenhouse effect (Carvalho et al., 2010). In addition to organic matter, microorganisms also play a fundamental role in soil quality, as it is a complex microbiome that contains thousands of bacterial and fungal strains, which coexist by exploiting a variety of organic carbon sources (Fierer, 2017). The scarcity of this element is found mainly in agricultural soils (Bonanomi et al., 2018).

In this study we found that in clay soils, monoculture caused damage to microbial biomass, causing a reduction in the release of carbon by microorganisms as the years of monoculture progressed. The activity of microorganisms is directly related to the availability of carbon in the soil, therefore, as the total supply of carbon is reduced in the soil, there is also a reduction in the carbon of the microbial biomass, as found in this study. Therefore, adopting practices that aim to improve soil health with organic matter is a knowledge recognized by many who practice sustainable agriculture worldwide. However, for others, this approach still represents a promising avenue to ensure the functionality and health of the agricultural system in the future (Monther et al., 2020).

The increase in the population of total and sporulating bacteria in the oldest area (20 years of monoculture) observed in the present study is likely due to the constant application of biological products in the soil studied. Because of the increasing problems caused by soil-dwelling pathogens in melon plants cultivated in areas with longer periods of melon monoculture, farmers in the region frequently use biological products to foster soil health.

These products are mainly composed of bacteria from the *Bacillus* genus, and this justifies the greater quantity of total and sporulating bacteria found in cultivated soils compared to those in native areas. It is important to highlight that in sandy soils, there is less availability of organic matter and, consequently, lower conditions of activity and survival of microorganisms. Therefore, the application of microorganisms in the soil, as well as practices that favor their multiplication, must be constant to avoid the reduction of microorganism populations in the soil. The practice of using microorganisms in the soil is common in melon monoculture areas in Brazil.

In this study, the significantly higher quantities of fungi observed in monoculture areas are likely due to the increase in root diseases caused by soil-borne fungi, such as *Fusarium* spp., *Macrophomina phaseolina*, *Rhizoctonia solani*, and *Monosporascus cannoballus* (Medeiros et al., 2015; Dantas et al., 2013; Stewart et., 2015). Furthermore, this high disease pressure leads to the intense use of *Trichoderma* spp. in the soil, where application occurs weekly in most melon monoculture areas in the Northeast Region of Brazil.

The observed decline in soil metrics, such as enzyme activity and soil carbon stocks, paralleled a reduction in bacterial diversity under monoculture. This suggests that alterations in the soil bacterial community may be driving the observed changes in soil quality. Importantly, the identification of a conserved set of bacterial taxa responding to monoculture indicates predictable community responses. This predictability provides a valuable opportunity to develop targeted cultivation practices that promote the resilience of microbial populations most vulnerable to monoculture. Furthermore, it is noteworthy to point out that all the variables analyzed guided us to the same conclusion - the longer the melon monoculture period, the lower the microbial activity in the soil. These findings suggest that it is possible to evaluate microbial indicators of soil health using less costly techniques, such as measuring microbial biomass carbon, soil enzyme activity, and quantifying cultivable microorganisms.

FINAL CONSIDERATIONS

Although monoculture is widespread in melon production areas in Brazil and generates vital jobs for the local community, it reduces the activity of microorganisms that are important for soil health, challenging the sustainability of this system over time. Our results indicate that if growers continue to use monoculture for long periods of time, it is necessary to adopt practices that favor the activity of these microorganisms to maintain the sustainability of this cultivation system. Ideally, such practices need to foster the increase of organic matter in the soil to favor the beneficial soil-dwelling microorganisms.

Acknowledgments:

This work was supported through funding from the National Council for the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) Grant/Grant Numbers 307860/2022–1 and 140602/2021-6. This research was partially funded by the USDA Specialty Crop Block Grant Program (SCBGP) – Grant Award #23DAG0087AA and the USDA-NIFA Hatch Grant – Project #1017940. The company Alltech Crop Science for the support, and donation of equipment and reagents to carry out the research, through technical cooperation agreement number 05/2022, process: 23091.009382/2022-40. The authors also thank Fazenda Agrícola Famosa for providing some data needed for this research.

Credit authorship contribution statement:

TRCA: Investigation, Data curation, Writing – original draft. **APM:** Investigation, Writing – review & editing. **JLSS:** Investigation, Data Analysis, Writing – review & editing. **IVPS:** Investigation. **FÉRO:** Investigation. **JLR:** Investigation, Data curation. **EFS:** Data curation, Supervision. **JSSL:** Data curation, Formal analysis. **BS:** Investigation, Data curation, Data Analysis, Formal analysis, Writing – review & editing. **WLS:** Investigation, Funding

acquisition, Supervision, Writing – review & editing. **MMQ:** Investigation, Funding acquisition, Supervision, Conceptualization, Writing – original draft, review & editing.

Funding sources:

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of Competing Interest:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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ARTICLE II

POTENTIAL OF COMBINED USE OF BACTERIA AND METABOLITES IN REDUCTION OF ROOT ROT IN MELON AND INCREASING SOIL ENZYMATIC ACTIVITY

