BACTERIOMA DA SEMENTE: ESTRUTURA DA COMUNIDADE E IMPACTOS NA GERMINAÇÃO, CRESCIMENTO E PROTEÇÃO DO MILHO (*Zea mays* L.)

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CAMPOS DOS GOYTACAZES - RJ
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Aos meus maiores mestres: meu pai Antônio e minha mãe Regina pelo apoio, amor e sabedoria.

Dedico

"Não devemos ter medo dos confrontos.
Até os planetas se chocam e do caos nascem as estrelas."

(Charlie Chaplin)

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BIOGRAFIA

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RESUMO

SANTOS, Lidiane Figueiredo dos, D.Sc., Universidade Estadual do Norte Fluminense Darcy Ribeiro, novembro de 2020. **Bacterioma da semente: estrutura da comunidade e impactos na germinação, crescimento e proteção do milho (*Zea mays* L.)**. Orientador: Fabio Lopes Olivares.

As plantas hospedam comunidades microbianas diversas e complexas, que juntas compõe o chamado "microbioma". A origem desses micro-organismos é amplamente discutida, mas comumente atribuída ao solo. Recentemente, a microbiota transmitida pela semente também passou a ser considerada fonte essencial para o microbioma vegetal. Nesse sentido, esta tese apresenta dois capítulos dedicados a revisar os conceitos e as funcionalidades dos microbiomas e discutir os fatores envolvidos na sua modulação. Os objetivos dos outros três capítulos foram: (i) caracterizar o bacterioma da semente de milho SHS 5050 e DKB 177; (ii) avaliar o papel das bactérias transmitidas por sementes em processos de germinação, crescimento e biocontrole; e (iii) identificar o impacto da inoculação microbiana na estrutura do bacterioma da raiz de milho. Para alcançar esses objetivos, diversas abordagens foram utilizadas, incluindo sequenciamento de nova geração, contagem em meio de cultura e PCR em Tempo Real, microscopia, análises bioquímicas e ensaios de germinação, crescimento e biocontrole. Brevemente, os resultados indicam que a remoção parcial da microbiota transmitida pela semente através da desinfestação com hipoclorito de sódio afeta, negativamente, a germinação e o crescimento das plântulas de milho SHS 5050 e altera a estrutura da comunidade associada às raízes emergidas. A recomposição parcial do bacterioma, principalmente com isolados do gênero *Burkholderia*, restaura parcialmente o fenótipo das sementes desinfestadas. Na variedade de milho DKB 177, a perturbação da comunidade bacteriana da semente reduz o desempenho das plântulas e a tolerância a fungos fitopatogênicos transmitidos pela semente. A inoculação microbiana também altera a estrutura do bacterioma da raiz de milho SHS 5050 ao interagir com membros-chave da comunidade, como *Burkholderia*. Além disso, o impacto da inoculação é intensificado nas comunidades que tiveram membros parcialmente removidos pela desinfestação. Pela primeira vez na literatura, mostrou-se o efeito da desinfestação de sementes e da inoculação microbiana sobre o bacterioma e os potenciais táxons bacterianos responsáveis pela germinação, crescimento e tolerância a fungos fitopatogênicos.

Palavras-chave: Bacterioma da semente, biocontrole, bioinoculante, desinfestação, sequenciamento rRNA 16S.

ABSTRACT

SANTOS, Lidiane Figueiredo dos, D.Sc., Universidade Estadual do Norte Fluminense Darcy Ribeiro, November, 2020. **Seed bacteriome: community structure and impacts on maize germination, growth, and protection (*Zea mays* L.)**. Advisor: Fabio Lopes Olivares.

The plants host diverse and complex microbial communities, which together make up the so-called "microbiome". The origin of these microorganisms is widely discussed but is commonly attributed to the soil. Recently, the seed-transmitted microbiota has also come to be considered an essential source for the plant microbiome. In this sense, this thesis presents two chapters dedicated to review the concepts and functionalities of microbiomes and to discuss the factors involved in their modulation. The objectives of the other three chapters were: (i) to characterize the SHS 5050, and DKB 177 maize seed bacteriome; (ii) evaluate the role of seed-borne bacteria in germination, growth, and biocontrol processes; and (iii) identify the impact of microbial inoculation on the structure of the maize root bacteriome. To achieve these goals, several approaches were used, including new generation sequencing, counting in culture medium and Real-Time PCR, microscopy, biochemical analyzes and germination, growth, and biocontrol tests. Briefly, the results indicate that the partial removal of the seed-transmitted microbiota thorough disinfection with sodium hypochlorite negatively affects the germination and growth of SHS 5050 maize seedlings and changes the community structure associated with the emerged roots. The partial rebuilding of the bacteriome, mainly with isolates of the *Burkholderia* genus, partially restores the disinfected seeds phenotype. In the maize variety DKB 177, disturbance of the seed bacterial community reduces seedling performance and tolerance to phytopathogenic fungi transmitted by the seed. Microbial inoculation also alters the structure of the SHS 5050 maize root bacteriome by interacting with key members of the community, such as *Burkholderia*. In addition, the impact of inoculation is intensified on communities that have had members partially removed by disinfection. For the first time in the literature, the effect of seed disinfection and microbial inoculation on the bacteriome and the potential bacterial taxa responsible for germination, growth, and tolerance to phytopathogenic fungi was shown.

