



MAYANNA KARLLA LIMA COSTA

**BIONEMATÓCIDAS NO CONTROLE BIOLÓGICO DE
PRATYLENCHUS: EFICIÊNCIA EM MILHO, CANA-DE-AÇÚCAR E
FEIJÃO-FAVA E IMPACTOS NA COMUNIDADE BACTERIANA DA
RIZOSFERA**

**TERESINA - PI
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Tese apresentada à Universidade Federal do Piauí, como parte das exigências do Programa de Pós-Graduação em Agronomia, na área de concentração de manejo e conservação do solo, como requisito para obtenção do título de Doutora em Agronomia.

Orientador

Prof. Dr. Ademir Sérgio Ferreira de Araujo

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**TERESINA-PI
2025**

2025

Às minhas Marias, raízes do meu coração.
Aos meus Osanos, pai e irmão, pelo exemplo e
afeto que compartilham.
À minha irmã Clotilde, pela amizade eterna e
cumplicidade.

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“O que nos ajuda a estabelecer as prioridades certas e a gerir bem o nosso tempo é a consciência de que só temos de trabalhar com o dia de hoje, pois o passado está irremediavelmente perdido, e o futuro é apenas uma possibilidade”

Dorothey Kelley

RESUMO

O controle de nematoides fitoparasitas do gênero *Pratylenchus* continua sendo um dos principais desafios para a produção agrícola sustentável, especialmente em culturas de importância agrícola como milho, feijão-fava e cana-de-açúcar. O uso de microrganismos como agentes de biocontrole representa uma alternativa eficiente ao manejo químico convencional, por sua capacidade de suprimir patógenos e, ao mesmo tempo, contribuir para a saúde do solo. Este trabalho avaliou o desempenho de diferentes microrganismos benéficos no controle de *Pratylenchus* spp. em sistemas agrícolas tropicais, com foco nos efeitos sobre o crescimento vegetal, nodulação e estrutura da comunidade bacteriana do solo. No Capítulo I, realizado em condições de campo, foi avaliada a eficácia da cepa AP-3 de *Bacillus subtilis* na supressão de *Pratylenchus* spp. em milho e feijão-fava. A inoculação reduziu significativamente a população de nematoides nas raízes (~90% em milho e ~40% em feijão-fava), além de estimular o crescimento radicular e aumentar os atributos de nodulação no feijão-fava, especialmente na ausência de fertilizantes químicos. Os resultados indicam que *B. subtilis* atua tanto por na supressão de *pratylenchus* spp quanto por efeitos positivos no desenvolvimento das plantas. No Capítulo II, em experimento conduzido em casa de vegetação, foram testadas cinco formulações microbianas contendo *B. subtilis*, *B. amyloliquefaciens*, *B. licheniformis*, *Pseudomonas oryzihabitans* e *Purpureocillium lilacinum* no controle de *Pratylenchus zaeae* na cana-de-açúcar. O tratamento com *Pseudomonas oryzihabitans* apresentou a maior eficiência, reduzindo em até 81% a densidade de nematoides nas raízes e 64% no solo. Além disso, essa inoculação aumentou a atividade enzimática (fosfatase, urease e β -glucosidase) e a biomassa microbiana. A análise da microbiota por sequenciamento do gene 16S rRNA indicou que a estrutura da comunidade bacteriana foi mantida, embora *Pseudomonas oryzihabitans* tenha promovido maior riqueza e diversidade microbiana. Por outro lado, as coinoculações apresentaram menor diversidade e menor eficácia no controle de *Pratylenchus zaeae*, possivelmente devido à competição entre os microrganismos. No Capítulo III, avaliou-se o efeito dos bionematicidas sobre a comunidade de bactérias promotoras de crescimento vegetal (PGPB) na rizosfera da cana-de-açúcar. A inoculação com *Pseudomonas oryzihabitans* aumentou a diversidade de PGPB e favoreceu a presença de grupos especialistas, além de promover redes microbianas mais complexas. Em contraste, os tratamentos com *B. amyloliquefaciens* e as coinoculações reduziram a diversidade e as interações, indicando efeitos seletivos no recrutamento microbiano. Apesar dessas diferenças, a estrutura geral da comunidade bacteriana permaneceu estável entre os tratamentos. Os resultados obtidos demonstram que a aplicação de bionematicidas pode ser uma estratégia eficaz e ecologicamente segura para o manejo de nematoides do gênero *Pratylenchus*. Além de controlar os patógenos, esses microrganismos contribuem para o desenvolvimento das plantas e a preservação da funcionalidade da microbiota do solo, com destaque para os grupos benéficos como os PGPB.

Palavras-chave: *Pratylenchus* spp., controle biológico, rizobactérias promotoras de crescimento, diversidade microbiana, atividade enzimática do solo, 16S rRNA, agricultura sustentável.

ABSTRACT

The control of plant-parasitic nematodes of the genus *Pratylenchus* remains one of the main challenges for sustainable agricultural production, especially in economically important crops such as maize, lima bean, and sugarcane. The use of microorganisms as biocontrol agents provides an effective alternative to conventional chemical management, as they can suppress pathogens while promoting soil health. This study evaluated the performance of different beneficial microorganisms in the control of *Pratylenchus* spp. in tropical agricultural systems, with a focus on plant growth, nodulation, and the structure of soil bacterial communities. In Chapter I, conducted under field conditions, the effectiveness of the *Bacillus subtilis* strain AP-3 in suppressing *Pratylenchus* spp. in maize and lima bean was assessed. Inoculation significantly reduced nematode populations in the roots (~90% in maize and ~40% in lima bean) and also stimulated root growth and increased nodulation traits in lima bean, particularly in the absence of chemical fertilizers. The results indicate that *Bacillus subtilis* acts both by suppressing *Pratylenchus* spp. and by promoting plant development. In Chapter II, a greenhouse experiment tested five microbial formulations containing *B. subtilis*, *B. amyloliquefaciens*, *B. licheniformis*, *Pseudomonas oryzihabitans*, and *Purpureocillium lilacinum* for the control of *Pratylenchus zaeae* in sugarcane. *Pseudomonas oryzihabitans* was the most effective treatment, reducing nematode density by up to 81% in roots and 64% in soil. This inoculation also enhanced enzymatic activities (phosphatase, urease, and β -glucosidase) and microbial biomass. Microbiota analysis based on 16S rRNA gene sequencing showed that bacterial community structure was preserved, although *Pseudomonas oryzihabitans* promoted greater richness and microbial diversity. In contrast, co-inoculations resulted in lower diversity and reduced efficacy in controlling *P. zaeae*, possibly due to microbial competition. In Chapter III, the impact of bionematicide application on the community of plant growth-promoting bacteria (PGPB) in the sugarcane rhizosphere was evaluated. Inoculation with *Pseudomonas oryzihabitans* increased PGPB diversity and favored the presence of specialist groups, in addition to promoting more complex microbial networks. In contrast, treatments with *B. amyloliquefaciens* and co-inoculations reduced microbial diversity and interactions, indicating selective effects on PGPB recruitment. Despite these differences, the overall structure of the bacterial community remained stable across treatments. The results demonstrate that the use of bionematicides can be an effective and environmentally safe strategy for managing *Pratylenchus* spp. In addition to controlling pathogens, these microorganisms contribute to plant development and help preserve the functional integrity of soil microbiota, particularly beneficial groups such as PGPB.

Keywords: *Pratylenchus* spp., biological control, plant growth-promoting rhizobacteria, microbial diversity, soil enzymatic activity, 16S rRNA, sustainable agriculture.

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1 INTRODUÇÃO GERAL

A cana-de-açúcar (*Saccharum* spp), o feijão-fava (*Phaseolus lunatus*) e o milho (*Zea mays*) são culturas agrícolas de grande relevância econômica, embora inseridas em contextos produtivos distintos. A cana-de-açúcar, cultivada globalmente, é essencial para a produção de açúcar e bioetanol, desempenhando papel importante na mitigação de emissões de gases de efeito estufa (LIU et al., 2024). Neste contexto, o Brasil sobressai como líder mundial tanto em produção quanto em exportação da cultura, reforçando sua relevância econômica e ambiental no cenário internacional (CONAB, 2024).

O feijão-fava, embora apresente menor relevância econômica global em relação à cana-de-açúcar, é essencial para a segurança alimentar em regiões tropicais e subtropicais, principalmente na América do Sul e África, devido ao seu alto valor nutricional, rico em proteínas e fibras e seu papel na subsistência rural (ADEBO et al., 2023). No Nordeste brasileiro, integra sistemas agrícolas desde o período pré-colonial, sendo fundamental na dieta e geração de renda local (COSTA et al., 2023).

Já o milho destaca-se como uma das principais commodities agrícolas do mundo, com ampla distribuição geográfica e importância estratégica na alimentação humana e animal, além de ser matéria-prima para diversos setores industriais. No Brasil, tem papel central na rotação de culturas e no sistema produtivo de grãos, contribuindo diretamente para a sustentabilidade e a produtividade das áreas cultivadas (MAPA, 2023).

Essas culturas, entretanto, compartilham desafios fitossanitários importantes, sobretudo devido à ocorrência de nematoides fitoparasitas. Esses organismos representam pragas severas, responsáveis por perdas anuais na produção vegetal estimadas em até 173 bilhões de dólares (LI et al., 2025). Dentre eles, destacam-se os nematoides do gênero *Pratylenchus*, amplamente distribuídos em solos agrícolas, causadores de lesões radiculares que comprometem a absorção de água e nutrientes (SMILEY et al., 2021), podendo resultar em reduções anuais de produtividade e prejuízos bilionários (THOMPSON et al., 2021).

Na cana-de-açúcar, *Pratylenchus zeae* é um dos principais agentes associados aos danos radiculares, prejudicando o vigor das plantas e causando perdas significativas na produtividade dos canaviais (ARAÚJO et al., 2025). No feijão-fava,

entretanto, os efeitos da infestação por nematoides permanecem pouco estudados. Em sistemas tradicionais, a limitada adoção de tecnologias de controle aumenta as perdas produtivas (COSTA et al., 2023). Essa escassez de informações representa uma lacuna importante quanto a estratégias sustentáveis específicas para o controle de nematoides nessa cultura

Diversas estratégias têm sido empregadas para controlar nematoides fitoparasitas, principalmente nematicidas químicos e práticas culturais (DESAEGER et al., 2017; DINARDO-MIRANDA et al., 2019). Apesar da eficácia imediata, o uso contínuo desses químicos pode gerar resistência e reduzir a biodiversidade do solo ao eliminar espécies não-alvo (FORGHANI; HAJIHASSANI, 2020).

Nesse contexto, o controle biológico surge como uma alternativa sustentável, alinhada às demandas por práticas agrícolas menos agressivas ao ambiente e baseia-se na utilização de microrganismos antagonistas capazes de suprimir nematoides por mecanismos diretos ou indiretos, incluindo a produção de metabólitos antimicrobianos e indução de resistência vegetal. (MAZZUCHELLI et al., 2020; SAAD et al., 2022).

Entre esses microrganismos, destacam-se bactérias do gênero *Bacillus*, como *Bacillus subtilis*, amplamente reconhecidas pelo seu potencial antagonista contra nematoides fitoparasitas em diferentes culturas agrícolas, incluindo gramíneas e leguminosas (Mazzuchelli et al., 2020; COSTA et al., 2023). Tais bactérias atuam principalmente por meio da produção de compostos antimicrobianos e indução de resistência sistêmica nas plantas, reduzindo a capacidade infectiva dos nematoides e favorecendo o desenvolvimento vegetal.

Além das bactérias do gênero *Bacillus*, outros microrganismos têm se destacado na formulação de bionematicidas pelo seu potencial no controle sustentável de nematoides. Espécies como *Pseudomonas oryzae* e o fungo *Purpureocillium lilacinum* atuam por diferentes mecanismos, incluindo antibiose, produção de sideróforos e enzimas líticas (Machado, 2022).

Embora promissora na supressão de nematoides em diversas culturas, a aplicação de bionematicidas ainda envolve efeitos pouco compreendidos sobre a microbiota nativa da rizosfera. Microrganismos introduzidos podem interagir de diversas formas com a microbiota residente, promovendo desde o enriquecimento de

populações benéficas até alterações na diversidade local, dependendo das relações ecológicas estabelecidas (CIRVILLERI et al., 2020).

Estudos recentes indicam que a introdução de bionemáticas pode influenciar significativamente a microbiota da rizosfera, promovendo interações complexas com a comunidade microbiana residente. Por exemplo, Zhou et al. (2022) observaram que solos com maior diversidade bacteriana são mais eficazes na supressão de nematoides fitoparasitas, melhorando o desempenho vegetal. Além disso, Topalović et al. (2020) demonstraram que a introdução de bactérias antagonistas, como *Devosia*, *Rhizobium* e *Streptomyces*, fortalece características supressivas naturais do solo e diminui as infestações por nematoides. Esses achados sugerem que o aumento na diversidade bacteriana é um fator-chave para reduzir a capacidade infectiva desses parasitas.

Dessa forma, o uso criterioso de bionemáticas surge como uma alternativa promissora ao controle químico tradicional, representando uma estratégia eficaz e sustentável para o manejo de *Pratylenchus spp.* e promoção da saúde do solo (FAVORETTO et al., 2024).

Diante desse cenário, as hipóteses do trabalho foram:

(i) A aplicação de *Bacillus subtilis* controla eficientemente as populações de *Pratylenchus* no feijão-fava, resultando em melhorias no desenvolvimento radicular e aumento da nodulação e da biomassa vegetal;

(ii) Formulações contendo múltiplos microrganismos controlam eficazmente *Pratylenchus zae* na cana-de-açúcar e podem afetar a microbiota rizosférica;

(iii) A aplicação de diferentes bionemáticas modula de forma distinta o recrutamento de bactérias promotoras do crescimento vegetal (PGPB) na rizosfera da cana-de-açúcar

2 REVISÃO DE LITERATURA

2.1 Nematoides do gênero *Pratylenchus*: Biologia, impactos e estratégias de controle.

Os nematoides do gênero *Pratylenchus* pertencem ao filo Nematoda, classe Chromadorea e ordem Tylenchida, sendo amplamente reconhecidos entre os principais nematoides fitoparasitas de importância agrícola. Esses organismos

endoparasitas migradores penetram nas raízes e alimentam-se das células do córtex, interferindo diretamente na funcionalidade do sistema radicular e, conseqüentemente, na absorção de água e nutrientes (CHAUDHARY et al., 2020).

Estima-se que mais de 100 espécies tenham sido descritas no gênero, embora apenas cerca de 20 sejam consideradas economicamente relevantes, devido à ampla distribuição geográfica, à capacidade de parasitar diversas culturas e ao alto potencial de prejuízo ao desenvolvimento vegetal (CASTILLO; VOVLAS, 2007; JONES et al., 2013; SHAO et al., 2023).

As espécies de *Pratylenchus* ocupam o terceiro lugar em impacto econômico nas culturas agrícolas ao redor do mundo, atrás apenas dos nematoides-das-galhas (*Meloidogyne* spp.) e dos nematoides de cisto (*Heterodera* e *Globodera* spp.) e sua relevância agrônômica decorre principalmente da capacidade de adaptação a diferentes ambientes, de regiões frias a tropicais (CASTILLO; VOVLAS, 2007, FATEMI e JUNG, 2023).

Esse impacto é amplamente evidenciado em culturas de grande importância econômica e espécies como *Pratylenchus brachyurus*, *P. zaeae* e *P. penetrans* estão associadas a perdas significativas em soja, milho, cana-de-açúcar, batata, trigo e frutíferas, podendo comprometer mais de 50% da produtividade, especialmente sob condições de estresse abiótico (BUCKI et al., 2020; NGUYEN et al., 2024).