ABSTRACT

The present study aims to assess the effect of formulations based on bacteria, *Trichoderma,* and metabolites in the management of soil-borne pathogens of melon plants. A pilot greenhouse study was conducted, and the treatments that provided the lowest disease severity underthose conditions were tested in the field. The experiment was carried out in areas with a history of diseases caused by soil-borne pathogens, with a randomized block experimental design, with six treatments and seven replicates: T1 = (*Bacillus subtilis* and *B. licheniformis* + Metabolites 1), T2 = (*B. subtilis*, *Lactobacillus plantarum* and *Enterococcus faecium* + Metabolites 2), T3 = (Metabolites 1 + metabolites 2), T4 = (*B. subtilis*, *B. licheniformis* + *L. plantarum* and *E. faecium* + Metabolites 1 + Metabolites 2), T5 = standard management (*T. asperelum*, *B. subtilis*, metabolites 1 and metabolites 2), and T6 = Control. The following parameters were evaluated: plant stem diameter, shoot dry mass, disease severity, number of fruits per plant, fruit weight and ºbrix, and soil enzymes. Treatments 2 and 3 of the first experiment, and treatments 1 and 4 of the second experiment demonstrated better performance in reducing disease severity, with reductions of 50% compared to the control. For this parameter, none of the treatments showed great prominence; however, treatment 4 showed promising signs, such as greater activity of soil enzymes, a greater number of fruits per plant, and was one of those that provided the greatest reduction in the severity of the disease caused by soil-borne pathogens in melon.

Keywords: Soil-borne pathogens; Biological control; Plant disease; *Cucurbitaceae*.

Declarations

Funding

This work was supported through funding from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) Grant/Award Numbers 307860/2022–1 and 140602/2021-6. This research was partially funded by the USDA Specialty Crop Block Grant Program (SCBGP) – Grant Award #23DAG0087AA and the USDA-NIFA Hatch Grant – Project #1017940. The authors thank Fazenda Agricola Famosa for providing some of the data for this work.

Informed consent

All authors consented to the publication of this study.

Conflicts of interest

The authors declare no conflicts of interest.

Ethics approval

The article does not contain studies with human participants or animals performed by any of the authors, and complies with the Ethical Rules applicable to the 'Journal of Plant Pathology'.

Data availability

The analyzed data that support the conclusions of this study are available in the article.

Authors contributions

Conducted the experiments: [T.R.C.A], [A.P. M], [L.F.B.E], [D.C.C.A], [S.M.S.F], [V.M.G.S], [J.C.F], and [V.M.A.F], Coordinated the research project: [M. M. Q. A], [W.L.S], and [T.R.C. A]. Wrote the paper: [T.R.C.A], [J. L. S. S], [W.L.S], and [M. M. Q. A]. Performed the statistical analysis: [J.L.S.S]. Approved the final version of the manuscript: [M. M. Q. A], and [W.L.S]. All authors have read and approved the manuscript.

Acknowledgments

This work was supported through funding from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) Grant/Award Numbers 307860/2022–1 and 140602/2021-6. This research was partially funded by the USDA Specialty Crop Block Grant Program (SCBGP) – Grant Award #23DAG0087AA and the USDA-NIFA Hatch Grant – Project #1017940. The authors thank Fazenda Agricola Famosa for disponible the farm for the experiment. The authors thank the Alltech Crop Science the donation of equipments for the analysis.

INTRODUCTION

Brazil stands out in the international trade of many fruits, and melon is one of the main crops produced in the country. The brazilian production of this fruit crop in 2023 was 862,000 tons, of which 167,000 tons were destined for export (HF Brasil 2024). The Northeast Region is the main producer in the country, contributing to more than 90% of the national production, and the state of Rio Grande do Norte holds more than 70% of this total. (IBGE 2025; HF Brasil 2024). The edaphoclimatic conditions propitious for melon production, such as high temperatures (25 to 35 ºC), low rainfall (300 to 500 mm/year), and high levels of solar radiation, are responsible for the expansion of this crop in the region, and for the excellent quality of the fruits (Rocha 2009; Figueiredo et al. 2017). However, one of the main problems that make melon production challenging is root system diseases caused by soil-borne pathogens (SBP). Environmental conditions favorable to phytopathogens and the continuous use of monoculture by melon growers directly influence the increase of SBP populations, increasing the severity of diseases and force farmers to cease production in highly affected areas (Medeiros et al. 2015).

The fungi *Rhizoctonia solani*, *Monosporascus cannoballus, Fusarium* spp., and *Macrophomina phaseolina* are among the main SBP that attack melon plants (Medeiros et al. 2015; Dantas et al. 2013; Stewart et al. 2015; Porto et al. 2016). These pathogens produce resistance structures, which allow them to survive in the soil for long periods of time, in addition to having a wide host range that makes management difficult and agricultural production a challenge (Porto et al. 2016). There are no chemical fungicides registered for melon field applications in the Ministério da Agricultura, Pecuária e Abastecimento (Brazilian Department of Agriculture) (AGROFIT 2025). Furthermore, European countries that import melons produced in Brazil have strict regulations that restrict the use of agrochemicals in melons and other agricultural products, due to environmental issues and risks to human health, which makes it even more difficult to control these pathogens (Borges et al. 2024). This way, there is a pressing need to develop new management strategies targeting SBP to help to sustainably reduce the use of agrochemicals (Borges et al. 2024). This will allow the continuous cultivation of melon crops in the Northeast Region of Brazil, facilitating the export of Brazilian melons to European countries, and guaranteeing the employability of the sector. Thus, the use of products based on microorganisms and/or metabolites is a much neededalternative in reducing diseases caused by SBP (Lahlali et al. 2022).

Antagonistic microorganisms release compounds that act on pathogens, compete for

space and nutrients, and produce metabolites and enzymes that degrade the cellular components of pathogens (Tuzun, 2001), reducing the incidence or severity of target diseases (Coêlho et al. 2018). Biocontrol agents such as bacteria of the genus *Bacillus* and fungi of the genus *Trichoderma* are widely used in the formulation of products that act in the control of PHS and are alternatives to chemicals (Coêlho et al. 2018; Bedendo 2011). Bacteria from the genus *Bacillus* can form endospores under unfavorable conditions (Lima 2011); besides, they can form biofilm on the root, which is bacterial cells wrapped in polysaccharides, proteins, and DNA, aiming to promote growth and protect the roots against attack of SBP (Beauregard et al. 2013). Fungi of the genus *Trichoderma* are widely known as biological control agents, they act to protect plant roots against pathogens through competition, mycoparasitism, and antibiosis (Lorito et al. 2010; Druzhinina et al. 2011).