Keywords: Seed bacteriome, biocontrol, bioinoculant, disinfection, 16S rRNA sequencing.

INTRODUÇÃO GERAL

Introdução Geral

É crescente a busca por práticas agrícolas inovadoras, com abordagens que utilizam micro-organismos associados às plantas para superar o desafio de alcançar a produtividade das culturas e, simultaneamente, garantir a sustentabilidade ambiental (Singh et al., 2020). Assim como os humanos e outros eucariotos, as plantas coevoluíram com micro-organismos, incluindo bactérias, arqueas e fungos (Trivedi et al., 2020). Coletivamente, essas assembleias microbianas compõe o chamado "microbioma", que desempenha funções-chave no crescimento e na saúde das plantas (Berg et al., 2020). Portanto, é fundamental compreender a estrutura dos microbiomas e os fatores que o alteram. Até o momento, estudos direcionados para as frações bacterianas do microbioma ou do "bacterioma" são de longe os mais abundantes e demonstram que a composição e a atividade das comunidades variam de acordo com o genótipo da planta, interações micróbio-micróbio, práticas agrícolas e condições ambientais (Dastogeer et al., 2020).

Uma vez que o genótipo da planta hospedeira molda a comunidade microbiana, é preciso caracterizá-la em diferentes espécies agrícolas e avaliar sua importância para processos de germinação, crescimento e biocontrole. Para isso, é essencial saber a origem (ou "as origens") do microbioma vegetal. Os pesquisadores comumente escolhem o solo como a principal fonte de inóculo (Johnston-Monje et al., 2016). Mas estudos recentes apontam que a microbiota transmitida pela semente tem sido considerada uma fonte essencial para o microbioma da planta. Por muitos anos, sementes saudáveis foram consideradas como "estéreis" e sua microbiota rotulada como "patogênica" para as plantas (Berg e Raaijmakers, 2018). Por conta disso, diversos protocolos fitossanitários foram desenvolvidos nas últimas décadas, incluindo métodos físicos, químicos e biológicos de desinfestação (Berg e Raaijmakers, 2018). Contudo, esses procedimentos não removem todos os micro-organismos da semente, o que torna o conceito de "sementes livres de germes" utópico.

Ao contrário do que se pensava, as sementes são colonizadas por diversos micro-organismos benéficos, isso inclui bactérias que vivem na superfície e nos tecidos internos das sementes (Nelson et al., 2018); e que colonizam a raiz da planta após a germinação (Johnston-Monje et al., 2016; Compant et al., 2019). Como o bacterioma da semente representa os colonizadores iniciais das plântulas antes de recrutar micróbios do ambiente circundante, ele pode desempenhar papéis importantes na montagem e na função do microbioma vegetal (Nelson et al., 2018). Pesquisas com microbioma de sementes precisam ser realizadas, inicialmente, em condições experimentais axênicas, sem a contribuição da comunidade

microbiana do solo-rizosfera. Também é possível utilizar protocolos de desinfestação química amplamente difundidos (geralmente a base de álcool e hipoclorito de sódio) para reduzir o número de táxons da semente e comparar fenótipos da planta com as sementes naturais (não desinfestadas). Com essas abordagens, é possível caracterizar a estrutura do bacterioma da semente e suas funções.

Compreender o papel de bactérias nativas da semente na montagem do microbioma vegetal pode identificar os fatores que determinam o estabelecimento bem-sucedido de cepas microbianas introduzidas (Dini-Andreote e Raaijmakers, 2018). O uso de bioinoculantes na agricultura não é algo novo, mas que ainda enfrenta desafios ao transferir micróbios do laboratório para o campo sem considerar a complexidade e o comportamento ecológico da microbiota natural (Sessitsch et al., 2019). O encontro de um bioinoculante com a comunidade residente é marcado pela competição de recursos e nichos (Mawarda et al., 2020). O resultado dessa competição pode ser a resistência da comunidade ao inoculante, que não terá nenhum efeito, ou o estabelecimento da bactéria inoculada, que pode impactar a estrutura da comunidade de forma transitória ou permanente (Mawarda et al., 2020). Impactos na estrutura do microbioma caracterizam o negligenciado efeito “indireto” ou “não-alvo” dos bioinoculantes e podem resultar no crescimento vegetal e/ou no controle de patógenos (Sessitsch et al., 2019).

Para aumentar a produtividades das culturas, agricultores e cientistas tentam otimizar ou alterar o microbioma do solo e da rizosfera (Arif et al., 2020). Talvez a resposta que procuram não esteja só nesses compartimentos, mas sim nas bactérias nativas da semente. Tecnologias sustentáveis podem ter mais sucesso se os esforços forem concentrados em caracterizar, isolar ou “perturbar” (por inoculação) membros do bacterioma associado às sementes (Johnston-Monje et al., 2016).