No Cerrado brasileiro, *Pratylenchus brachyurus* é amplamente disseminado e ameaça culturas de segunda safra. Segundo Paes et al. (2024), cultivares de feijão-caupi como a 'BRS Guariba' apresentaram alta multiplicação do nematoide (R = 8,34), superando o milho (R = 3,43), também suscetível. A sucessão da soja por essas culturas elevou as populações do patógeno e reduziu a sua produtividade.

Já na cana-de-açúcar, *Pratylenchus zaeae* é um dos principais agentes de lesões radiculares, causando perdas de produtividade entre 10% e 40%, com efeitos negativos sobre o crescimento e a qualidade tecnológica dos colmos (BARBOSA et al., 2013; ROSA DE ARAÚJO et al., 2025). Estudos conduzidos na Ásia também confirmam reduções significativas de rendimento associadas à alta densidade populacional desse nematoide em áreas comerciais (JAYAKUMAR, 2019).

Na batata, *Pratylenchus penetrans*, em associação com *Verticillium dahliae*, está diretamente envolvido na síndrome da murcha precoce, provocando perdas de até

70% na produtividade em cultivares suscetíveis (FIGUEIREDO et al., 2022; UC IPM, 2016). Em frutíferas como a macieira, sua ocorrência está fortemente relacionada à doença de replantio, comprometendo o desenvolvimento radicular e o vigor de pomares jovens (SHOKOOHI et al., 2023).

O mecanismo de patogenicidade desses nematoides reforça seu potencial destrutivo. O parasitismo envolve a migração intercelular no córtex radicular e a liberação de enzimas hidrolíticas, como pectinases e celulases, que degradam as paredes celulares vegetais e provocam lesões internas nos tecidos (VICENTE et al., 2019). Diferentemente de nematoides sedentários, como *Meloidogyne* spp., que formam galhas visíveis, *Pratylenchus* spp. causam danos mais sutis e de difícil detecção inicial, acumulando-se com o tempo.

Como resultado, observa-se o declínio progressivo das plantas, caracterizado por clorose, murchamento, menor vigor e redução do crescimento da parte aérea. Além disso, as lesões radiculares criadas por esses nematoides tornam-se pontos de entrada para patógenos oportunistas, como *Fusarium* spp. e *Rhizoctonia* spp., agravando os sintomas e comprometendo ainda mais a saúde vegetal (VIKETOFT et al., 2020).

Diante desses impactos, diferentes estratégias de manejo têm sido adotadas para mitigar os danos e preservar a produtividade das culturas. Entre as alternativas convencionais, destacam-se os nematicidas químicos, como abamectina e fluopiram, que atuam por contato ou de forma sistêmica.

Estudos demonstram que esses produtos são eficazes na redução inicial das populações de nematoides, mas sua ação é geralmente limitada às primeiras semanas após a aplicação, sendo influenciada por características como textura do solo e forma de incorporação (FASKE e Starr, 2006; FASKE e HURD, 2015). Além disso, o uso contínuo dessas moléculas pode comprometer a diversidade microbiana, afetar a atividade de enzimas do solo e gerar impactos ambientais e toxicológicos relevantes (ZHANG et al., 2014; LI et al., 2020).

Em razão dessas limitações, muitos países têm promovido a redução do uso de moléculas mais agressivas, incentivando o desenvolvimento de práticas de controle mais sustentáveis. Nesse contexto, estratégias culturais têm ganhado destaque. A rotação de culturas com espécies não hospedeiras, como *Crotalaria breviflora* e *C.*

spectabilis, tem se mostrado eficaz na redução da densidade de *Pratylenchus* spp. no solo, além de favorecer a atividade de microrganismos antagonistas naturalmente presentes no ambiente (SILVA et al., 2018).

Adicionalmente, o uso de adubos verdes, como *Crotalaria spectabilis*, *C. ochroleuca* e *C. breviflora*, pode modificar as propriedades físicas e biológicas do solo, contribuindo para a supressão indireta de nematoides fitoparasitas e para o estímulo de organismos antagonistas, especialmente bacterívoros e fungívoros, que atuam na regulação da comunidade edáfica (HINDS et al., 2016).

Esses resultados reforçam a importância de práticas culturais e físicas no contexto do Manejo Integrado de Nematoides (MIN), que se consolida como uma estratégia promissora para o controle sustentável dessas pragas. Essa abordagem combina práticas químicas, físicas, culturais e biológicas, fundamentadas em diagnósticos precisos das espécies presentes e no monitoramento contínuo da dinâmica populacional (NICOL et al., 2011).

Entre as práticas biológicas integradas ao MIN, destaca-se o uso de microrganismos benéficos, como *Bacillus* e *Pseudomonas* spp., que atuam por diferentes mecanismos, incluindo o parasitismo de ovos, a produção de metabólitos com ação nematicida e a indução de resistência sistêmica nas plantas (VAGELAS e GOWEN, 2012; WALIA et al., 2000; HABAZAR et al., 2021; ANTIL et al., 2023).

O controle eficaz de espécies do gênero *Pratylenchus* requer, portanto, a combinação de múltiplas práticas adaptadas às condições locais, considerando fatores como tipo de solo, clima e sistema de cultivo. Assim, a adoção crescente de tecnologias baseadas em microrganismos, especialmente os bionematicidas, representa um avanço relevante no contexto do manejo sustentável, e será discutida com maior profundidade no tópico seguinte.

2.2 Bionematicidas no controle biológico de nematoides fitoparasitas

No cenário de crescente busca por estratégias sustentáveis e adaptadas à complexidade do ambiente agrícola, os bionematicidas vêm ganhando destaque como ferramentas promissoras no Manejo Integrado de Nematoides. No Brasil, observa-se um crescimento consistente desse mercado: de acordo com dados do sistema

AGROFIT atualizados até março de 2024, há 785 biopesticidas registrados, sendo 78 deles classificados como nematocidas microbiológicos, o que representa aproximadamente 9,9% do total (MAPA, 2024). Esse avanço reflete o interesse crescente por alternativas mais seguras e ambientalmente responsáveis no controle de nematoides fitoparasitas.

No cenário internacional, o setor de biopesticidas também vem se expandindo. Em 2023, esse mercado movimentou cerca de US\$ 7,54 bilhões, com estimativas apontando para US\$ 8,73 bilhões em 2024 e podendo alcançar US\$ 28,61 bilhões até 2032. Entre os produtos biológicos, os bionemáticos estão entre os que apresentam as maiores taxas de crescimento, com estimativa de 16% ao ano no período de 2024 a 2032 (FORTUNE BUSINESS INSIGHTS, 2024).

Entre os microrganismos mais utilizados na formulação de bionemáticos destacam-se as bactérias dos gêneros *Bacillus* e *Pseudomonas* (MIGUNOVA e SASANELLI, 2021), além de fungos nematófagos como *Purpureocillium lilacinum* (RIGOBELLO et al., 2024). Esses microrganismos atuam por diferentes mecanismos, como a produção de enzimas e metabólitos com efeito nematocida, competição por recursos e espaço, e indução de resistência nas plantas hospedeiras (MIGUNOVA e SASANELLI, 2021).

No caso de *Bacillus subtilis*, trata-se de uma das espécies mais utilizadas comercialmente, em razão de sua robustez, capacidade de formar esporos resistentes e ampla atividade antagonista (CAWOY et al., 2011). Essa bactéria forma biofilmes protetores nas raízes e produz compostos como subtilina, bacitracina, bacilina, subtenolina e bacilonicina (KILLANI et al., 2011), além de voláteis com ação nematocida como 2-nonanona e dissulfeto de dimetila (KAVITHA et al., 2012). Também possui potencial de induzir resistência sistêmica em plantas hospedeiras (VLAMAKIS et al., 2013).

Estudos demonstram sua eficácia na redução de populações de *Meloidogyne incognita* e *Pratylenchus spp.* em culturas como soja, tomate, milho e cana-de-açúcar, com efeitos sustentados ao longo de múltiplos ciclos de cultivo (OLIVEIRA et al., 2019; FERNANDES et al., 2018; FAVORETTO et al., 2024; SCHOEN-NETO et al., 2019).

Outro grupo bacteriano com grande potencial é o das *Pseudomonas spp.*, que se destacam pela capacidade de colonizar a rizosfera, formar microcolônias e

biofilmes na superfície das raízes, dificultando a penetração dos nematoides e promovendo a liberação de compostos antimicrobianos (COUILLEROT et al., 2009; SINGH e SIDDIQUI, 2010).

Diversas cepas do gênero têm demonstrado eficácia contra nematoides fitoparasitas como *Meloidogyne incognita*, *M. javanica*, *M. graminicola* e *Pratylenchus loosi* (JAHANBAZIAN et al., 2015; KHAN et al., 2016; RAHANANDEH et al., 2012). Em experimentos com tomate em casa de vegetação, reduções superiores a 40% em juvenis, fêmeas, galhas radiculares e massas de ovos foram observadas com o uso de *Pseudomonas fluorescens* (ANWAR-UL-HAQ et al., 2011).

Outras espécies, como *Pseudomonas putida* e *Pseudomonas aeruginosa*, também apresentaram efeito supressivo sobre nematoides como *Globodera rostochiensis* e *M. incognita* (TRIFONOVA et al., 2014; KHAN et al., 2008). Compostos bioativos como o ciclodipeptídeo cyclo (L-Pro–L-Leu), isolado de *Pseudomonas putida*, têm mostrado atividade nematicida significativa (ZHAI et al., 2019), embora mais estudos em campo sejam necessários para validar sua eficácia.

No grupo dos fungos entomopatogênicos e nematófagos, *Purpureocillium lilacinum* tem se destacado como um dos agentes biológicos mais utilizados no controle de nematoides fitoparasitas. Seu mecanismo de ação envolve a produção de enzimas hidrolíticas, como quitinases, serina proteases e fosfolipases, que promovem a penetração nos ovos dos nematoides e a degradação das estruturas embrionárias (YANG et al., 2015).

Estudos genômicos revelaram genes associados à virulência, como proteases, hidrolases e enzimas de adesão (PRASAD et al., 2015; XIE et al., 2016a). Além disso, *Purpureocillium lilacinum* produz metabólitos secundários com atividade nematicida, como leucinostatinas e lilacinas (CHEN e HU, 2021), e pode atuar também sobre fêmeas por meio de aberturas naturais (MOOSAVI e ZARE, 2020).

Sua eficácia foi demonstrada contra *Meloidogyne spp.*, *Pratylenchus thornei* e *Globodera rostochiensis* (KEPENEKCI et al., 2018; ISAAC et al., 2021; ZHANG et al., 2023). Aplicações na forma de microescleródios têm mostrado resultados superiores ao uso de conídios (SONG et al., 2016). A quantidade de produtos comerciais à base de *Purpureocillium lilacinum* é atualmente superior à de outros fungos utilizados no

controle de nematoides, com isolados disponíveis em países da América, Europa, Ásia e África (ABD-ELGAWAD e ASKARY, 2018).

No entanto, certos isolados apresentam potencial patogênico em humanos, sendo relatadas infecções oportunistas como onicomicose e infecções profundas em indivíduos imunocomprometidos (DE HOOG et al., 2000) o que reforça a necessidade de seleção criteriosa das cepas utilizadas (LUANGSA-ARD et al., 2011).

Apesar do seu potencial, o desempenho dos bionematicidas pode variar conforme o tipo de solo, a microbiota nativa e as condições ambientais (AYAZ et al., 2024; AL RAISH; SOURANI; ABU-ELSAOUD, 2025). Algumas questões como a estabilidade da formulação, a persistência no ambiente e a capacidade de colonização eficaz ainda representam desafios importantes para sua adoção em larga escala (MACHADO, 2022; WEND et al., 2024). Avanços na tecnologia de aplicação, no conhecimento genômico das cepas e na regulamentação específica serão fundamentais para superar essas limitações (ABD-ELGAWAD, 2024; AYAZ et al., 2024).

Dessa forma, os bionematicidas representam uma abordagem promissora no contexto da agricultura sustentável, especialmente no manejo de nematoides fitoparasitas como *Pratylenchus* spp. Para garantir sua adoção eficaz no campo, é fundamental compreender não apenas suas interações com a microbiota rizosférica nativa, mas também sua persistência, desempenho sob diferentes condições edafoclimáticas e compatibilidade com práticas agrícolas regionais.

2.3 A microbiota da rizosfera no controle biológico de nematoides fitoparasitas.

A rizosfera, camada de solo diretamente influenciada pelas raízes, forma um microambiente dinâmico onde plantas, microrganismos e nematoides interagem de forma complexa, afetando diretamente a saúde vegetal e a produtividade agrícola. Moldada pelos exsudados radiculares que atuam como sinalizadores químicos e fontes de nutrientes (MOHANRAM e KUMAR, 2019; BAIS et al., 2006), essa região concentra uma diversidade microbiana única, que inclui bactérias, fungos, nematoides e outros organismos, que podem estabelecer relações tanto benéficas quanto prejudiciais com as plantas (RAAIJMAKERS et al., 2009; MENDES et al., 2013).

O microbioma rizosférico desempenha função crucial no controle natural de nematoides fitoparasitas por meio de múltiplos mecanismos: antagonismo direto (como a produção de compostos nematocidas), competição por sítios de colonização e nutrientes, e indução de respostas de defesa nas plantas (BERENDSEN et al., 2012; MENDES et al., 2013).

A composição dessas comunidades microbianas é profundamente influenciada pela química dos exsudados radiculares, que determinam quais grupos se estabelecem e prosperam na rizosfera (BAIS et al., 2006). Em solos com histórico de supressividade natural, observa-se predominância de táxons microbianos como Actinobacteria e Firmicutes, conhecidos por seu potencial no controle de fitonematoides e na promoção da saúde do solo (BARROS et al., 2022).

A introdução de microrganismos no solo por meio de produtos biológicos representa um fator adicional na dinâmica ecológica da rizosfera, podendo desencadear interações complexas com a microbiota nativa, desde sinergias funcionais até competição por nichos ecológicos e recursos (BERENDSEN et al., 2012).

Abordagens baseadas em técnicas de sequenciamento de nova geração (NGS), como metabarcoding, têm sido utilizadas para avaliar essas alterações, revelando tanto efeitos positivos quanto negativos (KAUL et al., 2021). Alguns estudos mostram que cepas introduzidas de *Bacillus subtilis* e *Trichoderma harzianum* conseguiram se integrar à comunidade microbiana nativa sem causar desequilíbrios duradouros, promovendo inclusive o enriquecimento de grupos benéficos (GANUZA et al., 2019; POVEDA e EUGUI, 2022).

Por outro lado, há registros de mudanças transitórias na estrutura da microbiota e na abundância relativa de certos grupos funcionais, o que reforça a necessidade de monitoramento ambiental contínuo (POVEDA e EUGUI, 2022). Embora técnicas de sequenciamento de nova geração (NGS) e análises de comunidades microbianas tenham sido aplicadas para caracterizar alterações na microbiota rizosférica após aplicação de microrganismos, ainda são poucos os estudos que avaliam detalhadamente os impactos sobre diversidade, abundância relativa e estrutura comunitária em campo (TOPALOVIC et al., 2020).

Muitas investigações focam nas respostas da planta ou na redução do nematoide, sem contemplar plenamente os efeitos sobre a ecologia microbiana do solo (TOPALOVIĆ et al., 2020). Além disso, diversos antagonistas microbianos que demonstram potencial para controle de nematoides em condições de laboratório e estufa não mantêm o mesmo desempenho em campo, especialmente quando aplicados em diferentes regiões geográficas (TOPALOVIĆ et al., 2020; POVEDA; KAUL et al., 2021; POVEDA e EUGUI, 2022).