Several studies have shown the efficiency of these microorganisms in controlling PHS. For example, Samaras et al. (2021) verified a positive effect of *Bacillus subtilis* against *Fusarium oxysporum*, *Pythium ultimum,* and *Rhizoctonia solani*, the three main SBP that cause damage to tomato plants. Gabardo et al. (2020) reported that *T. asperellum* demonstrated potential as an antagonist to the fungi *Sclerotium rolfsii* and *R. solani* by producing toxic metabolites. Regarding the SBP that causes damage to melon plants, just a few studies have effectively demonstrated the efficiency of the use of biological products with action on these microorganisms, although the use of products based on microorganisms in melon production in Brazil is increasing. Given the need for sustainable and efficient tools for the management of root diseases, the present study aimed to verify the effect of products based on bacteria, *Trichoderma,* and metabolites in the management of SBP in melon plants.

MATERIAL AND METHODS

The experiments were carried out at Fazenda Agrícola Famosa, located in the municipality of Icapuí/CE, from October to December 2024, and the analyses were performed at the Laboratório de Microbiologia e Fitopatologia, of the Departamento de Ciências Agronômicas e Florestais of the Universidade Federal Rural do Semi-Árido, located in Mossoró, Brazil.

A pilot study was performed with 30 treatments, composed of 6 alternative products, and their associations. Combinations of up to 4 products were tested, verifying the interaction between bacteria (*Bacillus*, *Lactobacillus,* and *Enterococcus*), the fungus *Trichoderma*, and fermentation metabolites (produced by bacteria from genus *Bacillus* and yeasts), on the severity

of root system rot.

The associations were: T1= *Trichoderma asperellum;* T2= *Bacillus subtilis;* T3= *B. subtilis* + *B. Licheniformis;* T4= *Bacillus subtilis* + *Lactobacillus plantarum*+ *Enterococus faecium;* T5= Fermented metabolites 1; T6= Fermented metabolites 2; T7= *T. asperellum* + *B.subtilis;* T8= *T. asperellum* + *B.subtilis* e *B.licheniformis;* T9= *T. asperellum* + B. *subtilis, L. plantarum* e *E. Faecium;* T10= *T. asperellum* + Metabolites 2; T11= *T. asperellum* + Metabolites Metabolites 1; T12= *B.subtilis* + *B. Licheniformis*; T13= *B.subtilis* + *L. plantarum* e *E. Faecium;* T14= *B.subtilis* + Metabolites 2; T15= *B.subtilis* + Metabolites 2; T16= *B.subtilis* e *B. licheniformis* + *L. plantarum* e *E. Faecium;* T17= *B.subtilis* e *B. licheniformis* + Metabolites 2; T18= *B.subtilis* e *B. licheniformis* + Metabolites 1; T19= *B.subtilis, L. plantarum* e *E. faecium* + Metabolites 2; T20= *B.subtilis, L. plantarum* e *E. faecium* + Metabólitos 1; T21= Metabolites 1 + Metabolites 2; T22= *T. asperellum* +*B.subtilis* + *L. plantarum* e *E. Faecium;* T23= *T. asperellum* + *B.subtilis* + *L. Plantarum* + *E. faecium*+ Metabolites 2; T24= *T. asperellum* + *B.subtilis* + *L. Plantarum* + *E. faecium*+ Metabolites 1; T25= *T. asperellum* + *B.subtilis* e *B. licheniformis* + *L. plantarum* e *E. Faecium;* T26= *T. asperellum* + *B.subtilis* e *B. licheniformis* + *L. plantarum* e *E. faecium* + Metabolites 2; T27= *T. asperellum* + *B.subtilis* e *B. licheniformis* + *L. plantarum* e *E. faecium* + Metabolites 1; T28= *B.subtilis* e *B. licheniformis* + *L. plantarum* e *E. faecium*+ Metabolites 1+ Metabolites 2; T29= *T. asperellum* + *B.subtilis* + Metabolites 1 + Metabolites 2; T30= Control. These associations were tested in order to find an efficient and economically viable combination for pathogen management.

This studywas performed in greenhouse conditions, with soil from an area with a history of diseases caused by SBP, including *Fusarium*, *Macrophomina*, *Monosporascus,* and *Rhizoctonia*. The melon Gália, variety SV4974, was used in this pilot study and the 5 treatments that presented the lowest severity of the disease were selected for the field experiment (supplementary fig 1). In the field experiment, the experimental design used was randomized blocks with 6 treatments (Table 1) and 7 replications. experiment was repeated at the same time and under the same conditions.

For this essay, commercial products based on microorganisms and metabolites were used. The metabolites are of industrial origin and obtained through the natural fermentation of beneficial microorganisms, along with amino acids and nutrients. The products were applied via soil, following the recommendations of the manufacturer.