Esta tese investiga a estrutura e a função do bacterioma da semente e seus principais objetivos foram: (i) discutir a base conceitual dos microbiomas vegetais, incluindo estrutura, papel funcional e fatores envolvidos na sua modulação; (ii) caracterizar o bacterioma da semente de milho (variedades SHS 5050 e DKB 177); (iii) avaliar o papel das bactérias transmitidas por sementes em processos de germinação, crescimento e biocontrole; e (iv) identificar o impacto da inoculação microbiana na estrutura do bacterioma da raiz de milho. A tese está organizada em cinco capítulos compilados a partir de artigos independentes. Cada capítulo contém introdução e discussão, bem como detalhes dos métodos utilizados. No final da tese são apresentadas as principais conclusões do trabalho.

Referências

Arif I, Batool M, Schenk PM (2020) Plant Microbiome Engineering: Expected Benefits for Improved Crop Growth and Resilience. Trends Biotechnol. doi:10.1016/j.tibtech.2020.04.015.

Berg G, Raaijmakers JM (2018) Saving seed microbiomes. The ISME Journal 12 (5):1167-1170.

Berg G, Rybakova D, Fischer D, Cernava T, Vergès M-CC, Charles T, Chen X, Cocolin L, Eversole K, Corral GH, Kazou M, Kinkel L, Lange L, Lima N, Loy A, Macklin JA, Maguin E, Mauchline T, McClure R, Mitter B, Ryan M, Sarand I, Smidt H, Schelkle B, Roume H, Kiran GS, Selvin J, Souza RSCd, van Overbeek L, Singh BK, Wagner M, Walsh A, Sessitsch A, Schloter M (2020) Microbiome definition re-visited: old concepts and new challenges. Microbiome 8 (1):103.

Compant S, Samad A, Faist H, Sessitsch A (2019) A review on the plant microbiome: Ecology, functions, and emerging trends in microbial application. Journal of advanced research 19:29-37.

Dastogeer KMG, Tumpa FH, Sultana A, Akter MA, Chakraborty A (2020) Plant microbiome–an account of the factors that shape community composition and diversity. Current Plant Biology 23:100161.

Dini-Andreote F, Raaijmakers JM (2018) Embracing Community Ecology in Plant Microbiome Research. Trends in Plant Science 23 (6):467-469.

Mawarda PC, Le Roux X, Dirk van Elsas J, Salles JF (2020) Deliberate introduction of invisible invaders: A critical appraisal of the impact of microbial inoculants on soil microbial communities. Soil Biology and Biochemistry 148:107874.

Nelson EB, Simoneau P, Barret M, Mitter B, Compant S (2018) Editorial special issue: the soil, the seed, the microbes and the plant. Plant and Soil 422 (1):1-5.

Sessitsch A, Pfaffenbichler N, Mitter B (2019) Microbiome Applications from Lab to Field: Facing Complexity. Trends in Plant Science 24 (3):194-198.

Singh BK, Trivedi P, Egidi E, Macdonald CA, Delgado-Baquerizo M (2020) Crop microbiome and sustainable agriculture. Nature Reviews Microbiology 18 (11):601-602.

Sivakumar N, Sathishkumar R, Selvakumar G, Shyamkumar R, Arjunekumar K (2020) Phyllospheric Microbiomes: Diversity, Ecological Significance, and Biotechnological Applications. In: Yadav AN, Singh J, Rastegari AA, Yadav N (eds) Plant Microbiomes for Sustainable Agriculture. Springer International Publishing, Cham, pp 113-172.

Trivedi P, Leach JE, Tringe SG, Sa T, Singh BK (2020) Plant-microbiome interactions: from community assembly to plant health. Nature Reviews Microbiology 18 (11):607-621.

CHAPTER 1:

Plant microbiome structure and benefits for sustainable agriculture

Chapter 1: Manuscript published

Plant microbiome structure and benefits for sustainable agriculture

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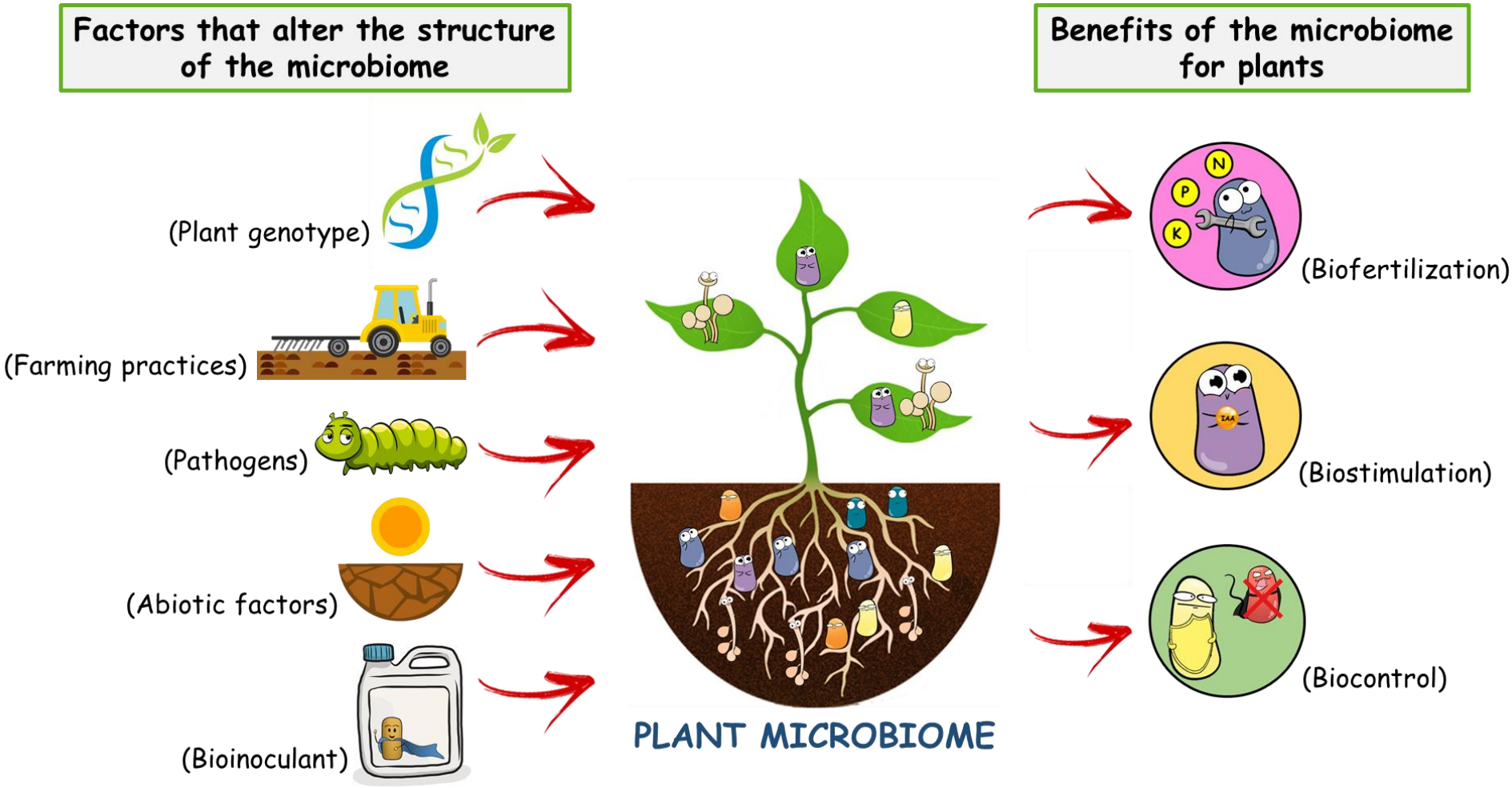
Resumo

As plantas são colonizadas por uma infinidade de micro-organismos, coletivamente denominados “microbioma”, e que desempenham papéis importantes nos nichos que habitam. O potencial do microbioma, aliado a necessidade de produzir alimentos de forma mais sustentável, torna seu estudo promissor. Apesar do crescente reconhecimento da ampla capacidade funcional da microbiota nativa para a planta, nossa compreensão acerca do modo como as comunidades microbianas se estruturam e dos fatores que podem alterá-la é limitada. Nesta revisão, mostramos como as pesquisas têm caracterizado a microbiota de diferentes nichos da planta e quais metodologias têm utilizado para isso. Demos ênfase ao impacto do genótipo da planta, interações entre táxons microbianos, práticas agrícolas e condições ambientais no estabelecimento ou enriquecimento de micro-organismos com efeitos benéficos para crescimento, desenvolvimento e saúde das plantas. Ao final, propomos um modelo que converte o conhecimento acerca dos microbiomas em produto biotecnológico. Uma primeira abordagem é utilizar fatores que influenciam a microbiota para estimular seus integrantes benéficos. A exemplo desses fatores tem-se o melhoramento de plantas, uso de bioinoculantes, de práticas agrícolas apropriadas e das condições adversas do meio. Por outro lado, micro-organismos com papéis-chave no microbioma podem ser isolados e passar a compor novos bioinoculantes. Independentemente da abordagem a ser utilizada, inovações com o uso de microbiomas representam o futuro da agricultura sustentável.

Palavras-chave: endófitos; bioinoculante; bactérias benéficas; biota vegetal; bacterioma; insumos biológicos.