Essa inconsistência pode estar associada à dificuldade desses microrganismos em sobreviver e se estabelecer em comunidades microbianas complexas e ambientalmente diversas, ou à sua limitada capacidade de colonizar as raízes das plantas sob condições naturais adversas (BERENDSEN et al., 2012; KAUL et al., 2021).

Sabe-se que práticas de manejo que favorecem a diversidade microbiana, como rotação de culturas e adição de matéria orgânica, podem amplificar a supressividade natural do solo (MENDES et al., 2013). Evidências experimentais reforçam esse papel da microbiota: por exemplo, a transferência do microbioma do solo de áreas não infestadas para áreas infestadas reduziu significativamente a infecção por nematoides das galhas na planta hospedeira, sendo que o solo não infestado apresentava maior diversidade microbiana do que o solo infestado (KAUL et al., 2021).

Além disso, a transferência da microbiota da rizosfera de uma cultura para outra pode impedir que os nematoides fitoparasitas interajam com a planta hospedeira. No entanto, o efeito supressivo da própria microbiota rizosférica da planta tende a ser mais eficaz do que o da microbiota de outra cultura, oferecendo uma proteção superior contra a invasão por nematoides (ELHADY et al., 2018) e a adição de matéria orgânica, podem amplificar a supressividade natural do solo (MENDES et al., 2013).

Da mesma forma, a seleção de consórcios microbianos nativos, já adaptados às condições edafoclimáticas locais, pode potencializar a eficácia do controle biológico de nematoides, ao favorecer o estabelecimento de uma microbiota funcional, resiliente e com ação supressiva no solo (TOJU e TANAKA, 2019).

Portanto, compreender as interações entre bionematicidas e a microbiota do solo é essencial para o desenvolvimento de estratégias sustentáveis de manejo de fitonematoides. Avaliações que integrem dados sobre diversidade, abundância,

estrutura e funcionalidade microbiana são fundamentais para garantir que os benefícios do biocontrole sejam acompanhados pela manutenção da saúde e do equilíbrio ecológico da rizosfera.

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CAPÍTULO I – A *Bacillus subtilis* strain mitigates the root-lesion nematode *Pratylenchus* in a field study with maize and lima bean.

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Abstract

This study assessed the potential of a *B. subtilis* strain in controlling *Pratylenchus* in the roots of lima bean and maize under field conditions. Maize (*Zea mays* L.) and lima bean (*Phaseolus lunatus* L.) were submitted to three treatments: *B. subtilis* (strain AP-3) alone (a) or in combination with chemical fertilization (NPK) (b), and a treatment without *B. subtilis* inoculation (c). The inoculation of *B. subtilis* in both maize and lima bean seeds resulted in a decrease in the active forms of *Pratylenchus spp.* in their respective roots, reducing *Pratylenchus spp.* in maize (~90%) and lima bean (~40%) roots. *B. subtilis* also stimulated higher nodule number, biomass, and size, and increased the specific nodulation in lima bean. Therefore, the inoculation of *B. subtilis* can potentially to control *Pratylenchus* in the roots of maize and lima bean under field conditions and stimulate the nodulation in lima bean.

Keywords: *Saccharum officinarum*; plant microbes interaction; microbial ecology

1 Introduction

The control of pathogenic nematodes is a challenging issue to agriculture worldwide, and the most used methods include solarization (Gill et al., 2017), chemical pesticides (Desaeger et al., 2017) and the biological control with rhizobacteria. Among these methods, the biological control is more suitable and effective to agriculture since it involves various biological and biochemical mechanisms (Saad et al., 2022), such as production of antibiotics and metabolites, disruption of nematode signalization, and niche exclusion (Subedi et al., 2020).

The biological control of nematodes includes *Pseudomonas fluorescens*, *Bacillus subtilis*, and *B. velezensis* (Xiang et al., 2017; Borrajo et al., 2022). For instance, *B. subtilis* and *B. velezensis* strains showed high potential to reduce the population of *Meloidogyne* and enhance the growth of cotton.

As an important rhizobacterium, *B. subtilis* takes an advantage as this genus presents high potential to produce antibiotics (Mazzuchelli et al., 2020), including fengycin, surfactin, and iturin, which collectively contribute to its remarkable pathogen-controlling capabilities (Hashem et al., 2019). In addition, *B. subtilis* can disrupt the orientation by nematode reducing its ability of migration (Araujo et al., 2005).

Therefore, previous studies have reported *B. subtilis* efficiently controlling nematodes in roots of sugarcane (Mazzuchelli et al., 2020), soybean (Gardiano-Link et al., 2022), and common bean (Oliveira et al., 2017).

The genus *Pratylenchus* is well-known for causing root lesions in important crops, such as soybean and sugarcane (Severino et al., 2010; Oliveira et al., 2019). Previous studies have reported the adverse effects of *Pratylenchus* on maize growth and yield (Silva et al., 2017; Fabiyi et al., 2020), but its impact on lima bean growth remains unexplored.

Likewise, the potential of *B. subtilis* in controlling *Pratylenchus* in maize and lima bean is poorly understood. Considering the previous findings of *B. subtilis* controlling *Pratylenchus* in crops, we hypothesized that inoculating *B. subtilis* in lima bean and maize could effectively control *Pratylenchus* in their roots. Thus, this study aimed to evaluate the impact of *B. subtilis* on the population of *Pratylenchus* in the roots of lima bean and maize.

2 Material and methods

The study was conducted under field conditions at Federal University of Piauí, Teresina, Piauí, Brazil (05 ° 05'05"S and 42 ° 05'W, 72 m), from February to June 2023. The climate in this area is classified as Aw'(Köppen), with average annual temperatures of approximately 27 ° C and precipitation of around 1500 mm.

The soil in the experimental field is classified as fluvic Neossol and exhibits the following main properties: clay content - 14%, silt content - 16%, sand content - 70%, pH - 6.0, organic matter - 6.2 g kg⁻¹, P - 2.0 mg dm⁻³, K - 5.0 mg dm⁻³, and basis saturation (V) - 68%.

Before the experiment, soil samples were collected (0–20 cm depth) and processed to evaluate the population *Pratylenchus spp.* (root-lesion nematode) according to Jenkins (1964). The initial population of juveniles was ~1000 *Pratylenchus* per 100 g of soil.

The experiment involved the cultivation of maize (*Zea mays L.*) and lima bean (*Phaseolus lunatus L.*) using a randomized complete block design. Three treatments were applied with four replicates. The treatments included the inoculation of *B. subtilis* alone or in combination with chemical fertilization (NPK). A treatment without *B. subtilis* inoculation was used as control. *Bacillus subtilis* (strain AP-3) were grown on agar solid nutrient at 28 ° C for one week according to methodology proposed by Araujo et al. (2005). After one week, the cells were scraped and transferred to 100 mL of sterile distilled water and the mixture was stirred to form a homogeneous suspension and its concentration was determined and adjusted to 1.0 × 10⁸ cells per ml.

At the sowing, seeds of maize and lima bean were sown in experimental plots (12.8 m²), consisting of four rows (4.0 m), with the two central rows considered as the useable area. Before the inoculation in the seed, a suspension was prepared mixing 100 mL of the concentrated suspension (1.0 × 10⁸ cells per mL) to 10 L of water.

To proceed the inoculation, we applied 100 mL of the suspension in the seeds of maize and lima bean (three seeds of each genotype) directly into each furrow which contained the seeds. The spacing between rows was 0.80 m, while the spacing between plants was 0.5 m.

During the flowering period, which varied according to maize and lima bean development, all plants were sampled individually. The roots were carefully separated

from the shoots, and to eliminate excess soil, all roots were immersed in water. The weight of the roots was determined through gravimetry.

For the lima bean plants, all nodules present in the roots were collected and measured in terms of number, biomass, size (the ratio between nodule biomass and number), and specific nodulation.

To extract the nematodes, the roots were washed with water and then sieved using mesh sizes of 100 and 500 (pore sizes of 0.841 mm and 0.046 mm, respectively).

The retained material was combined with the corresponding root samples for nematode extraction. The root material was homogenized using a blender, and nematodes were extracted through centrifugal flotation in a sucrose solution containing kaolin, following the method described by Coolen and D'Herde (1972).

To estimate the total number of *Pratylenchus* per gram of lima bean and maize root, a Peters counting chamber and a microscope (60x) were employed.

The statistical analyses were performed using R software (version 4.2.3) and RStudio software (version 2023.06.0–421). The data were analyzed by analysis of variance (ANOVA) preceded by the F test ($p < 0.05$), and Dunnett's test was performed to assess statistical differences between treatments ($p < 0.05$).

3 Results and discussion

The inoculation of *B. subtilis* decreased the active forms of *Pratylenchus spp.* in roots of maize and lima bean (Fig. 1). Furthermore, the combined application of NPK and *B. subtilis* did not show any significant difference compared to *B. subtilis* alone. Compared to the treatment without *B. subtilis* (control), we observed a higher efficacy in reducing *Pratylenchus spp.* in maize roots (~90%) compared to lima bean roots (~40%) when *B. subtilis* was inoculated.

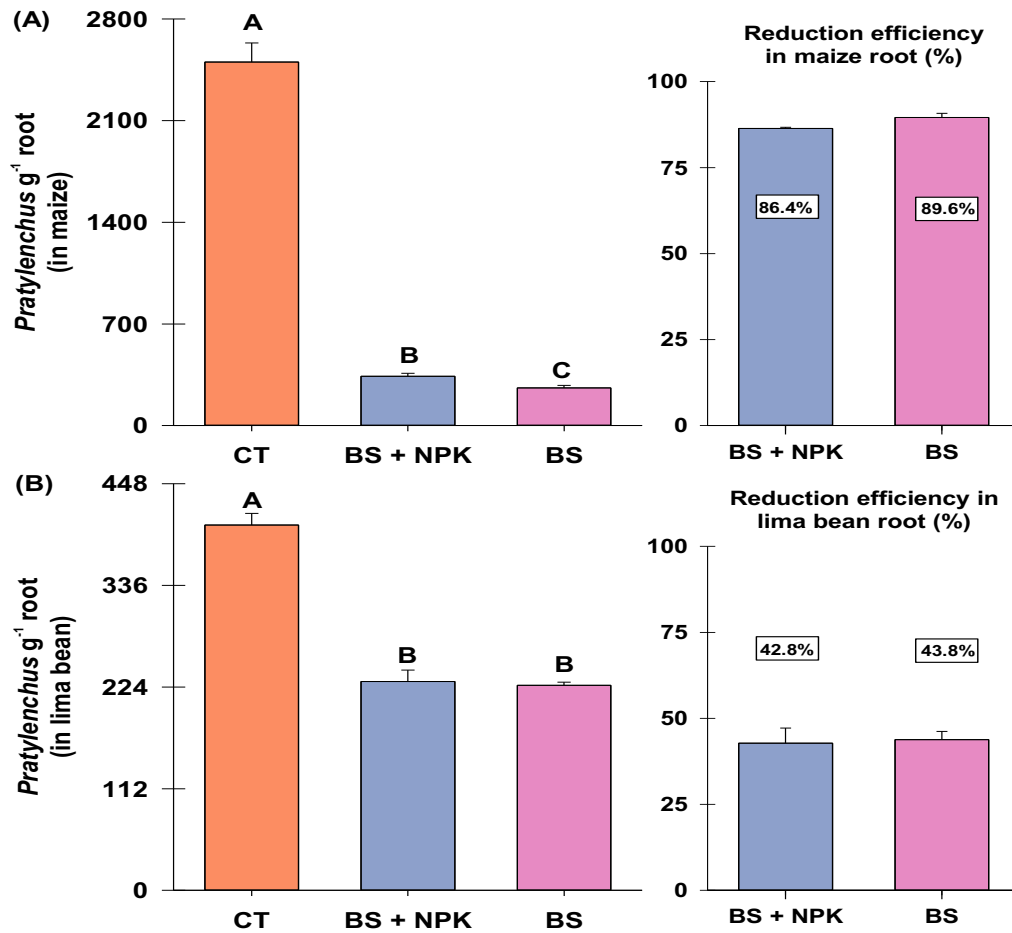


Figure 1. Population of *Pratylenchus* and reduction efficiency by *Bacillus subtilis* in maize (A) and lima bean (B) roots.

In maize and lima bean, the inoculation of *B. subtilis* (without NPK) promoted the greatest root length and biomass (Fig. 2). It was observed 35% and 50% increase in root dry weight of the maize and lima bean inoculated with *B. subtilis* (without NPK), respectively. When assessing nodulation traits, the results demonstrated that the highest nodule number, biomass, and size were observed in plants inoculated solely with *B. subtilis* (Fig. 3). Specific nodulation was also greatest when *B. subtilis* was inoculated alone. Interestingly, a decrease in nodulation was observed when *B. subtilis* was combined with NPK.

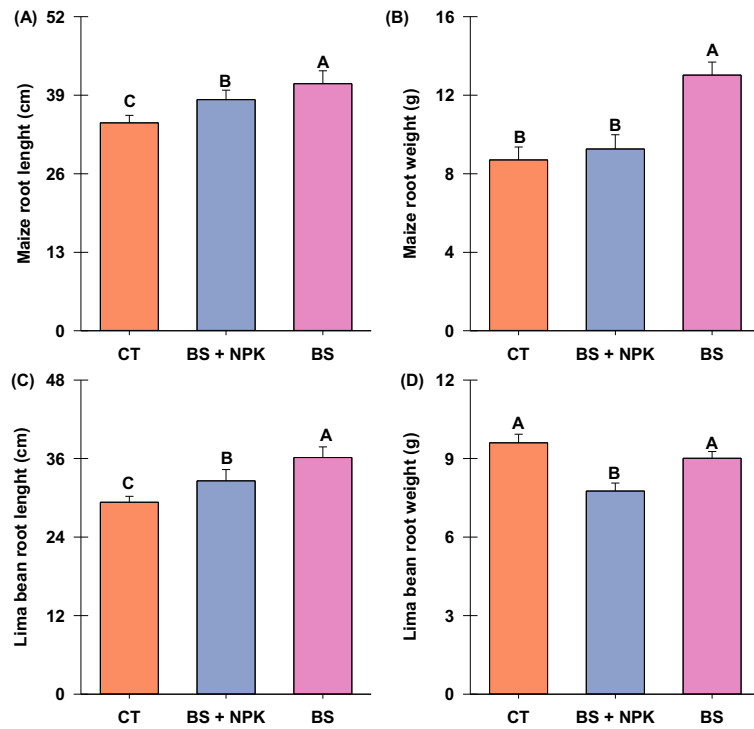


Figure 2. Root length and weight of maize (A, B) and lima bean (C, D) stimulated by *Bacillus subtilis*.