Table 1 Treatments applied to soil cultivated with Gália melon aiming at managing root diseases

Treatment	Composition and dosage
T1	*B. subtilis* and *B. licheniformis* (1kg/ha) + Metabolites 1 (natural fermentation of microorganisms, enriched with amino acids and macronutrients) (1L/ha);
T2	*B. subtilis*, *Lactobacillus plantarum* an *Enterococcus faecium* (1kg/ha) + Metabolites 2 (natural fermentation of beneficial microorganisms) (1L/ha);
T3	Metabolites 1 (natural fermentation of microorganisms, enriched with amino acids and macronutrients) (1L/ha) + Metabolites 2 (natural fermentation of beneficial microorganisms) (1L/ha);
T4	*B. subtilis* + *B. licheniformis* + *L. plantarum* and *E. faecium* (1kg/ha) + Metabolites 1 (natural fermentation of microorganisms, enriched with amino acids and macronutrients) (1L/ha) + Metabolites 2 (natural fermentation of beneficial microorganisms) (1L/ha);
T5	*T. asperelum* (300mL/ha), *B. subtilis* (1L/ha), Metabolites 1 (natural fermentation of microorganisms, enriched with amino acids and macronutrients) (1L/ha) + Metabolites 2 (natural fermentation of beneficial microorganisms) (1L/ha) - Farm management;
T6	Control

The area with a history of root diseases in melon plants was cultivated with Gália melon, and treatments were applied weekly, at the dosages recommended by the manufacturers, as mentioned in Table 1.

After preparing the soil, organic compost (cow manure, sorghum, and monoammonium phosphate) was added, and the planting lines were covered with white polyethylene mulch, which helps to reduce the population of plants produced in the area and maintains soil moisture. The seedlings were transplanted 10 days after sowing, and then a non-woven fabric blanket was placed over the seedlings, which helps to reduce the attack of pests, such as the leafminer (*Liriomyza huidobrensis* Blanchard). Irrigation was carried out via drip irrigation, with the nutrient solution containing macro and micronutrients needed for crop development. During the period of opening of the first flowers, when the plants were approximately 22 days after transplanting (DAT), the non-woven fabric blanket was removed for pollinators, bees, to have access to the flowers to perform pollination. The crop cycle was 60 DAT, when the following parameters were evaluated:

Stem diameter: The plant stem diameter was measured using a digital caliper, model MTX-316119.

Shoot dry mass: The base of the stem was separated from the root, and the shoot was placed in Kraft paper bags and dried in a forced air oven (± 65 ºC) until a constant weight was observed.

Disease severity (SEV): The roots were separated from the shoot and washed in running water for evaluation using the score scale modified by Ambrósio et al. (2015), where: 0 = plant without symptoms; 1 = less than 3% of infected tissue; 2 = 3-10% of infected tissue; 3 = 11-25% of infected tissue; 4 = 26-50% of infected tissue; and 5 = more than 50% of infected tissue.

Number of fruits per plant: The number of fruits on each plant of the treatments and replicates was counted.

Fruit weight and ºBrix: The fruits were weighed on a digital scale, model Vonder 3885001010, and the ºBrix of each fruit was measured using a digital refractometer, model MA871.

Soil enzymatic activity: The activities of the enzymes β-glucosidase, arylsulfatase, and acid phosphatase were determined. This analysis was based on the determination of p-nitrophenol formed after the addition of specific substrates for each enzyme. To quantify the activity of β-glucosidase, arylsulfatase, and acid phosphatase, p-nitrophenyl-β-d-glucopyranoside (PNG), p-nitrophenyl sulfate (PNS), and disodium p-nitrophenyl phosphate (P-NPP) were added as substrates for each enzyme, respectively. These determinations were performed directly on air-dried soil samples and expressed in μg p-nitrophenol g^{-1} soil h^{-1} (Tabatabai 1994).

The data were subjected to the Shapiro-Wilk normality test ($p<0.05$) and Bartlett homogeneity of variances test ($p<0.05$) to fulfill the ANOVA presumptions. Subsequently, parametric variance analysis was performed, and the means were compared by the Tukey test at 5% probability. All statistical analyses were performed on R (R Core Team 2022) using RStudio as a graphical interface (RStudio Team 2024).

RESULTS

In the pilot test carried out in green house conditions, it was demonstrated that for the severity of the disease, the best results were obtained in treatments 18 (*B. subtilis* + *B. licheniformis* + metabolites 1), 19 (*B. subtilis* + *L. plantarum* + *E. faecium* + metabolites 2), 21 (metabolites 1 + metabolites 2) and 28 (*B. subtilis* + *B. licheniformis* + *L. plantarum* + *E.*

faecium + metabolites 1 + metabolites 2), reducing 62.5% in treatments 18, 19 and 28, and 68.75% in treatment 21 (supplementary figure 1) compared with the control. The main genera found causing rot in the root system were *Fusarium* and *Macrophomina*.

In the first experiment, treatments 2 (*B. subtilis* and *B. licheniformis* + metabolites 1) and 3 (metabolites 1 + metabolites 2) were those that provided the largest stem diameters, with 10.6 and 9.7 mm, respectively, promoting increases of 23.5% (T2) and 16.5% (T3) compared to the control (8.1 mm). However, treatments 1, 4, and 5 did not differ from treatment 3 (Figure 1A). In experiment 2, there was no statistical difference between the treatments (Figure 1A).

Observing the results of the shoot dry mass, it is noted that there was no statistical difference between the treatments, for both experiments. However, treatment 4 (*B. subtilis* + *B. licheniformis* + *L. plantarum* and *E. faecium* + Metabolites 1 + Metabolites 2) showed an increase of approximately 24% in the dry mass of the aerial part of the melon plant compared to the control (Figure 1B).

For the SEV of the disease in the first experiment, treatments 1, 2, 3, and 4 did not present a statistical difference. However, treatments 2 (*B. subtilis* + *B. licheniformis* + metabolites 1) and 3 (metabolites 1 + metabolites 2) provided 50% reduction in the SEV of the disease, and treatments T1 (*B. subtilis*, *L. plantarum* and *E. faecium* + metabolites 2) and T4 (*B. subtilis* + *B. licheniformis* + *L. plantarum* and *E. faecium* + metabolites 1 + metabolites 2) promoted a 20% reduction in the SEV of the disease, all compared to the control (Figure 1C).