Graphical Abstract
(Resumo Gráfico)



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Review article

Plant microbiome structure and benefits for sustainable agriculture

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ABSTRACT

Plants are colonized by a multitude of micro-organisms, collectively called "microbiome," and which play essential roles in the niches they inhabit. The potential of the microbiome, combined with the need to produce food more sustainably, makes its study promising. Despite the growing recognition of the vast functional ability of the native microbiota as an intricate component of the plant fitness, our understanding of how microbial communities are structured, and the intrinsic and environmental factors that can modulate microbial network is partial understood. In this review, we explore how research has characterized the microbiota of different plant niches and the methodological approaches that they have used to access this. We consider the impact of the plant's genotype, interactions between microbial taxa, the influence of the agricultural practices, as well as the environmental conditions that influence the establishment and enrichment of micro-organisms with beneficial effects on plant growth, development, and health. Ultimately, we suggested a model route that converts current knowledge about microbiomes into a potential biotechnological product applied to agriculture systems. One proposed approach is the use of the driven factors that influence the microbiota community composition to stimulate its beneficial members. Some examples of these influential factors are the use of bioinoculants, proper agricultural practices, and certain environmental conditions. On the other hand, micro-organisms with critical roles in the microbiome can be isolated, formulated, and become a new biological product. Regardless of the approach to be used, innovations with the use of microbiomes represent the future of sustainable agriculture.

1. Introduction

During evolution, micro-organisms began to colonize higher organisms, establishing symbiotic relationships that range from mutualistic to antagonistic [1]. In humans, the symbiotic relationship of the intestinal microbiota has been extensively studied, which performs a series of metabolic functions, including absorption, synthesis, and breakdown of compounds, and which is associated with host health in many instances [1]. Plants' body can be considered a complex interplay of ecological niches that harbor in their rhizosphere, surface-tissues (rhizoplane and phylloplane), and inner-tissues (endosphere), a great diversity of micro-organisms with which they establish a broad range of beneficial, neutral, and harmful interactions [2].

In the last 500 million years, with the evolution and diversification of animals and plants, micro-organisms have developed several mechanisms to invade and proliferate within eukaryotic cells and tissues [3]. The high adaptation of the microbiota (mainly bacteria) to all types of ecological niche, can be attributed to their high genetic and metabolic diversity [3]. In these environmental niches that they inhabit, symbionts provide a variety of benefits to the host. In the case of plants, the microbiota associated acts in the acquisition of soil nutrients, tolerance to abiotic stresses, and disease control, all related to the promotion of plant growth and the consequent increase of ecological fitness under the natural environment or food, fiber, and energy production under agricultural systems [4].

Given the potential of micro-organisms to improve the efficiency of plant production, several pieces of research have been carried out to discover which variables act in the modulation of symbiotic interactions [5]. What is known so far is that there are several routes for the evolution of bacterial symbiosis. Most of the time, this symbiosis emerges from ancestors of the environment, bacteria with characteristics that can benefit the host, or through horizontal transfer of genes that encode symbiotic features [5]. According to Sachs et al. [5], bacterial symbiosis can also arise from parasitic ancestors, through the vertical transmission

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of genetic material. However, these mechanisms are still not well understood.

Native microbial community of plants is called "microbiome," which is defined as the "set of genes found in association with the organisms that colonize a given environment" [6]. The microbiome dynamically interacts with the plant host to create synergistic relationships, which, in turn, influence the host's physiology [7]. Several studies are driven to explore how the microbiome is built and what is the driving forces that affect its dynamics to shape plant performance in the ecosystem.

The potential of the microbiome has stimulated studies to identify the biotic and abiotic factors that shape its taxonomic and functional structure in plants. These factors are related to the plant genotype, the interaction between microbial communities, the adoption of specific agricultural practices, and certain environmental conditions. However, little attention has been given to the interaction between "native" plant micro-organisms and the introduced as "microbial inoculants" [8], herein defined as products based on micro-organisms formulations that favor plant growth and health [9].

In recent years, most of the initiatives developed for plant growth promotion and disease control technological bio-products have appeared as bioinoculants formulations containing a single selected cultivable micro-organism [10]. The use of these individual microbial strains has faced variable success, attributed to the complexity of native microbial communities and their interactions with the environment [10]. Thus, the effect of bioinoculants on the balance of non-cultivable microbial plant communities has not yet been elucidated, but it can be attributed to 1) its direct impact on plant metabolism; 2) its effect on the displacement of the microbiome structure; 3) combination of both. However, most studies negligence the impact of bioinoculants on bacterial microbiome composition and dynamics. Understanding the bioinoculant-microbiome interaction will elucidate its effects on the host plant.

The new sequencing generation technologies, combined with bioinformatics tools, have revealed the taxonomic structure and the relative abundance of micro-organisms in plant microbiome research [8]. With these independent-cultivation techniques, it is possible to understand how the microbiome is structured in different ecosystems and their role in these environments [7]. Advances in this area will undoubtedly promote benefits to the "health" of plants. It is believed that, shortly, it will be possible to manipulate the bacterial community of plants to promote their best development, in addition to isolating the "central microbiome" or the "specific microbiome" of cultures for the formulation of new bioinoculants [11].

This review aims to describe the location, benefits, and factors that alter the structure of the plant microbiome. Ultimately, a model that converts this knowledge into biotechnological agricultural applications will be considered.