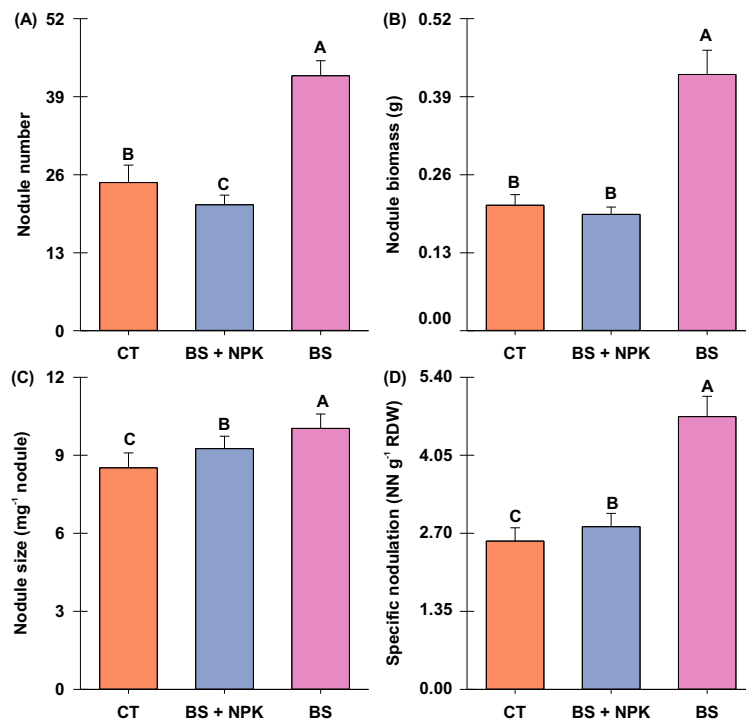


Figure 3. Nodule number (A), biomass (B), and size (C), and specific nodulation (D) in roots of lima bean stimulated by *Bacillus subtilis*.

This study applied *B. subtilis* as potential biocontroller of root-lesion nematode in maize and lima bean. In line with the hypothesis, *B. subtilis* effectively controlled *Pratylenchus* in roots of both plant species. In contrast, the combination of NPK and *B. subtilis* did not show any significant effect, which suggests that the chemical fertilization does not enhance the effectiveness of *B. subtilis* in reducing the *Pratylenchus* population. Although there are not reports about the interaction of *B. subtilis* and chemical fertilization on nematode control, a previous study has found that the chemical fertilization in soybean and maize contributed to decrease the population of *B. subtilis* in soil (Bueno et al., 2022). Thus, the application of NPK probably harmed the *B. subtilis* in controlling the *Pratylenchus* in roots of maize and lima bean.

This study showed a high efficacy of *B. subtilis* in reducing the population of *Pratylenchus* in the roots of maize and lima bean. The control of *Pratylenchus* by *B. subtilis* can start by the disruption of nematode migration (Araujo et al., 2021). Thereafter, once the ability of *Pratylenchus* to migrate is disrupted, *B. subtilis* can control the nematode by several mechanisms, such as producing antibiotics (Hashem et al., 2019) and siderophores (Maheshwari et al., 2019).

These results are promising since previous studies have reported an average of 50% in the reduction of *Pratylenchus* with the inoculation of *B. subtilis* in sugarcane and common bean (Wepuhkhulu et al., 2011; Mazzuchelli et al., 2020).

Our results showed that the reduction of *Pratylenchus* by *B. subtilis* was more pronounced in maize roots (~90%) as compared to lima bean roots (~40%). This different efficacy of *B. subtilis* in suppressing *Pratylenchus*, comparing maize and lima bean roots, suggests that this bacterium can present a varied response according to different plant species.

Since the population of *Pratylenchus* is reduced, the root growth of maize and lima bean was stimulated by *B. subtilis*. Indeed, we observed that *B. subtilis* suppresses *Pratylenchus* and stimulates root development in maize and lima bean, being observed that these plants exhibit longer (~20%) and heavier (~50%) roots than those plants without inoculation. A previous studies have reported *B. subtilis* inhibiting *Meloidogyne* and increasing the root biomass in cowpea (Abd-El-Khair et al., 2019).

Hashem et al. (2019) reported that the positive effect of *B. subtilis* in stimulating the root growth is due its ability to produces a wide range of growth-promoting

phytohormones and to solubilize phosphates. Interestingly, Santos et al. (2020) observed higher root growth in plants inoculated with *B. subtilis* that were attacked by pathogens, such as *Fusarium* and *Rhizoctonia*. It is possible that the positive action of the *B. subtilis* observed in our study reflects the combination of inhibition of nematode growth, through the release of extracellular enzymes with nematicide action, with the release of growth-stimulating substances in the rhizosphere of these plants.

Regarding to nodulation in lima bean, plants that were inoculated solely with *B. subtilis* exhibited the highest nodule number, biomass, and size, which suggests that *B. subtilis* inoculation, without chemical fertilization, positively influenced nodulation traits in lima bean. In addition, the specific nodulation was stimulated by *B. subtilis*.

The higher nodule number, biomass, and size indicate an enhanced ability of lima bean to fix nitrogen (Brito et al., 2023), which is essential for their growth and development. Interestingly, the higher specific nodulation (the ratio of nodulation and root growth) suggests a positive effect of *B. subtilis* on nodule initiation and development (Gulden and Vessey, 1998). It is important since the lima bean may have its natural nodulation faster with the inoculation of *B. subtilis*. A previous study has also reported higher specific nodulation in soybean with the inoculation of *B. subtilis* (Araujo et al., 2021).

4 Conclusion

This study shows that *B. subtilis* can effectively to control *Pratylenchus* in the roots of maize and lima bean under field conditions. In addition, *B. subtilis* promotes higher root growth and stimulates the nodulation in lima bean.

This study suggests the recommendation of inoculating *B. subtilis* as potential to control *Pratylenchus*, being an economically and environmentally strategy to decrease the negative effect of root-lesion nematode on maize and lima bean.

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CAPÍTULO II – Inoculation of microorganisms for the *Pratylenchus zae* control in sugarcane: potential and response of rhizosphere bacterial communities

Abstract

Sugarcane is a globally important crop for ethanol production and is vital in meeting bioenergy demands. However, its productivity is compromised by plant-parasitic nematodes, particularly *Pratylenchus zae*, which damage root systems and reduce yields. Although chemical nematicides are still widely used, they raise environmental and toxicological concerns. In this study, we evaluated the efficacy of microbial inoculants (*Bacillus subtilis*, *Bacillus amyloliquefaciens*, *Pseudomonas oryzihabitans*, *Bacillus subtilis* + *Bacillus licheniformis*, and *Purpureocillium lilacinum* + *Bacillus subtilis* + *Bacillus licheniformis*) for suppressing *P. zae* in sugarcane under greenhouse conditions and assessed their effects on the rhizosphere bacterial microbiota. Among treatments, *P. oryzihabitans* was the most effective, reducing nematode populations by 64% in the rhizosphere soil and 81% in the roots. *B. amyloliquefaciens* also showed strong efficacy, with reductions of 63% and 72%, respectively. In contrast, the co-inoculation of *B. subtilis* and *B. licheniformis* had the lowest suppression rates (47% in soil and 30% in roots). In addition to nematode control, some treatments improved soil health indicators such as microbial biomass and enzymatic activity, without causing significant shifts in overall bacterial community structure. Although no major structural changes were observed for the treatment with *P. oryzihabitans*, which showed the highest nematode suppression, it also led to the greatest bacterial diversity in the rhizosphere. These findings suggest that microbial inoculants are promising alternatives to chemical nematicides, contributing both to nematode management and the maintenance of soil health in sugarcane production systems.

Keywords: Root lesion nematode, Nematode suppression, 16S rRNA gene sequencing, Microbial diversity

1. Introduction

Sugarcane is one of the most important crops worldwide, being cultivated in more than 100 countries. Despite being important in producing sugar, sugarcane has critical implications as a crop for ethanol production, and thus for the reduction of greenhouse gas emissions from fossil fuels (Raza et al. 2019).

One of the major challenges of sugarcane production is the attack by pathogens and parasites (Grandis et al. 2024). Specially, plant-parasitic nematodes (PPN) are microscopic worms belonging to the phylum Nematoda found in soils, rhizosphere and roots, and represent a significant challenge to crop production (Zhang et al. 2020). Indeed, the attack of PPN reduces the growth and productivity of crops, accounting for an estimated 12% of annual crop losses, equivalent to approximately 125 billion dollars (Mendoza-de Gives et al. 2022). In sugarcane, some nematode species promote a disruption of the root growth, which diminishes water and nutrient uptake efficiency (Khanna et al., 2021). In particular, the *Pratylenchus zaeae*, a well-known PPN, produces root lesions in sugarcane and causes significant productivity losses (Dinardo-Miranda et al. 2019; Bhuiyan et al. 2021). Consequently, the use of strategies to control *P. zaeae*, while maintaining the soil and plant health, are necessary to promote the sustainability of sugarcane production.

Several strategies have been applied in the sugarcane fields to control *P. zaeae*, including chemical and biological methods. Although highly effective in the short term, the chemical products have the potential to develop resistant nematode species and non-desired effects on the soil microbiome by eliminating non-target species (Seong et al. 2021). The use of biological strategies, mainly by inoculating beneficial microorganisms, has been stimulated as an ecological tool to control pathogens and allow the long-term development of sustainable agriculture. Yet, we are still trying to compare the efficiency of these microorganisms in controlling *P. zaeae* and verify their potential effect on the rhizosphere microbiome. Unlike chemical products, the use of microorganisms to control PPN offers several advantages, when preserving beneficial organisms and providing higher efficiency and persistence in ecosystems (Abd-Elgawad and Askary 2020; He et al. 2021), which eliminates the need for repeated

applications, as the introduced biological agents can sustain their populations and continue to suppress pests over time (Rahman et al. 2024).

The biological control of PPN within the rhizosphere often employs bacteria (Antil et al. 2023) and fungi (Poveda et al. 2020) that leverage natural modes of action, including competition, antibiosis, and plant growth promotion (Bhat et al. 2023). In controlling PPN, the biological strategy commonly involves *Bacillus subtilis* application, exhibiting high efficiency against root-lesion nematodes (Dinardo-Miranda et al. 2022; Mazzuchelli et al. 2020). Nevertheless, various other bacterial and fungal species hold the potential for controlling root-lesion nematodes while simultaneously contributing to plant growth (Rahman et al., 2024). For example, the fungi *Purpureocillium lilacinum* can control *P. zaeae* by secreting enzymes to degrade the nematode eggshell and deploying mechanisms involved in nematode parasitism (Moreno-Gavira et al. 2020).

However, little is known about the efficiency of distinct microorganisms to control *P. zaeae* in sugarcane roots and the potential changes in bacterial communities in the rhizosphere. The understanding of how these microorganisms affect the bacterial communities is important since bacteria play important functions in soil and rhizosphere by enhancing plant growth and pathogen control (Pantigoso et al. 2022). Thus, any potential changes in the bacterial communities promoted by inoculating microbial agents, such as beneficial bacteria or fungi, can potentially enhance or disrupt their microbial functions (Ahsan et al. 2024). Assessing the efficiency of each microorganism as a biological agent for bionematicides is crucial for its potential application in sugarcane fields to control *P. zaeae*.

Therefore, we hypothesized that: a) different microorganisms show distinct performance of control against *P. zaeae* in sugarcane roots, and b) these distinct microorganisms would change the rhizosphere microbiome.

2 Material and methods

2.1 Experimental site and treatments

The soil samples (0-20 cm depth) were collected from a sugarcane monoculture field in Teresina, Piauí, Brazil (04°52'56" S, 42°52'58" W; 68 m) with a 10-year crop history conducive to the establishment of *P. zaeae*. The sampling followed a systematic

methodology, with soil samples collected at regular intervals across the field to ensure spatial representativeness.

The climate is classified as tropical (Aw), with an average annual rainfall of 1,500 mm and an average temperature of 27°C. The soil is classified as Fluvisol (70% sand, 14% silt, and 16% clay). The main chemical parameters of the soil were as follows: pH – of 5.5; soil organic matter - 15 g kg⁻¹; P - 8 mg kg⁻¹; K - 127 mg kg⁻¹; base saturation (V) - 70%. The soil collected in the field was homogenized, and six samples were collected and submitted by extraction and following quantification of *P. zae* using the centrifugal flotation technique in a 45.4% sucrose solution (Jenkins, 1964). The initial population averaged around 1400 individuals per pot.

The experiment was conducted in a greenhouse belonging to Plant Science Department (Federal University of Piauí) from September to November 2023. The pots (25 L) were arranged in a completely randomized design with six replicates. The treatments consisted of commercial products containing microorganisms and one experimental strain of *B. subtilis* (AP-3) with potential for nematode suppression (Mazzuchelli et al. 2020, Costa et al., 2023). These microorganisms were applied in single, co-inoculation, and triple inoculation forms: i) *Bacillus subtilis* strain AP-3, ii) Nemacontrol™ (*Bacillus amyloliquefaciens*); iii) Biotrinsic™ (*Pseudomonas oryzihabitans*); iv) Quartzo™ (*Bacillus subtilis* + *Bacillus amyloliquefaciens*); v) Profix™ (*Purpureocillium lilacinum* + *Bacillus subtilis* + *Bacillus licheniformis*). Additionally, a control treatment (CK) was included. Additionally, a control treatment (CK), with no inoculation, was applied.

The suspensions of microorganisms used in treatments were prepared according to the concentrations recommended by commercial bionematicide manufacturers. A total of 3.5 mL of *Bacillus subtilis* (strain AP-3, concentration 1.0 x 10⁸ CFU/mL); 612.5 µL of *Bacillus amyloliquefaciens* (strain SIMBI BS 10, concentration 5.0 x 10⁹ CFU/mL); 157.5 mg of *Pseudomonas oryzihabitans* (strain SYM23945, concentration 1.0 x 10⁷ CFU/mL); 875 mg of a combination of *Bacillus subtilis* (strain FMCH002, concentration 1.0 x 10¹¹ CFU/mL) and *Bacillus licheniformis* (strain FMCH001, concentration 1.0 x 10¹¹ CFU/mL); and 262.5 mg of a triple mixture containing *Purpureocillium lilacinum* (strain CPQBA 040-11 DRM 10, concentration 9.0 x 10⁹ CFU/mL), *Bacillus subtilis* (strain ATCC 6051), and *Bacillus licheniformis* (strain

ATCC 12713) were used. Each suspension was diluted in 350 mL of water, and 3.73 mL of each suspension was sprayed directly into simulated planting furrows in individual pots.

Preliminary tests were conducted to evaluate the cell viability, survival under storage conditions, colonization capacity, and nematode control efficacy of *Bacillus subtilis* AP-3, following the recommendations provided by Embrapa (2023). Additionally, similar viability tests were performed for commercial bionematicides before application to ensure the presence of viable cells and guarantee their efficacy. These evaluations were essential to confirm the stability, safety, and functionality of all inoculants, thereby ensuring the reliability of the experimental results.

Before the planting, sugarcane stalks (variety RB92579) were placed in germination boxes (Gerbox) for a period of 10 days. Subsequently, these pre-germinated stalks were transferred to pots, containing infested soil with *P. zea*. Each experimental treatment was inoculated, using spray inoculation in the planting furrow, with a depth of 15 cm and a width of 10 cm. Daily irrigation was carried out using the gravimetric method based on soil water retention capacity, maintaining soil moisture at 70% of field capacity. All plants were harvested 65 days after treatments inoculation.

2.2 Soil sampling

Sugarcane plants were harvested from all treatments 65 days after planting. Roots were separated, and soil samples were collected for analysis of biological parameters. All analyses were subsequently conducted in the laboratory. The microbial biomass carbon (MBC) was assessed according to Vance et al. (1987), through the extraction of C from fumigated and unfumigated soils by potassium dichromate. An extraction efficiency coefficient of 0.41 was applied to convert the difference in C between fumigated and unfumigated soil in microbial biomass C. Soil respiration was estimated by quantifying the CO₂ released during seven days of soil incubation in a closed system. The CO₂ produced was captured in a NaOH (1 mol L⁻¹) solution and subsequently titrated with HCL (0.05 mol L⁻¹) (Anderson 1982).