In the second experiment, the treatments containing microorganisms and their metabolites did not differ from each other, but they differed from the control. Treatments 1 (*B. subtilis* + *L. plantarum* + *E. faecium* + metabolites 2) and 4 (*B. subtilis*, *B. licheniformis* + *L. plantarum* + *E. faecium* + metabolites 1 + metabolites 2) provided lower SEV of the disease, with a 50% reduction compared to the control (Figura 1C).

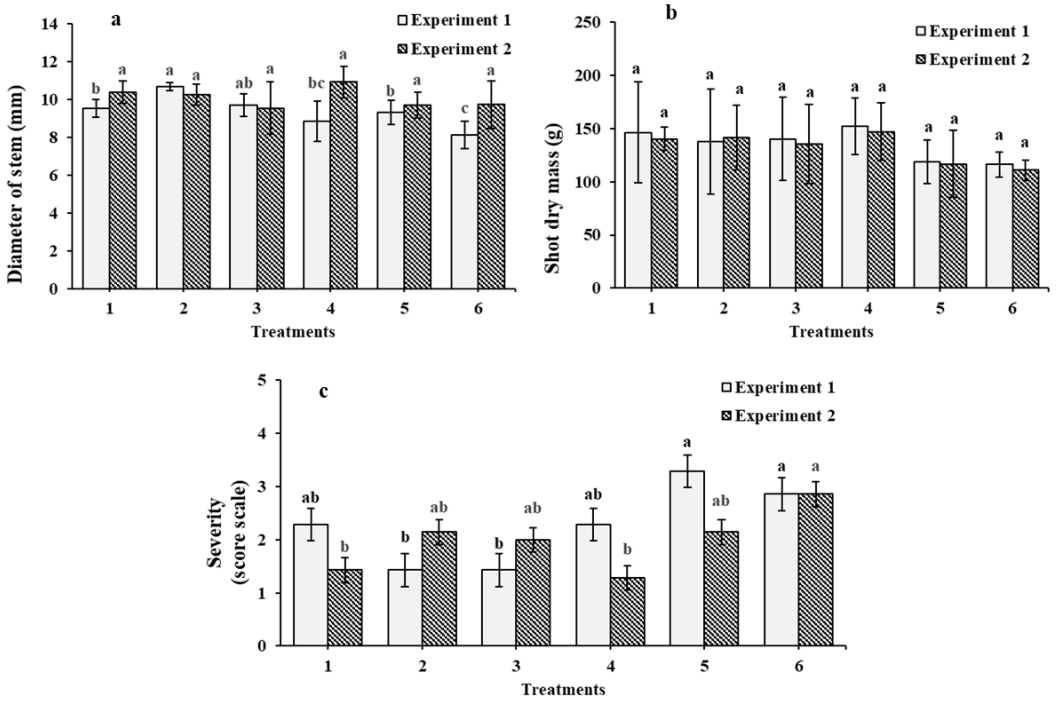

Fig 1 Stem diameter (A), shoot dry mass (B), and root rot severity (C) in melon plants. Cultivated soils were treated with the mix formulation of alternative products, being T1 = (*B. subtilis* + *B. licheniformis* + metabolites 1), T2 = (*B. subtilis* + *L. plantarum* + *E. faecium* + metabolites 2), T3 = (metabolites 1 + metabolites 2), T4 = (*B. subtilis* + *B. licheniformis* + *L. plantarum* + *E. faecium* + metabolites 1 + metabolites 2), T5 = standard management (*T. asperelum* + *B. subtilis* + metabolites 1 + metabolites 2), and T6 = Control

In the first experiment, for the number of fruits per plant, treatment 4 (*B. subtilis* + *B. licheniformis* + *L. plantarum* + *E. faecium* + metabolites 2 + metabolites 1) differed statistically from the other treatments, with an average of 3.4 fruits per plant (Figure 2A). In the second experiment, treatment 4 showed a statistical difference from most treatments, except for treatment 5 (*T. asperelum* + *B. subtilis* + metabolites 1 + metabolites 2), which was statistically equal to T4 and presented 3.3 fruits per plant. However, treatment 5 did not differ statistically from treatments T1, T2, and T3. The control and treatment 4 presented the lowest (2.8) and the highest number of fruits (3.4), respectively, in both experiments (Figure 2A). For the parameters of fruit weight and °Brix, there was no statistical difference between the treatments in both experiments (Figure 2B and 2C).