2. Concepts and evolution of studies in plant-microbiomes

The current microbiome (archaea and bacteria) emerged about 3.8 billion years ago, 2 billion years more than eukaryotic organisms [12]. However, the definition of "microbiome" has a shorter history. This term was first coined by Lederberg and McCray [13] as "the ecological community of commensal, symbiont or pathogenic micro-organisms, which occupy space in our body," where the authors referred to micro-organisms associated with humans. In 2002, "The Human Microbiome Project Consortium" [14] simplified the definition for "micro-organisms associated with humans." Currently, the term microbiome encompasses micro-organisms in any configuration. However, there is a problem with its meaning, which can be read as "micro-biome, " defined as the "set of resident micro-organisms that inhabit a given host/environment" or "microbi-ome," defined as the "set of genes found in association with the organisms that colonize a given environment." In this case, both definitions are accepted.

For many years, research on the microbial communities of the plant kingdom has focused on the study of specific micro-organisms, such as arbuscular mycorrhizal fungi and rhizobia [1]. Thus, a holistic view of microbial groups associated with plants is a new perspective [15]. The importance of research on plant microbiomes can be seen by the high number of publications on this topic, with numerous studies considering different plant compartments from the soil to plant continuum, studies showing changes in the microbiome modulated by environmental factors and its potential benefits for agriculture [8].

In Brazil, several research groups have contributed to important discoveries on microbiomes issues. About 3% of scientific publications in the plant microbiomes field are carried out by Brazilian research groups [8]. Brazilian contributions in scientific papers on the endosphere, rhizosphere, and phyllosphere compartments of plants correspond, respectively, to 4, 2, and 1% of the literature [8]. These studies have focused on the microbiome composition in different plant compartments and factors that influence the assembly of the microbiome, and the use of molecular tools to access microbial communities and its potential benefits for sustainable agriculture.

A recent Brazilian initiative was the creation of the "Brazilian Microbiome Project (BMP)" (http://www.brmicrobiome.org/), intending to organize data from different Brazilian microbiomes [16]. The results obtained at BMP are integrated into a larger project, called "Earth Microbiome Project (EMP)" (http://www.earthmicrobiome.org/), providing Brazilian microbiomes with a broader analysis [16].

From a global perspective to the plant microbiome theme, it is noted that recent initiatives have sought for its use in agricultural production. The first was launched by the White House, which created the US Microbiome on May 14, 2016, with investments of US $ 450 million to improve research and commercialization of microbiomes. A second initiative was created by the European Union Commission when launching the International Bioeconomy Forum (IBF) on October 13, 2016, where the use of microbiomes for food and nutritional security was the central theme [15].

From the works presented, the elucidation of taxonomic and functional patterns of micro-organism-micro-organism interactions and plant-micro-organism interactions is a direction for future research. It is worth mentioning that, together with the need to unveil the "secret" of microbiomes, the demand for new methodologies for their study has grown.

3. Methodologies used in the study of plant microbiomes

Classic microbiological approaches, such as the isolation and cultivation of micro-organisms from nutrients and growth conditions that vary according to the target organism, are essential for their genetic and physiological study [17]. Testing sole micro-organisms reveal their different life strategies and their influence on promoting plant growth [18]. However, cultivation-dependent techniques result in the loss of much of the microbial diversity network of the environment [17]. Thus, numerous independent-cultivation techniques have revolutionized science and allowed for a better understanding of plant-microorganism interactions [17]. Advances in DNA/RNA extraction procedures coupled with omics' analysis have been providing information about diversity, abundance, functional profile, and metabolites in different microbial communities [16,19,20].

Metagenomics, a term created in 1998 to represent the total genomes of microbial communities [21], initially consisted of using cloning DNA fragments obtained from environmental samples and analyzing libraries in search of new micro-organisms [21]. The advancement of this technique occurred with the development of next-generation sequencing technologies, which do not require cloning of DNA fragments and produce a higher amount of data [22]. Within the metagenomics, it is possible to sequence all the DNA of an environmental sample (Shotgun) and has its taxonomic and functional profile. However, most studies that unearthed the composition of microbiomes used sequencing with microbial markers for bacteria (rRNA 16S) and fungi (ITS).