The urease activity was determined according to Kandeler and Gerber (1988), using urea as a substrate and the amount of ammonia produced was measured by spectrophotometry. Arylsulfatase activity was determined following the methodology of

Tabatabai and Bremmer (1970). Soil samples (0.5 g) were incubated with 2 mL of acetate buffer (0.5 mol L⁻¹, pH 5.8) and 0.5 mL of p-nitrophenyl sulfate (PNS) for 1 h at 37°C. Subsequently, after filtering with CaCl₂ and NaOH.

The β-glucosidase (GLI) was determined according to the method described by Eivazi and Tabatabai (1988). Shortly, 0.5 g of soil was weighed and combined with 2 mL of MUB buffer (pH 6.5) along with 0.5 mL of a 25 mM solution of p-nitrophenol-β-D-glucopyranoside. The samples were then incubated at 37°C for 1 hour. After this incubation period, 0.5 mL CaCl₂ (0.5 M) and 2 mL Tris buffer at pH 12 were added. The activity of acid phosphatase was evaluated through colorimetric estimation of p-nitrophenol (Tabatabai and Bremmer 1969). All enzyme activities were ultimately quantified using spectrophotometric analysis.

2.3 Sampling and extraction of nematodes (roots and soil)

The number of *P. zaeae* individuals in 100 g of soil was determined by the centrifugal sugar-flotation method (Jenkins 1964). The nematode suspensions were sieved through mesh sizes of 100 and 400 (pore sizes of 0.841 mm and 0.038 mm, respectively). For the extraction of *P. zaeae* from roots, 10 g of root samples were weighed and homogenized in 100 mL of water using a blender.

The nematodes were separated using the centrifugal flotation method in a 45% sucrose solution containing 1.5 g of kaolin, following the protocol described by Coolen and D'Herde (1972). The nematode suspensions were sieved through mesh sizes of 100 and 500 (pore sizes of 0.841 mm and 0.046 mm, respectively), with the finer 500 mesh sieve ensuring the retention of nematode eggs and enhancing recovery efficiency from root samples.

The samples were stored at 10°C until the quantification. A Peters counting chamber and a microscope (60x) were used to estimate the population density of *P. zaeae* in 100 cm³ soil or per gram of sugar cane root.

2.4 DNA extraction and amplicon sequencing

DNA extraction was done using 0.5 g of rhizospheric soil samples and a DNA Isolation Kit (DNeasy PowerSoil Pro Kit; Qiagen, CA, USA). Quality of the DNA was checked using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA).

The primers 515F (5' -GTGYCAGCMGCCGCGGTAA-3') and 806R (5'-GGGACTACHVHHHTWTCTAAT-3'), along with dual-index barcodes for sample differentiation, were employed to amplify the V4 region of the 16S rRNA gene through polymerase chain reaction (PCR) (Caporaso et al. 2011). The PCR program consisted of an initial denaturation step at 94 °C for 3 min, followed by 35 cycles at 94 °C for 45 s, 50 °C for 60 s, and 72 °C for 90 s; a final extension of 10 min at 72 °C.

PCR products were purified using Agencourt AMPure XP beads (Beckman Coulter, Brea, CA, USA) and quantified with a Qubit fluorometer using the dsDNA BR Assay kit (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Subsequently, the libraries underwent paired-end sequencing utilizing the Illumina MiSeq Reagent Kit v2 (300-cycles, 2 × 150 bp) (Illumina) at the Genomic and Bioinformatic Facility Centre (CeGenBio) of the Federal University of Ceará, Brazil.

Microbial sequencing data were processed using USEARCH10 and QIIME2. Primer sequences and low-quality ends were removed, and paired-end reads were merged in USEARCH. The resultant sequences were quality-filtered (maximum expected error 1.0) and singletons were removed in USEARCH. Amplicon Sequence Variants (ASVs) were identified at 100% similarity using UNOISE3 in USEARCH with default parameters. Bacterial representative sequences were classified using SILVA (v13.8) databases in QIIME2 (Edgar 2016).

2.5 Data analyses

Data collected from the experiment, including nematode populations and soil biological indicators, were analyzed to evaluate the efficiency of different bionematicide treatments. Statistical analyses were performed using R software (version 4.3.1) in RStudio (version 2023.12.0 Build 369). First, the data were tested for normal distribution and homogeneity of variance using the Shapiro-Wilk and Levene tests, respectively. When necessary, data were transformed using $\log_{10}(x+1)$ to meet the assumptions of normality and homogeneity of variance.

Data were analyzed using one-way analysis of variance (ANOVA), followed by an F-test to determine significance at $p < 0.05$. Significant differences between treatments were further evaluated using the Scott-Knott test with a significance threshold of $p < 0.05$.

We calculated the nematode suppression efficiency for each treatment by determining the percentage reduction in *P. zaeae* population relative to the untreated control (CK). The following formula was used:

$$\text{Reduction efficiency (\%)}: \left(\frac{\text{CK} - \text{Treatment}}{\text{CK}} \right) \times 100$$

Where CK is the nematode population in the untreated control, and Treatment is the population in each biocontrol treatment. The percentages are shown in the right panels of Figure 1, illustrating each treatment's effectiveness in suppressing *P. zaeae* in sugar cane roots and soil.

For the microbiome data, alpha diversity metrics (observed richness, Shannon, and Chao1 indices) and beta diversity metrics were calculated using the Phyloseq package (McMurdie and Holmes, 2013, v.1.34.0) in R software (R Core Team, 2023). Alpha diversity was calculated and visualized using the `plot_richness` function in Phyloseq, providing insight into within-sample diversity.

Beta diversity was analyzed to explore differences in microbial community composition between bionematicide treatments, with results visualized using Non-Metric Multidimensional Scaling (NMDS) plots, focusing on the NMDS1 and NMDS2 axes.

Additionally, permutational multivariate analysis of variance (PERMANOVA) was performed using the `adonis2` function in the `vegan` package (Oksanen et al., 2024) with Bray-Curtis distances to assess changes in community structure between treatments. PERMANOVA tests were performed with 999 permutations to determine significant differences in beta diversity between treatments.

Spearman's correlations between *Pratylenchus zaeae* populations in soil and roots, enzymatic activities, soil attributes, and microbial communities were analyzed. Additionally, a Mantel test was performed to assess overall associations using the "linkET" package (Sun et al., 2022). All plots were visualized using the "ggplot2" package."

3 Results

All inoculated microorganisms were effective in suppressing *P. zaeae* populations in both soil and sugarcane roots (Fig. 1), with differences observed in their reduction

efficiencies when compared directly. Compared to the control, the highest reduction of the *P. zaeae* in both soil (~64%) and sugarcane roots (~81%) was found with the inoculation of *P. oryzihabitans*. The inoculation with *B. amyloliquefaciens* also showed a high efficiency in reducing *P. zaeae* (~63% and 72% in soil and roots, respectively). The lowest efficiency in controlling *P. zaeae* was found with the co-inoculation *B. subtilis* + *B. licheniformis* (47% and 30%, in soil and roots, respectively).

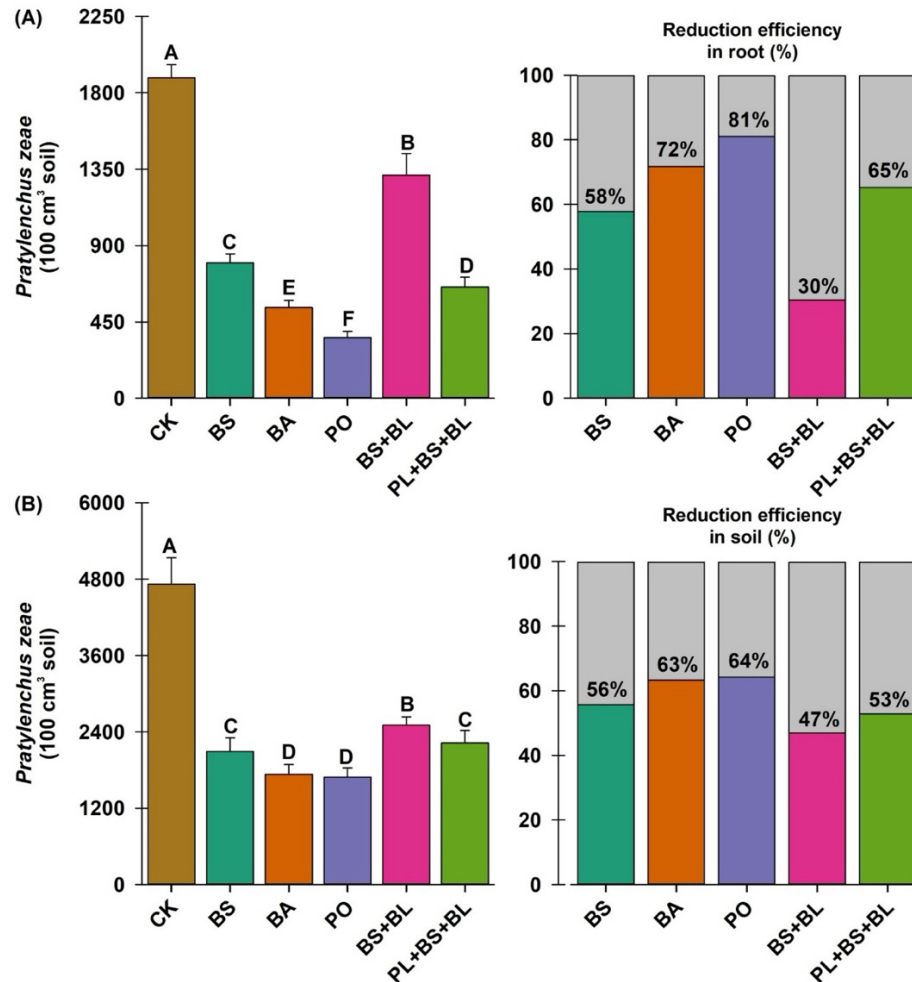


Fig. 1 Population of *Pratylenchus zaeae* in sugarcane roots and soil and reduction efficiency by the treatments of *Bacillus subtilis* (BS); *B. amyloliquefaciens* (BA); *P. oryzihabitans* (PO); *B. subtilis* and *B. licheniformis* (BS+BL); and *P. lilacinum*, *B. subtilis* and *B. licheniformis* (PL+BS+BL), compared to a control (CK)

Different letters represent significant differences by Scott-Knott' test ($p < 0.05$) and error bars represent the standard error of the mean (SE) for each treatment.

The inoculation of microorganisms had a positive effect on soil microbial biomass and enzymatic activity (Fig. 2). The non-inoculated soil showed low enzymatic activity and microbial biomass compared to those inoculated soils. The triple inoculation with *P. lilacinum*, *B. subtilis*, and *B. licheniformis* promoted an increase in urease, arylsulfatase, and soil respiration. The inoculation with *B. subtilis* also increased urease and β -glucosidase; while *P. oryzihabitans* increased urease, acid phosphatase, and soil respiration. The inoculation of *B. amyloliquefaciens* promoted the highest values for microbial biomass C.

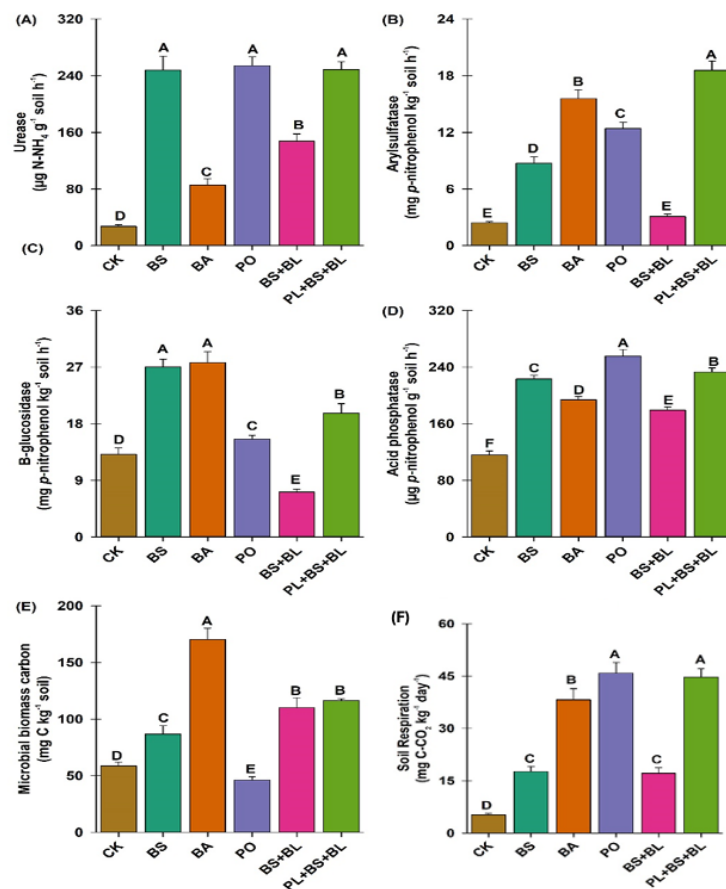


Fig. 2 (A-D) Soil enzyme activities in sugarcane soil inoculated with different bionematicides: (A) Urease, (B) Arylsulfatase, (C) β -glucosidase, and (D) Acid phosphatase, and (E) microbial biomass C, and (F) soil respiration in sugarcane soil after the application of different bionematicides: (E) Microbial biomass carbon, (F) Soil respiration. Treatments included *Bacillus subtilis* (BS), *B. amyloliquefaciens* (BA), *P. oryzihabitans* (PO), *B. subtilis* and *B. licheniformis* (BS+BL), *P. lilacinum*, *B. subtilis* and *B. licheniformis* (PL+BS+BL), and control treatment (CK).

Different letters indicate significant differences by Scott-Knott test ($p < 0.05$) and error bars represent the standard error of the mean (SE) for each treatment

The bacterial community was composed of Actinobacteriota, Proteobacteria, and Firmicutes as the top three dominant phyla (Fig. 3a). Other bacterial phyla were present in lower relative abundances, including Acidobacteriota, Chloroflexi, and Planctomycetota. At the genus level, *Bacillus* was more abundant in the soil and treatments involving *Bacillus* species (*B. amyloliquefaciens*; and co-inoculation *B. subtilis* and *B. licheniformis*). Furthermore, *Sphingomonas*, *Vicinamibacteraceae*, and *Gaiella* were observed to be relatively more prominent in the inoculated soils. Additionally, *Streptomyces* and *Thermoactinomyces* were abundant (Fig. 3b).