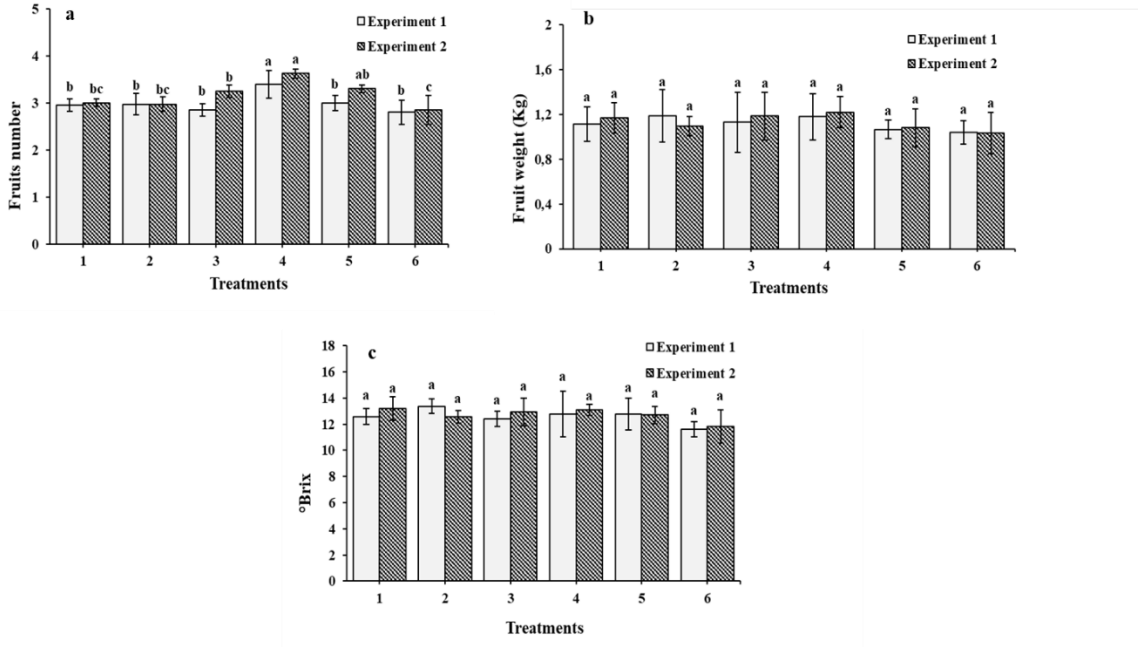

Fig 2 Weight (A), number of fruits per plant (B), and °Brix (C) of melon fruits. Cultivated soils were treated with the mix formulation of alternative products. T1 = (*B. subtilis* + *B. licheniformis* + metabolites 1), T2 = (*B. subtilis* + *L. plantarum* + *E. faecium* + metabolites 2), T3 = (metabolites 1 + metabolites 2), T4 = (*B. subtilis* + *B. licheniformis* + *L. plantarum* and *E. faecium* + metabolites 1 + metabolites 2), T5 = standard management (*T. asperelum* + *B. subtilis* + metabolites 1 + metabolites 2), and T6 = Control

For the β-glucosidase enzyme in experiment 1, treatment 4 (*B. subtilis* + *B. licheniformis* + *L. plantarum* + *E. faecium* + metabolites 1 + metabolites 2) was the one that promoted the greatest activity (44.3 µg p-nitrophenol g^{-1} h^{-1}), and differed only from the control treatment. The other treatments did not differ from the control. Experiment 2 presented the same behavior, but treatment 3 (metabolites 1 + metabolites 2) was inferior to treatment 4, which did not differ from the others (Figure 3A).

In the determination of Arylsulfatase, treatment 4 showed the best performance, differing from the other treatments in both experiments (Figure 3B). For acid phosphatase, in experiment 1, there was no statistical difference between treatments. In the second experiment, treatment 4 (*B. subtilis* + *B. licheniformis* + *L. plantarum* + *E. faecium* + metabolites 1 + metabolites 2) was superior to the control, with an average of 141.2 µg p-nitrophenol g^{-1} h^{-1}, but did not differ from the other treatments (Figure 3C).

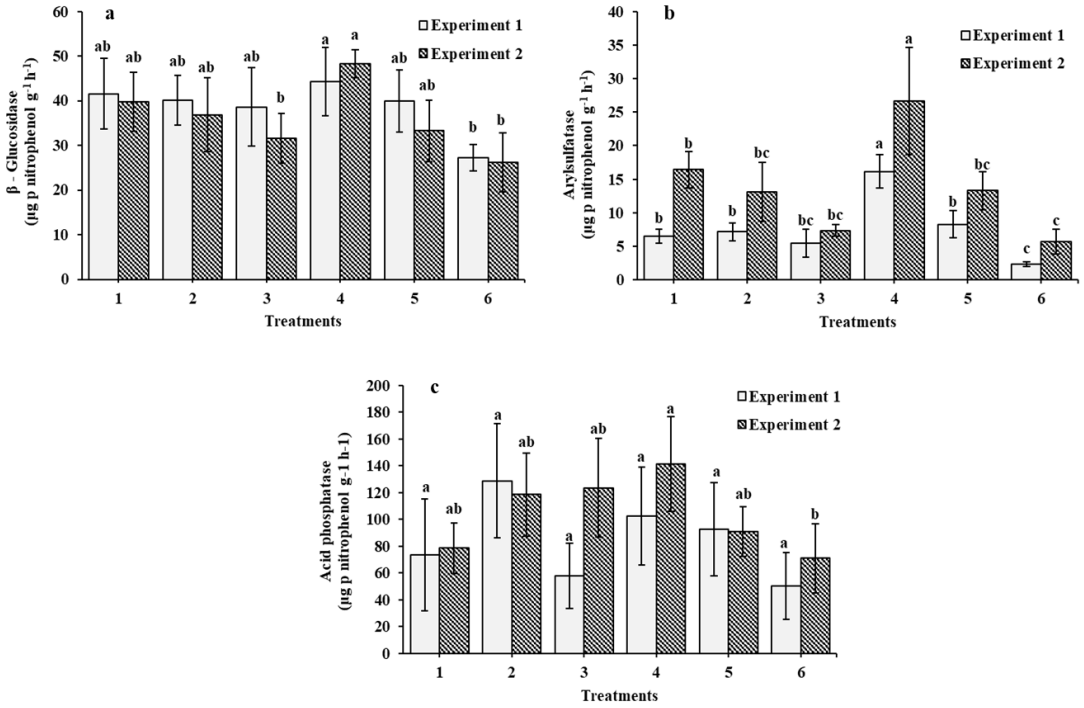

Fig 3 Activity of the enzyme β-glucosidase (A), Arylsulfatase (B), Acid phosphatase (C) of soils. Cultivated soils were treated with mix formulation of alternative products. T1 = (*B. subtilis* + *B. licheniformis* + metabolites 1), T2 = (*B. subtilis* + *L. plantarum* + *E. faecium* + metabolites 2), T3 = (metabolites 1 + metabolites 2), T4 = (*B. subtilis* + *B. licheniformis* + *L. plantarum* and *E. faecium* + metabolites 1 + metabolites 2), T5 = standard management (*T. asperellum* + *B. subtilis* + metabolites 1 and metabolites 2), and T6 = Control