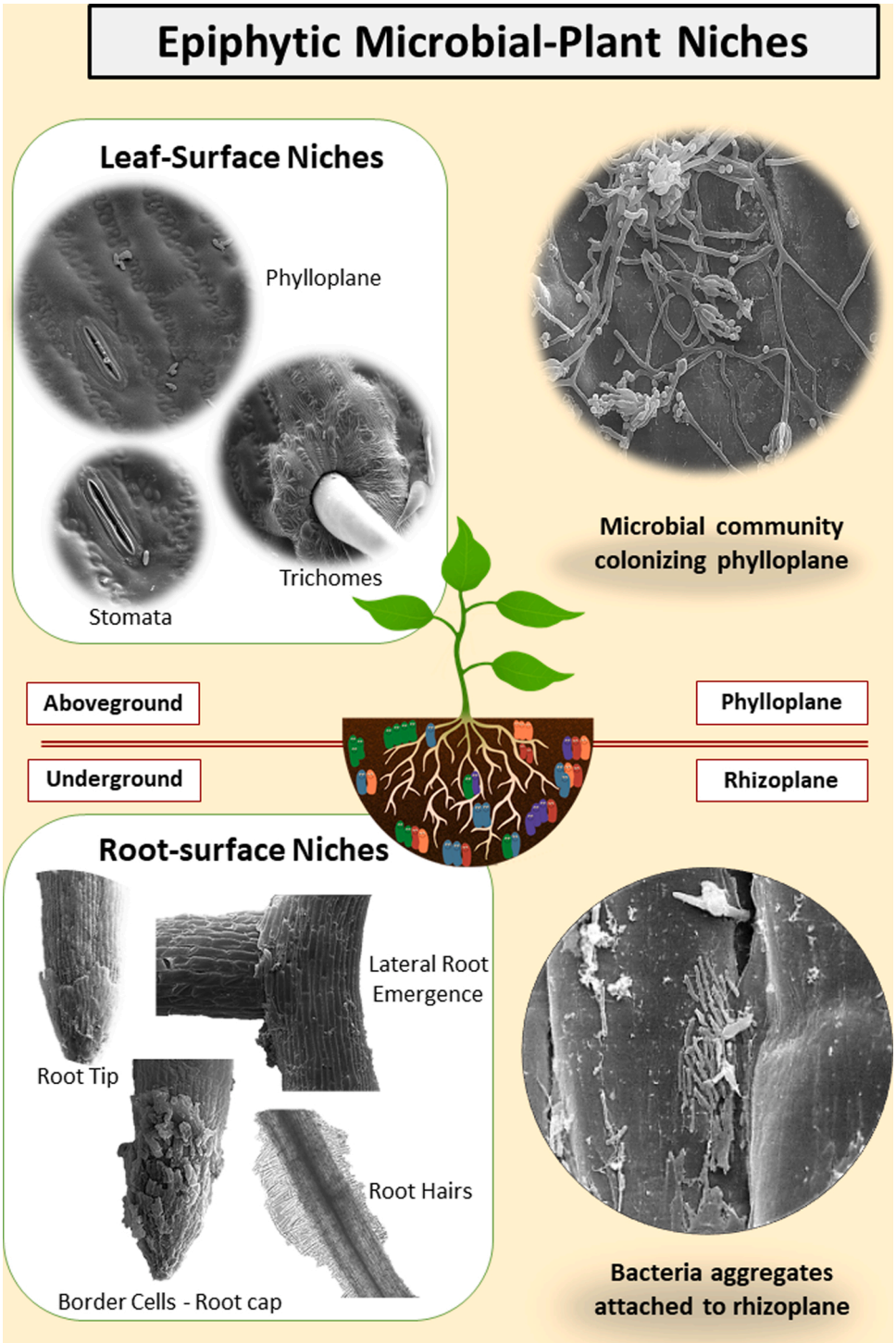

Fig. 1. Epiphytic microbial-plant niches at the underground (rhizoplane) showing some examples of root anatomy domains available to the microbial attachment (border cells, root tip zone, elongation zone, root hair zone). Aboveground leaf-surface niches exemplified by stomata complex, trichomes, and the periclinal cell-wall surface of epidermal cells available for phylloplane colonization.

By new generation sequencing, Cai and collaborators [23] concluded that the chemical composition of the soil influences fungi and bacteria rhizosphere composition of tomato and cucumber plants. Using this same technique, Hartman et al. [17] observed that rhizobia colonize 70 % of the root microbiome of *Trifolium pratense*. Other molecular biology platforms perform synthesis-based sequencing, pyrosequencing. When using this technique, Bergottini et al. [24] concluded that the microbial diversity of yerba mate roots was altered when the plant is submitted to different cultivation practices. Using the same approach, Shcherbakova et al. [25] observed that the inoculation of beneficial symbionts in chickpeas modified their rhizospheric microbiome and the composition of their root exudates.

Microscopic techniques combined with molecular biology tools also contribute to advances in the study of the structure of microbiomes [26, 27]. Among the methods used to evaluate these microbial communities, there is microscopy: 1) epifluorescence light microscopy; 2) interferential and differential contrast light microscopy; 3) bright-field light microscopy; 4) scanning electron (SEM) and 5) transmission electron