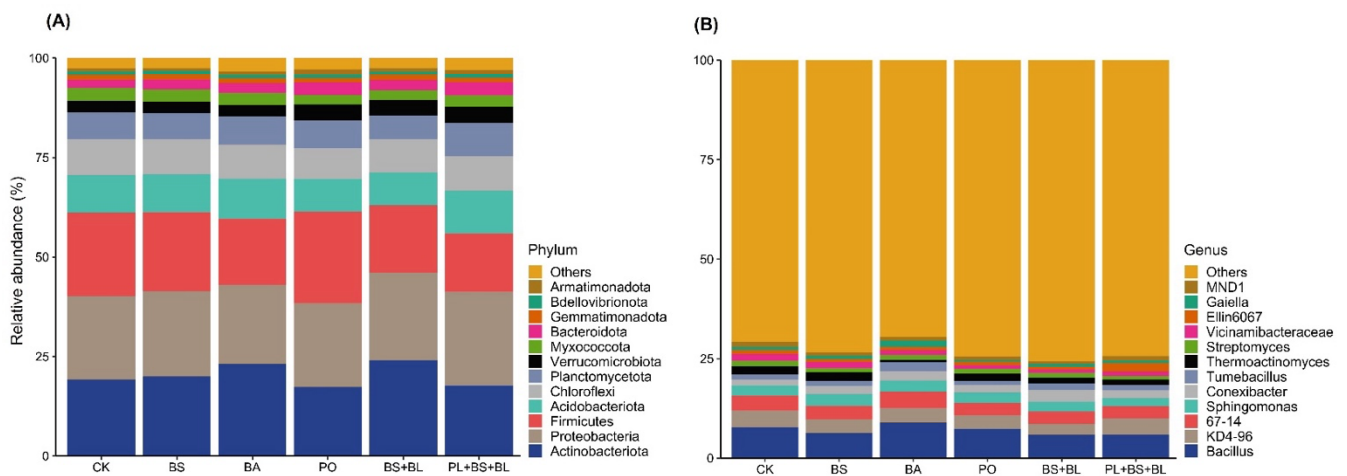


Fig. 3 Bacterial community analysis under different treatments. Relative abundance of bacterial phyla (A) and genera (B) across the control (CK), and the treatments inoculated with different microorganisms: *Bacillus subtilis* (BS); *B. amyloliquefaciens* (BA); *P. oryzihabitans* (PO); *B. subtilis* and *B. licheniformis* (BS+BL); *P. lilacinum*, *B. subtilis* and *B. licheniformis* (PL+BS+BL)

The highest values of chao1 and observed ASV were found with the inoculation of *P. oryzihabitans*, while the lowest values were found with co-inoculation (*B. subtilis* + *B. licheniformis*) and triple inoculation (*P. lilacinum* + *B. subtilis* + *B. licheniformis*) (Fig. 4a). In addition, the values of Shannon did not vary between treatments. The structure of bacterial communities did not show a clear clustering when comparing all treatments (Fig. 4b). The non-metric multidimensional scaling (NMDS) analysis of bacterial communities, with a stress value of 0.097, revealed no significant changes along the NMDS1 and NMDS2 axes.

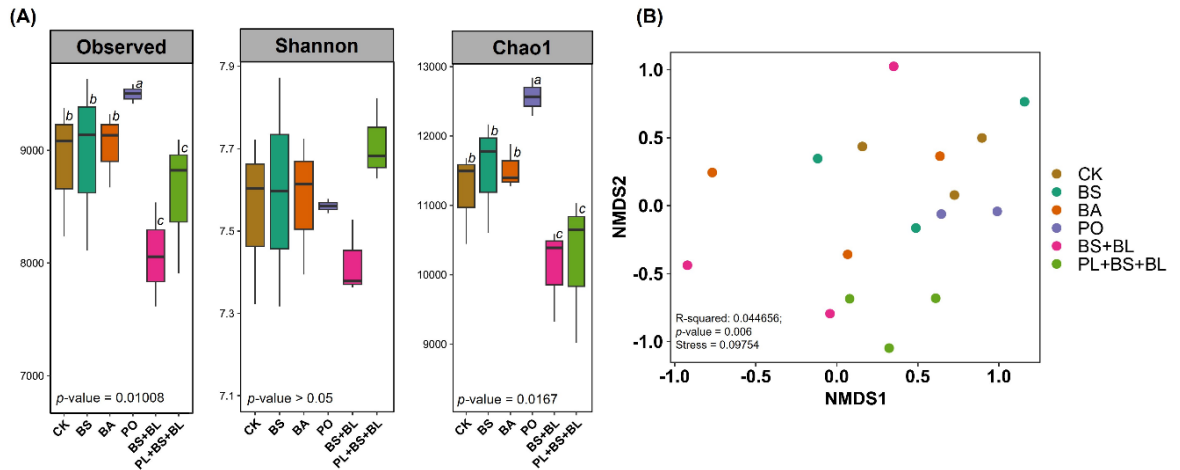


Fig. 4 Bacterial community analysis under different treatments. (A) Alpha diversity indices (Observed, Shannon, and Chao1) of bacterial communities. (B) Non-metric multidimensional scaling (NMDS) plot showing beta diversity of soil bacterial communities based on Bray-Curtis dissimilarities. Treatments included *Bacillus subtilis* (BS), *B. amyloliquefaciens* (BA), *P. oryzae* (PO), *B. subtilis* and *B. licheniformis* (BS+BL), *P. lilacinum*, *B. subtilis* and *B. licheniformis* (PL+BS+BL), and control treatment (CK).

The Mantel test and Spearman correlation results (Fig. 5) revealed significant associations between bionematicide treatments, *Pratylenchus zae* populations, soil enzymatic activity, and the genus-level microbial community composition. The inoculation of *B. amyloliquefaciens* showed a strong positive correlation with phosphatase ($r = 0.368$, $p = 0.001$). The Mantel test further confirmed a significant association between *B. amyloliquefaciens* and the genus-level microbial community composition, reinforcing its role in microbial structure shifts.

Spearman's correlation results confirmed strong negative associations between *P. zae* in roots and phosphatase (-0.82) and between *P. zae* in soil and phosphatase (-0.69). The Mantel test also highlighted a significant correlation between basal respiration and inoculation of *B. amyloliquefaciens* ($r = 0.395$, $p = 0.001$).

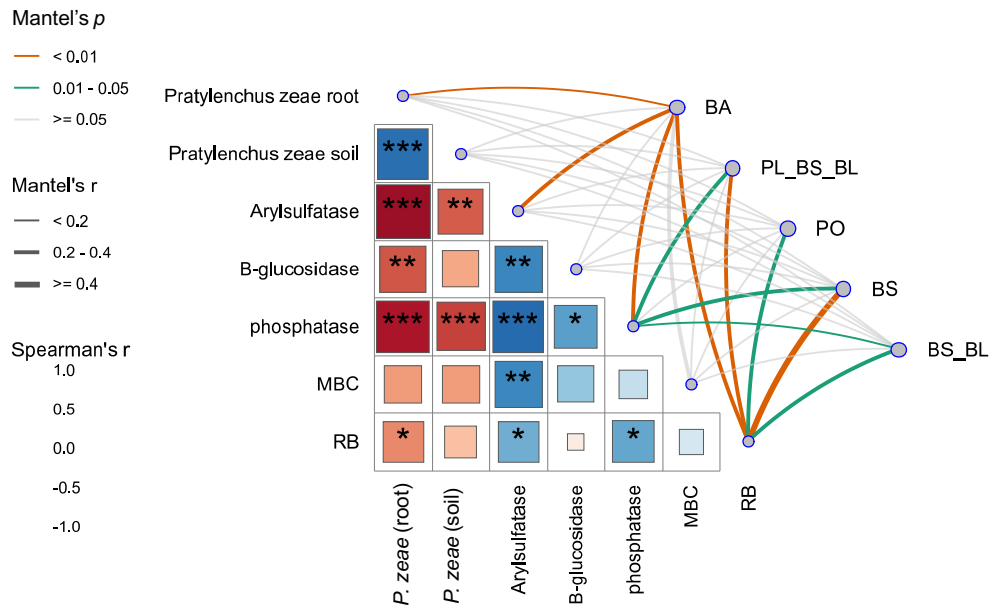


Fig. 5 Pairwise comparisons between *Pratylenchus zaeae* populations, soil biological properties, and microbial community composition across different bionematicide treatments. The color gradient denotes Spearman's correlation coefficients. The relationships between microbial community composition at the genus level (16S rRNA), *P. zaeae* populations in roots and soil, microbial biomass carbon (MBC), basal respiration (RB), and enzymatic activities were assessed through the Mantel test. Edge width corresponds to Mantel's r statistic for the corresponding distance correlations, and edge color denotes statistical significance based on 9,999 permutations. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

4. Discussion

This study provides new evidence that inoculating beneficial and efficient microorganisms can control *P. zaeae* in sugarcane roots, while boosting soil biological properties and preserving the original bacterial communities in the rhizosphere. The microorganism tested in this study effectively reduced the population of *P. zaeae* in both roots and soil, highlighting the potential of *P. oryzihabitans* in controlling *P. zaeae* in sugarcane roots (~81% of reduction). While no previous studies included *P. oryzihabitans* to control *P. zaeae* in sugarcane, it is known that *P. oryzihabitans* can efficiently control *Meloidogyne javanica* (root-knot nematode) in tomato (Vagela et al., 2007; Leontopoulos et al., 2017). *Pseudomonas* is a potential suppressor of nematodes by various mechanisms, such as competition for nutrients (Kamilova et al., 2005) and antibiosis (Raaijmakers and Mazzola, 2012). Interestingly, *Pseudomonas*

produces phanzine which is a known antibiotic against pathogens (Kerr, 2000) that can affect nematodes. Particularly, *P. oryzihabitans* act by disrupting the orientation and movement of nematodes (Vagela et al., 2007) and producing toxic metabolites with nematostatic effect (Leontopoulos et al., 2017). Thus, these biochemical properties observed in *P. oryzihabitans* contribute to reducing the infection and penetration of *P. zaeae*.

Similarly, the inoculation of *B. amyloliquefaciens* also promoted a significant decrease in the population of *P. zaeae*, which suggests its potential for nematode control. *Bacillus amyloliquefaciens* is known as an efficient rhizobacterium that enhances plant tolerance against pathogens (Dimopoulou et al., 2021; Kazerooni et al., 2021). This species produces antibiotics, such as surfactins, fengycin, iturin A, and bacillomycin D (Burkett-Cadena et al., 2008), which contribute to nematode suppression. Indeed, previous studies have reported *B. amyloliquefaciens* producing nematicidal compounds, reducing the abundance of root-lesion nematodes in soil and plants (Liu et al., 2013; He et al., 2013). In addition to its direct antagonistic effects, correlation analyses indicated that *B. amyloliquefaciens* also played a key role in shaping the microbial community at the genus level. Its association with increased microbial activity and basal respiration suggests that its inoculation not only contributes to nematode suppression but also enhances soil biological functions. These findings reinforce the idea that *B. amyloliquefaciens* may exert its effects through both direct and indirect mechanisms, promoting a more active and functionally stable microbiome that could contribute to long-term nematode control and soil health improvement.

Although presenting lower efficiency in controlling *P. zaeae*, *B. subtilis* is reported to act in controlling *Pratylenchus* by disrupting nematode migration and producing antibiotics (Hashem et al., 2019). Similarly, a recent study has shown *B. subtilis* reducing by ~50% of the population of *P. zaeae* in sugarcane roots (Mazzuchelli et al., 2020).

Interestingly, the co-inoculation *B. subtilis* + *B. licheniformis* showed lower efficiency in controlling *P. zaeae*. This suggests potential antagonistic interactions or reduced synergistic effects between the two bacterial species against pathogens (Lamichhane and Venturi, 2015). Probably, both bacterial species reduced efficiency in controlling *P. zaeae* due to their competition for resources or niches, limiting their

ability to produce antibiotics. Thus, such interactions can compromise the biocontrol potential against *P. zea*. The results have shown that these microorganisms have some antagonistic activity level against *P. zea*, potentially controlling its population in soil and sugarcane roots and alleviating the negative effect on plant growth.

The results of biological properties showed distinct responses to each inoculation, co-inoculation, and triple inoculation. For instance, the triple inoculation (*P. lilacinum*, *B. subtilis*, *B. licheniformis*) boosted urease and arylsulfatase. On the other hand, *P. lilacinum* can enhance enzymatic activity by interacting with the soil microbiome (Zhang et al., 2023) while *Bacillus* species directly produce these enzymes (Kim et al., 2005). Particularly, *B. subtilis* produces extracellular enzymes, such as urease (Kim et al., 2005) and β -glucosidase (Bagudo et al., 2014), which explain its potential to increase the activity of urease and β -glucosidase. These enzymes are essential for C and N mineralization, which include *B. subtilis* as a potential to improve soil fertility. In contrast, *P. oryzihabitans* enhanced phosphatase, which increases the P solubilization (Nosheen et al., 2018), while contributing to urease activity. Regarding microbial C, the inoculation of *B. amyloliquefaciens* increases the content of bioactive metabolites (Chowdhury et al., 2022), leading to the highest microbial biomass C. Therefore, inoculating these microorganisms collectively demonstrates distinct but complementary roles in enhancing soil health.

The bacterial community in inoculated soils was dominated by the phyla Actinobacteriota, Proteobacteria, and Firmicutes, which are often associated with healthy and functional soils. Actinobacteriota play a key role in organic matter decomposition and nutrient cycling, while Proteobacteria include bacteria that support nitrogen cycling and plant-microorganism interactions (Spain and Elshahed, 2009; Bao et al., 2021). The dominance of Firmicutes, particularly *Bacillus spp.*, highlights the success of the treatments in enriching the soil microbiota. The Mantel test highlighted significant associations between microbial genera composition and bionematicide treatments, reinforcing that the suppression of *P. zea* is closely linked to shifts in the genus-level microbiome. The positive correlation between microbial biomass, enzymatic activity, and *B. amyloliquefaciens* suggests that its inoculation suppressed nematodes and played a key role in microbial restructuring within the rhizosphere.

Interestingly, studies have shown that soils with reduced nematode populations, such as those treated with beneficial microorganisms, often exhibit higher bacterial biodiversity compared to nematode-infested soils (Zhou et al., 2019). In their analysis, the authors observed that non-infested soils were enriched with Proteobacteria (e.g., *Pseudomonas*) and Firmicutes (*Bacillus*), groups commonly associated with nematode suppression and soil health. In contrast, infested soils were dominated by Bacteroidetes, suggesting that the suppression of nematodes by microbial treatments may also indirectly foster the proliferation of beneficial bacterial taxa.

Less abundant phyla such as Acidobacteriota, Chloroflexi and Planctomycetota also contribute to microbial diversity and functional stability within the soil ecosystem (Kalam et al, 2020). Furthermore, the higher relative abundance of genera such as *Sphingomonas*, *Vicinamibacteraceae*, and *Gaiella* in inoculated soils suggests that the treatments not only suppressed nematode populations but also induced favourable shifts in the microbial community. *Sphingomonas* is known for its role in plant stress tolerance and degradation of complex organic compounds, while *Vicinamibacteraceae*, part of the phylum *Acidobacteriota*, may have benefited from the treatment-induced changes in the soil chemical and biological environment (Kalam et al., 2020; Wang et al., 2022).

The increased abundance of *Streptomyces* and *Thermoactinomyces* further highlights the functional activity of the microbiome in response to treatments. *Streptomyces*, a producer of antimicrobial compounds, is likely to contribute to pathogen suppression and competitive microbial interactions, while *Thermoactinomyces* may serve as an indicator of increased microbial activity, facilitating the degradation of recalcitrant compounds and improving overall soil health (Providenti et al., 1996; Dow et al., 2023).

Results showed that the inoculation of microorganisms decreased the abundance of *P. zae* while maintaining the bacterial community in the rhizosphere unchanged. Interestingly, the inoculation of *P. oryzihabitans* resulted in higher bacterial richness and diversity in the rhizosphere, which can be associated with greater reduction *P. zae* in roots and higher key enzymatic activities, such as β -glucosidase. This suggests that inoculating *P. oryzihabitans* contributes to increasing bacterial diversity in the rhizosphere and biological activity, which boosts the control of *P. zae* in

sugarcane roots. A recent study has observed that inoculating *Pseudomonas* species in wheat increases the microbial diversity in the rhizosphere and helps plants against pathogens (Poli et al., 2024). In contrast, the use of co-inoculation and triple inoculation promoted a decrease in bacterial diversity and richness in the rhizosphere. In addition, these treatments showed lower efficiency in controlling *P. zeae* (Fig. 1). This confirms that when in association, these microorganisms promote competition for the same resources or ecological niches (Lechón-Alonso et al., 2021), decreasing their potential beneficial effect against pathogens.