DISCUSSION

The use of biological products has become a common practice on melon farms in Brazil, including the multiplication of these products directly on the property. They are mainly used to manage root diseases, but they also help to promote plant growth and make nutrients available in the soil, due to the action of microorganisms. However, the understanding of the real effect of these microorganisms on the SBP that attack melons, as well as on plant development, is not yet fully understood. The present study worked with the formulations of biological products based on a mix of several bacteria, *Trichoderma,* and metabolites, aiming to clarify whether this combination reduces root rot caused by PHS in melon production areas that practice intensive continuous monoculture.

In both experiments, we were unable to, statistically, identify any treatment that stood out in terms of reducing the severity of the disease. We believe that this is due to the short

period of action of the microorganisms in their modes of action. Microorganisms are not like a chemical defense with an immediate effect. Biological control acts over time, as microorganisms need to establish themselves, compete, colonize, and interact with the soil and the plant to activate their defense mechanisms (Compant et al. 2019). In a short growing period, as is the case with a single melon cycle (60 days), it may not be enough time to obtain great results provided by the use of microorganisms, which act after their establishment in the soil. The reduction in SEV of root rot may be small at first glance, but it may gradually decrease, year after year, once the microorganisms establish themselves in the soil. In studies of Volynchikova (2022), it was observed that although *Pseudomonas* spp. strains demonstrated high *in vitro* antagonistic performance against *Phytophthora infestans*, their *in vivo* efficacy was limited. Barbieri (2023), when evaluating the performance of *T. harzianum*, *T. asperellum,* and *B. amyloliquefaciens* in soybean crops, found that under natural environmental conditions, there was no significant improvement in plant performance. The authors suggest that the time required for the establishment of microorganisms may not be compatible with the soybean crop cycle.

However, in both experiments of the present study, it was observed that the treatments containing bacteria and their metabolites stood out compared to the control, reducing the severity of the disease from 16.6 to 50%. The treatments were composed of a mix of bacteria and their metabolites, including *Bacillus* and *Lactobacillus*. Bacteria of the genus *Bacillus* present antagonistic activities that are often related to antibiotic properties, and the production of hydrolytic enzymes (chitinases, glucanases, cellulases, lipases, and proteases), which efficiently hydrolyze the main components of fungal and bacterial cell walls, in addition to the production of secondary metabolites (Miljaković et al. 2020). Several studies have already tested the efficiency of applying commercial products based on *Bacillus*, indicating it as an excellent alternative to the use of chemical products in agriculture for plant protection (Fira et al. 2018; Wang et al. 2018).

Although *Bacillus* is one of the best-known bacterial genera as a biocontrol agent, other genera are also efficient in suppressing pathogens, such as *Lactobacillus* and *Enterococcus* (Jaini et al. 2022). Reports in the literature show that specific metabolites of *L. plantarum* have antifungal potential against *Fusarium avenaceum* (Zhao et al. 2017). In study carried out by Sjogren et al. (2003) showed that the antimicrobial effect of metabolites extracted from *L. plantarum* is due to the detergent characteristics of the compounds, which disrupt the structure of the cell membranes of target organisms, such as phytopathogens.

Treatments containing bacteria and metabolites (treatment 4) also provided greater

quantities of fruits, and this may be due to the ability of fermenting microorganisms, such as *Bacillus* and *Lactobacillus*, to produce compounds such as organic acids, amino acids or phytohormones during the fermentation process, which stimulate plant development and, consequently, greater production (Spaepen et al. 2007; Ahemad e Kibret 2014). In this study, the same treatment (T4), which contained a mix of bacteria and metabolites, in addition to standing out for the greater number of fruits, was among the treatments that presented the lowest SEV of the disease. This treatment contained more than one species of *Bacillus*, in addition to *L. plantarum* and *E. faecium*. The genus *Bacillus* produces substances that combat fungi, such as iturins, bacillomycins, and surfactins (Ongena e Jacques, 2008). This is certainly the reason why this treatment is one of those that caused the lowest SEV of the disease. When the pathogen infects the host, the conducting vessels are obstructed, making it difficult to transport water and nutrients for the production and maintenance of fruits, and resources are redirected to the defense system, in function of the fruit (Agrios 2005; Huot et al. 2014). Biocontrol bacteria stimulate plant growth by improving nutrient availability, thus increasing the number of fruits and promoting a healthier environment.

Indicator enzymes of soil biological activity, such as β-glucosidase, arylsulfatase, and acid phosphatase, also showed greater activity in treatment 4, as the high presence of *Bacillus* in the soil is associated with greater production of these enzymes, which are part of its metabolism. (Moreira e Siqueira 2006). These enzymes play essential roles in the decomposition of organic matter, the release of nutrients such as carbohydrates, sulfates, and phosphates, and the maintenance of soil fertility (Burns et al. 2013). Thus, the greater quantity of *Bacillus* contributes to greater enzymatic and biological activities, improving the dynamics of nutrients in the soil. Caballero et al. (2022) found that the application of a fermented bioproduct based on *B. licheniformis* in agricultural soils promoted a significant increase in the activities of the enzymatic dehydrogenase, phosphatase, and β-glucosidase. Ng et al. (2022) also reported that the enzymatic activity of the soil studied increased significantly in treatments containing *B. subtilis* and *Pseudomonas fluorescens* compared to the control.

CONCLUSION

Although the use of a mix of bacteria, metabolites and the fungus *Trichoderma* to manage melon root rot caused by SBP did not show sufficiently expressive results to recommend a definitive strategy for melon producers in Brazil, treatment 4, which combined bacteria and metabolites, showed promising signs, for example, greater enzymatic activity in

the soil and a greater number of fruits. These findings suggest that this combination may be a viable alternative and deserves further investigation to optimize its efficacy and viability in agricultural practice. Thus, this study contributed to the understanding of the possibilities and limits of the use of bioproducts in the management of root diseases, encouraging future research that can consolidate more sustainable strategies for melon producers.

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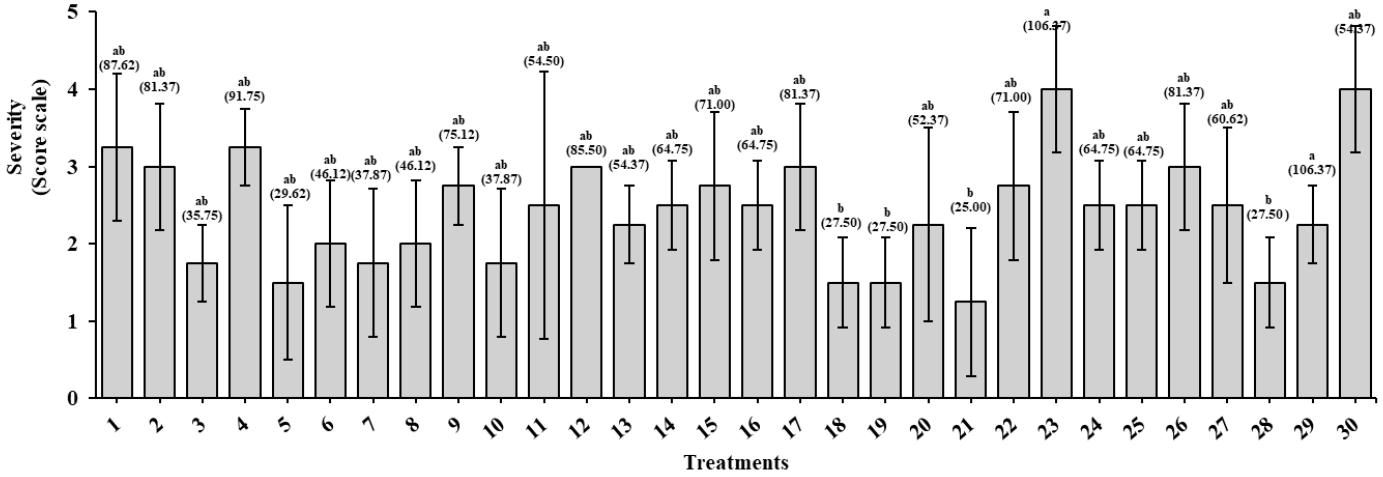
SUPPLEMENTARY



Supplementary figure 1 Disease severity of naturally infested melon plants and subjected to the application of alternative products and their associations. T1= *Trichoderma asperellum;* T2= *Bacillus subtilis;* T3= *B. subtilis* + *B. Licheniformis;* T4= *Bacillus subtilis* + *Lactobacillus plantarum*+ *Enterococus faecium;* T5= Fermented metabolites 1; T6= Fermented metabolites 2; T7= *T. asperellum* + *B.subtilis;* T8= *T. asperellum* + *B.subtilis* e *B.licheniformis;* T9= *T. asperellum* + B. *subtilis, L. plantarum* e *E. Faecium;* T10= *T. asperellum* + Metabolites 2; T11= *T. asperellum* + Metabolites 1; T12= *B.subtilis* + *B. Licheniformis*; T13= *B.subtilis* + *L. plantarum* e *E. Faecium;* T14= *B.subtilis* + Metabolites 2; T15= *B.subtilis* + Metabolites 2; T16= *B.subtilis* e *B. licheniformis* + *L. plantarum* e *E. Faecium;* T17= *B.subtilis* e *B. licheniformis* + Metabolites 2; T18= *B.subtilis* e *B. licheniformis* + Metabolites 1; T19= *B.subtilis, L. plantarum* e *E. faecium* + Metabolites 2; T20= *B.subtilis, L. plantarum* e *E. faecium* + Metabolites 1; T21= Metabolites 1 + Metabolites 2; T22= *T. asperellum* +*B.subtilis* + *L. plantarum* e *E. Faecium;* T23= *T. asperellum* + *B.subtilis* + *L. Plantarum* + *E. faecium*+ Metabolites 2; T24= *T. asperellum* + *B.subtilis* + *L. Plantarum* + *E. faecium*+ Metabólitos 1; T25= *T. asperellum* + *B.subtilis* e *B. licheniformis* + *L. plantarum* e *E. Faecium;* T26= *T. asperellum* + *B.subtilis* e *B. licheniformis* + *L. plantarum* e *E. faecium* + Metabolites 2; T27= *T. asperellum* + *B.subtilis* e *B. licheniformis* + *L. plantarum* e *E. faecium* + Metabolites1; T28= *B.subtilis* e *B. licheniformis* + *L. plantarum* e *E. faecium*+ Metabolites 1+ Metabolites 2; T29= *T. asperellum* + *B.subtilis* + Metabolites 1 + Metabolites 2; T30= Control.