Importantly, while diversity plays a critical role in maintaining a resilient soil ecosystem, the stability of microbial community structure is equally important. The non-significant changes in the structure of bacterial communities in the rhizosphere following the inoculation of different microorganisms suggest that although the rhizosphere can recruit some specific microbial taxa, the community structure remains unchanged. This reinforces the idea that inoculating these microorganisms could obtain a suitable control of *P. zeae* in sugarcane roots while maintaining the potential resilience of bacterial communities in the rhizosphere. This finding includes the use of microorganisms as a key component for the long-term sustainability of the sugarcane field.

Despite the significant findings of this study, particularly regarding the suppression of *P. zeae* in sugarcane by different microorganisms, certain limitations must be acknowledged. The focus on bacterial communities alone did not consider the broader impact of biological agent application on the entire soil microbiome, including protists, free-living nematodes, and fungi, which are critical for nutrient cycling and ecosystem stability. Future research should build on these findings by evaluating these groups, providing a more comprehensive understanding of the ecological effects of microbial inoculation.

Moreover, field trials are essential to validate the effectiveness of these microorganisms under diverse environmental conditions and cropping systems. These steps are essential for future research to ensure the long-term sustainability and applicability of microbial inoculation in agricultural systems.

5. Conclusions

The findings of this study provide new evidence that biological control using microorganisms can effectively mitigate the negative impacts of *P. zeae* on sugarcane without causing significant disruptions to the native soil microbiome. Among the treatments tested, the single inoculation with *P. oryzihabitans* emerged as the most effective, significantly reducing *P. zeae* population while enhancing microbial diversity and enzymatic activity in the soil. These findings suggest that *P. oryzihabitans* is a particularly promising biocontrol agent, offering dual benefits of nematode suppression and improved soil health.

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CAPÍTULO III – Bionematicides shape plant growth-promoting bacteria community in the sugarcane rhizosphere

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Abstract

Agricultural intensification and continuous monocropping have negatively impacted soil health and biodiversity, increasing the incidence of plant-parasitic nematodes while reducing beneficial microbial populations. Bionematicides containing microbial inoculants have emerged as a promising strategy for nematode control; however, their effects on plant growth-promoting bacteria (PGPB) in the rhizosphere remain poorly understood. This study aimed to evaluate the impact of different bionematicides on the recruitment of PGPB in the sugarcane rhizosphere. A greenhouse experiment was conducted using six microbial formulations containing *Bacillus subtilis*, *Bacillus amyloliquefaciens*, *Pseudomonas oryzihabitans*, *Purpureocillium lilacinum*, and *Bacillus licheniformis*. The PGPB community was assessed through 16S rRNA gene amplicon sequencing. Our results revealed that *Bacillus* and *Sphingomonas* were the most abundant PGPB across all treatments, with no major shifts in community structure between inoculated and non-inoculated rhizospheres. However, microbial diversity increased following inoculation with *P. oryzihabitans* and decreased with *B. amyloliquefaciens* and the multi-strain combination of *P. lilacinum*, *B. subtilis*, and *B. licheniformis*. Niche occupancy analysis showed a predominance of generalist over specialist PGPB, with *P. oryzihabitans* favoring the recruitment of specialists. Co-occurrence network analysis revealed a stable number of nodes across treatments, while microbial interactions (edges) were highest in the *P. oryzihabitans* treatment. These findings indicate that bionematicides influence PGPB diversity and interactions in the sugarcane rhizosphere, with *P. oryzihabitans* playing a key role in enhancing microbial specialization and network complexity. This study provides insights into the ecological impact of bionematicides and their potential to modulate beneficial microbial communities in agricultural soils.

Keywords: Bionematicides, sugarcane rhizosphere, microbial recruitment, *Pseudomonas oryzihabitans*, *Bacillus*, nematode suppression

1 Introduction

The intensification of agriculture and continuous monocropping have significantly decreased soil health and increased the incidence of pests, including plant-parasitic nematodes (Foley et al 2011). In addition, the practices in conventional agriculture have promoted losses of soil biodiversity, which negatively impacts the abundance of beneficial microbial genera (Birkhofer et al., 2021). Thus, ecological practices to control nematodes and, at the same time, maintain the abundance of beneficial microbes should be implemented in the agriculture.

In this context, the use of biological control of nematodes using microorganisms has been increased as a potential ecological strategy (Ramouthar and Bhuiyan, 2018). As well-known microorganisms found in soil, some microbial species, such as *Bacillus subtilis*, *Bacillus amyloliquefaciens*, and *Pseudomonas oryzae*, are used as bionematicides with potential efficiency against nematodes (Abdel-Salam et al., 2018, El-Nagdi et al., 2018, Sahebani and Gholamrezaee 2021).

On the other hand, some studies have shown these microbial genera promoting changes in the root exudates which drive the composition of microbial community within rhizosphere (Badri et al., 2009; Silva et al., 2018). For instance, Hernández-Montiel et al. (2022) reported that the application of *Bacillus amyloliquefaciens* in cowpea altered the composition of the microbial community in the rhizosphere, increasing the abundance of beneficial bacterial genera such as *Arthrobacter* and *Micromonospora*. These changes were associated with pathogen suppression and plant growth promotion.

Although these studies have reported effect of beneficial microbes in changing the core microbial community in the rhizosphere, little is known about the potential effect on the community of plant growth-promoting bacteria (PGPB). This is particularly important since PGPB promote plant growth by several mechanisms including pathogen suppression, such as nematodes (Gamalero and Glick, 2020). For instance, a study by Liu et al. (2022) demonstrated that the application of *Burkholderia vietnamiensis* B418 in watermelon not only inhibited root-knot nematode (*Meloidogyne incognita*) but also modified the rhizosphere microbial composition, enriching PGPB populations (Liu et al., 2022). Similarly, Sahebani and Gholamrezaee (2021)

highlighted the role of *Pseudomonas fluorescens* as a biocontrol agent against nematodes, promoting plant growth while suppressing soil-borne pathogens.

Thus, this is important to assess the effect of potential bionematicides on PGPB community in the rhizosphere of sugarcane, which is an important crop species to Brazil. In this study, we hypothesized that distinct bionematicides would affect differently the recruitment of PGPB by the rhizosphere of sugarcane. Thus, six distinct bionematicides containing bacteria and fungi were applied in sugarcane and the PGPB community by 16S rRNA amplicon sequencing, was assessed in the rhizosphere.

2 Material and methods

Soil samples (0-20 cm depth) were collected from a sugarcane field in Teresina, Piauí, Brazil (04°52'56" S, 42°52'58" W; 68 m). The region has a tropical climate (Aw), with an average annual rainfall of 1,500 mm and a mean temperature of 27°C. The soil is classified as Fluvisol, (70% sand, 14% silt, and 16% clay) and the main chemical properties are: soil pH - 5.5, soil organic matter content - 15 g kg⁻¹, P - 8 mg kg⁻¹, K - 127 mg kg⁻¹, and base saturation - 70%.

The soil was added to plastic pots, and the experiment was conducted in a greenhouse (from September to November 2023). The experiment was established in a completely randomized design with six replicates per treatment. The evaluated treatments included commercial microbial inoculants containing the following microorganisms: i) MicrosAtivos™ (*Bacillus subtilis*); ii) Nemacontrol™ (*Bacillus amyloliquefaciens*); iii) Biotrinsic™ (*Pseudomonas oryzihabitans*); iv) Quartzo™ (*Bacillus subtilis* + *Bacillus amyloliquefaciens*); v) Profix™ (*Purpureocillium lilacinum* + *Bacillus subtilis* + *Bacillus licheniformis*); vi) Control (CK), without microbial inoculation.

Microbial suspensions were prepared according to the manufacturer's specifications and diluted in 350 mL of water before being applied via spray inoculation into planting furrows (15 cm depth, 10 cm width) at the time of sugarcane planting.

To ensure viability and efficacy, preliminary tests were conducted to assess microbial stability, viability under storage conditions, and potential colonization capacity. Prior to planting, sugarcane stalks (variety RB92579) underwent pre-germination in germination boxes (Gerbox) for 10 days.

The pre-germinated stalks were subsequently transplanted into pots containing soil previously inoculated with *Pratylenchus zeae*. Irrigation was performed daily using the gravimetric method based on soil water retention capacity, maintaining soil moisture at 70% of field capacity. Plants were harvested 65 days after microbial inoculation and the rhizosphere soil was collected carefully collected and transferred to sterile Eppendorf tubes, which were immediately frozen at -80°C.

DNA was extracted from 0.5 g of rhizospheric soil samples using the DNeasy PowerSoil Pro Kit (Qiagen, CA, USA). The 16S rRNA gene V4 region was amplified using primers 515F (5'-GTGYCAGCMGCCGCGGTAA-3') and 806R (5'GGGACTACHVHHHTWTCTAAT-3') with dual-index barcodes (Caporaso et al., 2011). Sequencing was carried out using the Illumina MiSeq Sequencer (Illumina, San Diego, CA, USA) after Polymerase Chain Reaction (PCR). All raw sequences were processed using QIIME, following the UPARSE standard pipeline (Edgar, 2013). The amplicon sequencing variants (ASV) table was generated with a 97% similarity threshold. PGPB community was selected from ASV table according to the dataset proposed by Araujo et al. (2024).

Alpha diversity indices (observed richness and Shannon) were calculated using the Phyloseq package (McMurdie and Holmes, 2013) in R (R Core Team, 2023). Beta diversity was analyzed using Bray-Curtis dissimilarity and visualized through Non-Metric Multidimensional Scaling (NMDS). Differences in microbial community composition between treatments were evaluated using permutational multivariate analysis of variance (PERMANOVA) with 999 permutations (adonis2 function, vegan package; Oksanen et al., 2024).

The relative abundance of bacteria at the genus level was analyzed across the treatments. Bar charts were generated to display the relative abundances of the top eight genera. Mean relative abundances and standard deviations were calculated for each treatment, with error bars included in the plots to highlight treatment-specific variations. Niche occupancy was evaluated using the CLAM test, a Multinomial Species Classification Method (Chazdon et al., 2011), to compare microbial community abundance between the bionematicide treatments and the control.

This analysis was performed using the 'vegan' package and the 'clamtest' function in R software (Oksanen et al., 2024). Based on their distribution patterns,

microbial taxa were classified as specialists, generalists, or rare microorganisms in response to the applied treatments. The graphical representation of these classifications illustrates the distinct microbial niche preferences under bionematicide application compared to the untreated control.

To assess the complexity of PGPB community interactions in response to bionematicide application, network analysis was performed. Pairwise correlations between bacterial genera were estimated using the SparCC algorithm (Friedman & Alm, 2012). Only significant correlations ($p < 0.05$) with magnitudes >0.5 (positive correlation) or < -0.5 (negative correlation) were considered for network construction. Keystone taxa within each network were identified based on their number of connections and betweenness centrality.

Network visualization and analysis of topological properties were conducted using the Gephi interactive platform (Bastian et al., 2009). The following network parameters were calculated: number of nodes and edges, modularity, number of communities, average path length, network diameter, average degree, and clustering coefficient.

3 Results

In general, the relative abundance of PGPB genera showed *Bacillus*, *Sphingomonas*, *Streptomyces*, *Nocardioide*s, and *Paenibacillus* as the five most abundant across all treatments (Figure 1A). Particularly, *Bacillus* (~30 – ~40%) and *Sphingomonas* (~10 – ~15%) were the most relatively abundant PGPB genera (Figure 1B). The other PGPB genera, including *Streptomyces*, *Saccharopolyspora*, *Paenibacillus*, *Nocardioide*s, *Mycobacterium*, *Geobacillus*, and *Bradyrhizobium*, showed lower relative abundance (less than 10%).

The PGPB community structure separated bulk soil from the rhizosphere (Figure 2A). However, no clear separation was observed among rhizospheres in response to bionematicide application. PGPB diversity was highest following inoculation with *P. oryzihabitans* but decreased with *B. amyloliquefaciens* and the combination of *P. lilacinum*, *B. subtilis*, and *B. licheniformis* (Figure 2B). The PGPB richness was high with *P. oryzihabitans* inoculation but decreased with *P. lilacinum* + *B. subtilis* + *B. licheniformis* and *B. subtilis* + *B. licheniformis*.

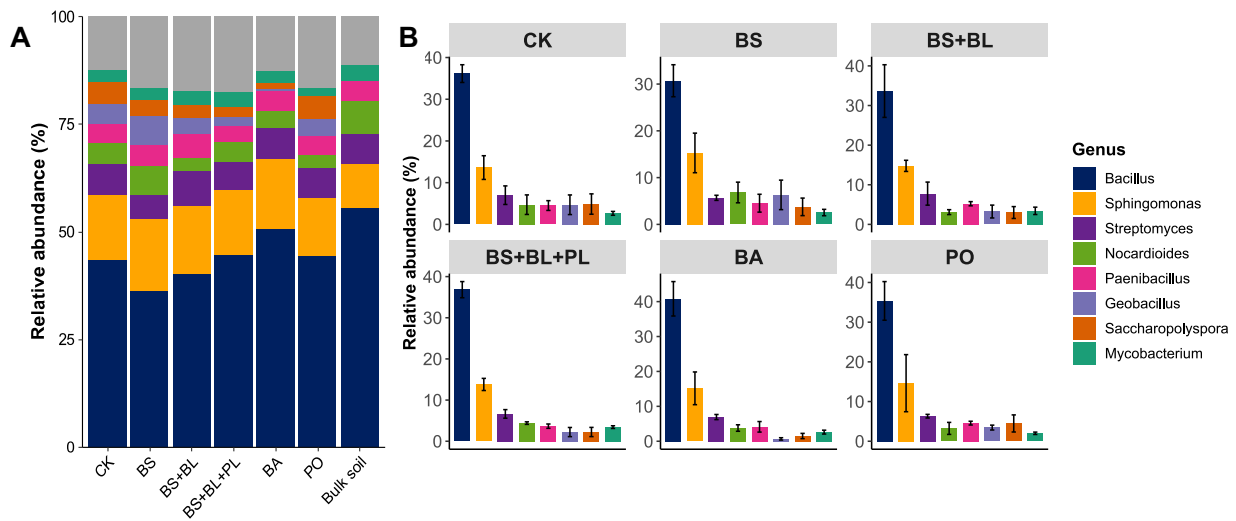


Figure 1: Effects of bionemacide treatments on the relative abundance of plant growth-promoting bacteria (PGPB) in the sugarcane rhizosphere. The bar plots display the relative abundance of the most representative PGPB genera across different bionemacide treatments. The treatments include *Bacillus subtilis* (BS), *Bacillus amyloliquefaciens* (BA), *Pseudomonas oryzihabitans* (PO), *Purpureocillium lilacinum* + *Bacillus subtilis* + *Bacillus licheniformis* (PL+BS+BL), and *Bacillus subtilis* + *Bacillus amyloliquefaciens* (BS+BL). The control treatment (CK) did not receive microbial inoculation. The bars represent the mean relative abundance \pm standard deviation.

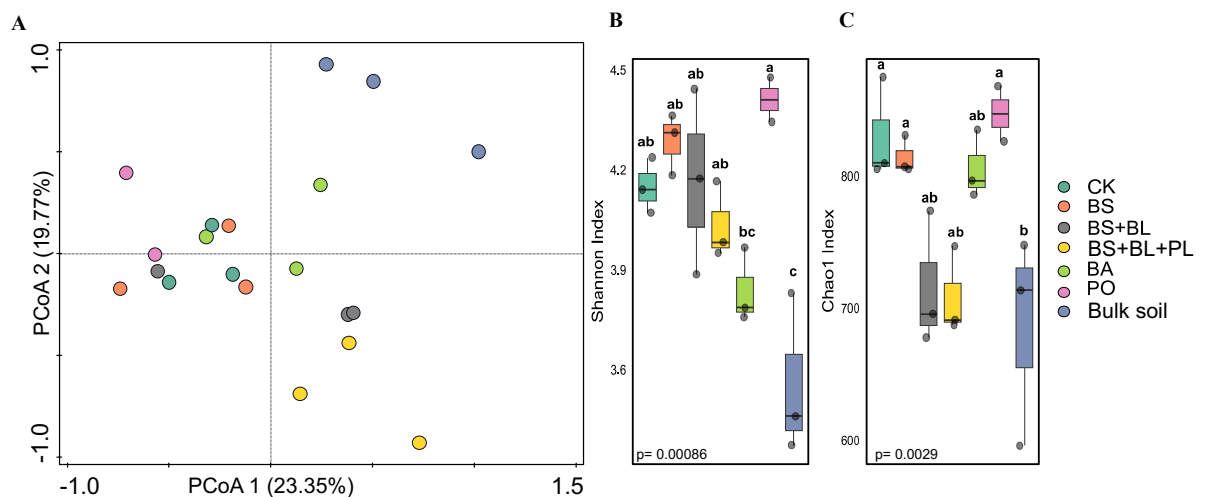


Figure 2: Beta diversity and alpha diversity indices of the PGPB community in the sugarcane rhizosphere under different bionemacide treatments: (A) Principal Coordinate Analysis (PCoA) based on Bray-Curtis dissimilarity showing the clustering of PGPB communities across treatments. Statistical significance was tested using PERMANOVA ($p < 0.05$); (B) Chao1 richness index representing the estimated bacterial richness across treatments; (C) Shannon diversity index displaying the overall diversity of bacterial communities in response to bionemacide application.

Niche occupancy analysis showed a higher proportion of generalist than specialist PGPB (Figure 3). The highest proportion of generalist PGPB was observed with *B. subtilis* (~59%), while the lowest was found with *P. lilacinum* + *B. subtilis* + *B. licheniformis* (~42%). Regarding specialist PGPB, the highest proportion was observed with *P. oryzihabitans* (~8%), whereas the lowest was found with *P. lilacinum* + *B. subtilis* + *B. licheniformis*.

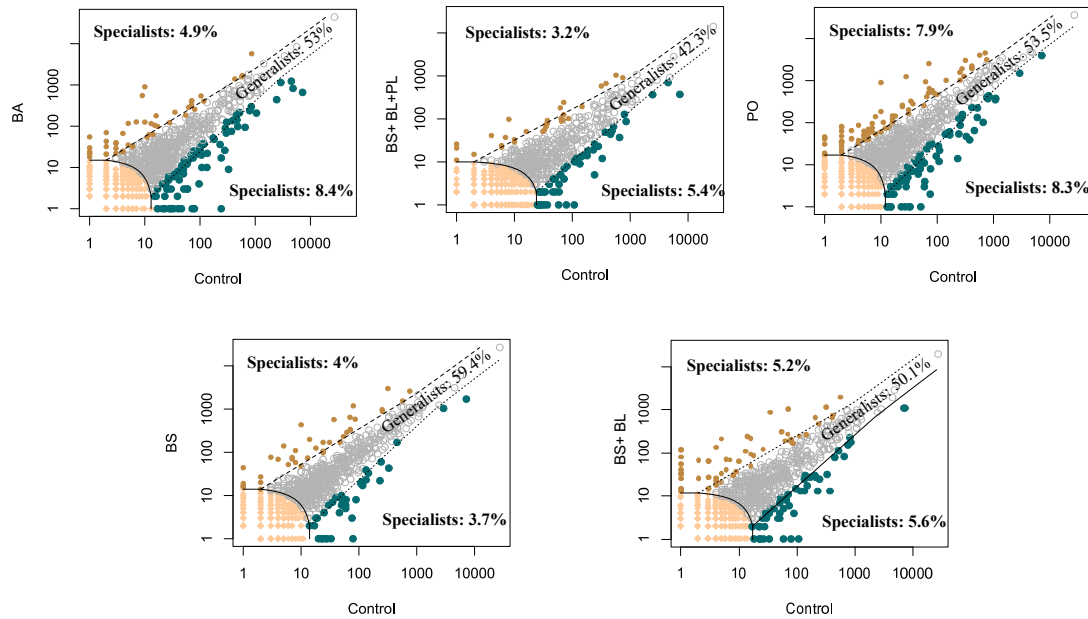


Figure 3: Niche occupancy of PGPB community in the sugarcane rhizosphere under bionematicide treatments.

The analysis of co-occurrence showed no significant differences in the number of nodes (ranging from 53 to 62) across all bionematicide treatments (Figure 4). However, the number of edges varied, with *P. oryzihabitans* promoting the highest number of edges (716 edges), compared to other treatments.

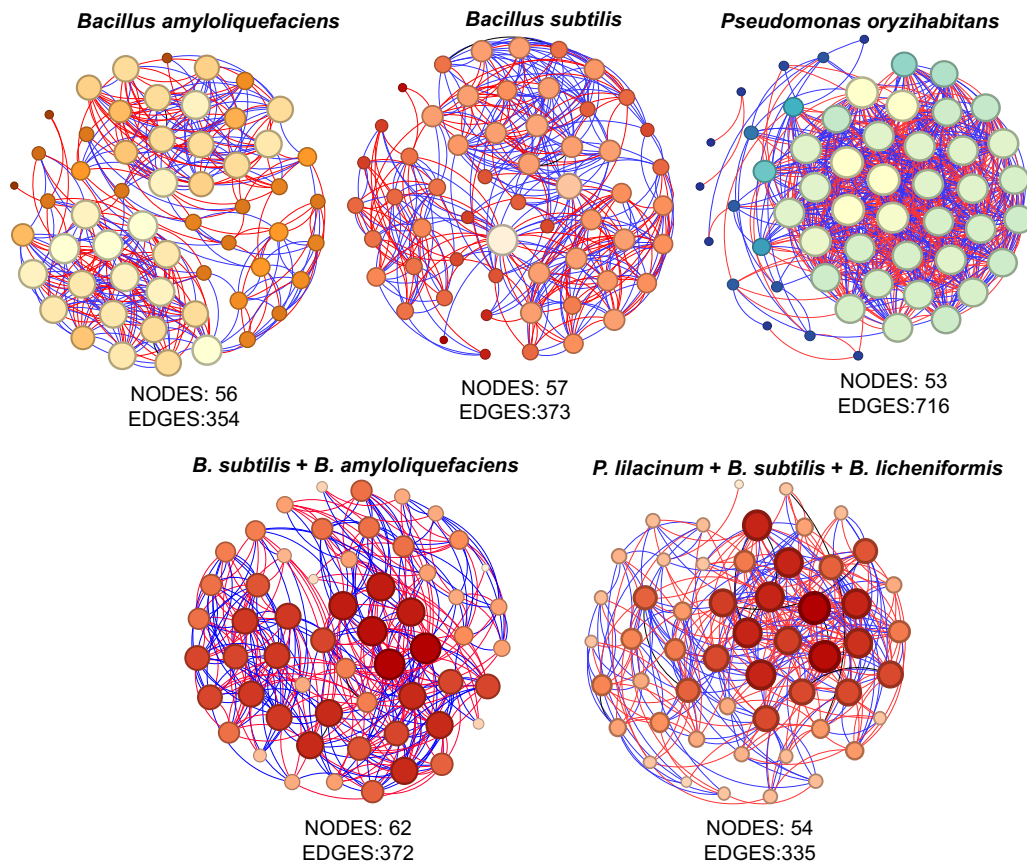


Figure 4: Co-occurrence network analysis of PGPB communities in the sugarcane rhizosphere under different bionematicide applications. The networks were inferred using SparCC correlations ($p < 0.05$; correlation magnitude > 0.5 or < -0.5). Nodes represent taxa at the ASV level, and edges indicate significant positive (blue) or negative (red) correlations. The size of each node is proportional to its number of connections (i.e., degree), while node colors highlight taxa with a higher number of correlations. Network complexity metrics, including the number of nodes, edges, modularity, and clustering coefficient, were calculated using Gephi.

4. Discussion

This study assessed the effect of distinct bionematicides on the PGPB community in the rhizosphere of sugarcane and showed different responses among PGPB genera. Regardless of the bionematicide applied, the rhizosphere of sugarcane exhibited a greater abundance of *Bacillus* and *Sphingomonas*. Interestingly, *Bacillus* and *Sphingomonas* were also abundant in the non-inoculated treatment.

Our results are consistent with previous studies that identified *Bacillus* and *Sphingomonas* as dominant taxa in the soil and rhizosphere following the application

of bionematicides, suggesting a synergistic interaction with biocontrol agents (Adam et al., 2014; Vinothini et al., 2024). For instance, Vinothini et al. (2024) applied the bionematicides *Bacillus velezensis* and *Trichoderma koningiopsis* against root-knot nematodes and found higher relative abundances of *Bacillus* (~18%) and *Sphingomonas* (~8%) in the tomato rhizosphere.

However, the highest abundance of *Bacillus* and *Sphingomonas* in the non-inoculated rhizosphere suggests that bionematicides application in sugarcane did not further enhance the recruitment of PGPB. Nonetheless, the increased abundance of *Bacillus* in the sugarcane rhizosphere is noteworthy, since this PGPB produces antibiotics and disrupts nematode orientation, contributing to nematode suppression (Mazuchelli et al., 2020; Dinardo-Miranda et al., 2023).

The structure of the PGPB community showed no significant differences between the inoculated and non-inoculated rhizosphere. Since microbial community structure refers to the composition and organization of a microbial assemblage (Liu et al., 2020), our results indicate that these bionematicides did not alter the structure of the PGPB community in the sugarcane rhizosphere. This is important because it suggests that these treatments can be applied to control nematodes without negatively affecting beneficial microbial groups.

In contrast, the application of bionematicides did affect PGPB diversity in the rhizosphere. Inoculation with *P. oryzihabitans* increased overall PGPB diversity, suggesting that this strain may act synergistically to enhance microbial diversity. For example, a recent review showed that *Pseudomonas* species can improve root activity (Khoso et al., 2024), which may help increase microbial diversity in the rhizosphere. Conversely, inoculation with *B. amyloliquefaciens* and the triple inoculation of *P. lilacinum*, *B. subtilis*, and *B. licheniformis* reduced PGPB diversity. This may indicate that introducing these microorganisms into the sugarcane rhizosphere can favor some PGPB genera while suppressing others.

Interestingly, the inoculation of *P. oryzihabitans* increased the proportion of specialist PGPB, suggesting that this microorganism may promote a more specialized community in the rhizosphere. *P. oryzihabitans* may support these specialists by producing growth-promoting metabolites (Xu et al., 2022). Enhancing the abundance

of specialist PGPB may, in turn, improve plant-beneficial processes such as pathogen suppression and plant growth promotion (Saeed et al., 2021).

The co-occurrence network analysis showed stability in the number of nodes across treatments, confirming that bionematicides did not significantly affect PGPB composition. However, the number of edges increased with the inoculation of *P. oryzihabitans*, indicating a greater number of microbial interactions in the rhizosphere of sugarcane (Guseva et al., 2022). This reinforces the positive effect of inoculating *P. oryzihabitans* in recruiting a more diverse, specialized, and interconnected PGPB community. These enhanced interactions may foster cooperation and symbiotic relationships among beneficial microbes, with implications for nematode control and sugarcane growth promotion.

5. Conclusions

Our findings demonstrate that the application of bionematicides did not significantly alter the overall structure of the PGPB community in the sugarcane rhizosphere. *Bacillus* and *Sphingomonas* remained dominant across all treatments, indicating their ecological stability in this system.

However, inoculation with *P. oryzihabitans* increased PGPB diversity and promoted the development of a more specialized community. In contrast, treatments with *B. amyloliquefaciens* and the combination of *P. lilacinum*, *B. subtilis*, and *B. licheniformis* reduced diversity, suggesting selective shifts in microbial composition.

Co-occurrence network analysis revealed stable microbial associations across treatments, with *P. oryzihabitans* notably enhancing network connectivity. These results suggest that, in addition to providing nematode control without disrupting beneficial microbial groups, *P. oryzihabitans* may contribute to strengthening microbial diversity and interaction networks, potentially enhancing the resilience and functional capacity of the rhizosphere.

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CONSIDERAÇÕES FINAIS

Este trabalho apresentou evidências relevantes sobre o uso de bionemáticas no manejo sustentável de *Pratylenchus* spp., com avaliações realizadas em culturas e condições distintas, permitindo examinar seus efeitos sobre a supressão do patógeno, o desenvolvimento vegetal e a composição da microbiota bacteriana do solo.

No **Capítulo I**, a aplicação da cepa AP-3 de *Bacillus subtilis* foi eficaz na redução de *Pratylenchus* spp. em raízes de milho (~90%) e feijão-fava (~40%), além de promover aumento no crescimento radicular e nos atributos de nodulação, especialmente quando aplicado sem fertilizantes químicos. Esses resultados indicam que *B. subtilis* atua por mecanismos múltiplos, combinando supressão direta de nematoides e estímulo ao crescimento das plantas.

O **Capítulo II** mostrou que *Pseudomonas oryzae* foi o tratamento mais eficiente no controle de *P. zae* na cana-de-açúcar, com reduções de até 81% na raiz e 64% no solo. A inoculação também elevou a atividade de enzimas do solo e a diversidade bacteriana, sem provocar alterações marcantes na estrutura geral da comunidade. Já os tratamentos com múltiplos microrganismos apresentaram menor eficiência, sugerindo competição entre os consórcios aplicados.

No **Capítulo III**, foi avaliado se diferentes bionemáticas modulam de forma distinta o recrutamento de bactérias promotoras de crescimento vegetal (PGPB) na rizosfera da cana-de-açúcar. Os resultados indicaram que essa modulação ocorreu principalmente em termos de diversidade, presença de táxons especialistas e conectividade entre gêneros bacterianos. *Pseudomonas oryzae* destacou-se por favorecer uma comunidade mais diversa e conectada. A estrutura geral da comunidade, contudo, permaneceu relativamente estável, indicando que os efeitos dos bionemáticas se concentraram mais em aspectos relacionados à composição do que a mudanças estruturais amplas.

As principais contribuições deste trabalho incluem dados inéditos obtidos sob diferentes condições experimentais e em distintas culturas agrícolas, como feijão-fava, milho e cana-de-açúcar. Destaca-se o registro de *Pratylenchus* em raízes de feijão-fava sob condições de campo no Brasil, com confirmação morfológica e impacto negativo no crescimento radicular e na nodulação, além da avaliação pioneira dos

efeitos de bionematicidas sobre a microbiota bacteriana da rizosfera da cana-de-açúcar. O estudo também comparou a eficácia de diferentes formulações microbianas, evidenciando seus efeitos sobre a diversidade e composição taxonômica, e mostrou que as combinações testadas não superaram o desempenho das formulações individuais.

Esses achados reforçam a importância de considerar não apenas a supressão do nematoide, mas também o equilíbrio microbiano do solo na escolha e aplicação de estratégias biológicas.

Entre as limitações, destacam-se a ausência de análises funcionais ou de outros grupos microbianos além das bactérias, e a necessidade de validação dos resultados em ambientes agrícolas sob diferentes condições temporais e de manejo.

Para avanços futuros, sugere-se realizar estudos em campo, avaliar a persistência dos microrganismos no solo ao longo do tempo, e explorar outras formulações. A ampliação das análises para outros domínios microbianos, como fungos e protistas, poderá contribuir para uma compreensão mais completa das interações ecológicas envolvidas. Com isso, o uso de bionematicidas poderá ser melhor ajustado aos princípios da agricultura sustentável, promovendo não apenas o controle de fitonematoides, mas também a preservação da biodiversidade do solo.