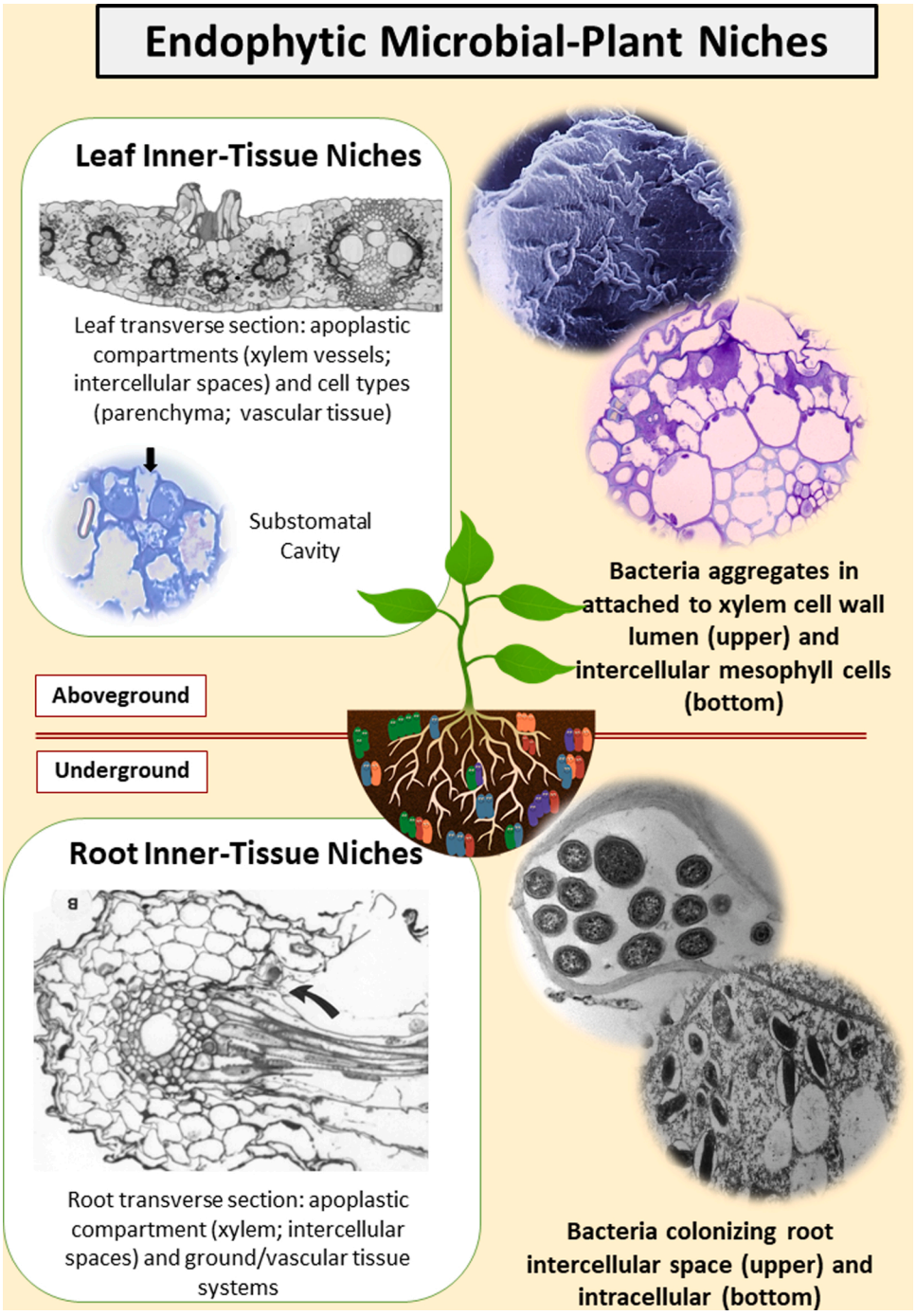

Fig. 2. Endophytic microbial-plant niches at the underground showing a transverse cross-section of the root tissue, also showing lateral root emergence (main entrance natural pathway for inner tissue) and internal domains that harbor endophytes. The main endophytic niche occupied is the apoplastic compartment, including intercellular spaces of ground tissue and vascular tissue. Aboveground leaf-transverse section showing the apoplastic compartments (intercellular mesophyll spaces, xylem lumen and substomatal cavity).

microscopy (TEM). These techniques make it possible to obtain visual evidence of the intimate colonization pattern of the microbial community associated with plant tissues [28]. Also, many other approaches are used in the study of plant microbiomes. For example the real-time PCR [26,29], automated version of ribosomal intergenic spacer analysis (ARISA) [29], fluorescence in situ hybridization (FISH), denaturing gradient gel electrophoresis (DGGE), terminal restriction fragment length polymorphisms (TRFLP), phospholipid and fatty acid analysis [30]. These methods analyze microbial diversity based on their genetic structure or differences in the lipid components of the membrane [30].

Given recent advances, it is believed that modern molecular methodologies can be used in conjunction with more conventional analyses [15]. Recently, microcosm systems have been used in combination with reference stocks of bacteria to assess the process of assembling the root microbiome and its effect on plant growth. Using microcosms, Hartman et al. [17] observed that the inoculation of *Flavobacterium* alone in *Trifolium pratense* reduced its growth. However, its co-inoculation with other members of the root microbiome reduced the negative effect of this single micro-organism application. Bodenhausen et al. [29], when evaluating the influence of the plant's genotype on the composition of the phyllosphere microbiome, used the plate counting method to enumerate the colony-forming units (CFU) of that compartment.

It is noteworthy that molecular techniques also have limitations, where the difficulty of amplifying the 16S rRNA gene from plants can be highlighted since plant tissues are rich in chloroplastic and mitochondrial DNA, similar to cyanobacterial DNA [15]. Nevertheless, in recent years the use of peptide nucleic acid (PNA), which blocks the amplification of contaminating sequences, has facilitated the sequencing of

amplicons in prokaryotes [15]. The PCR amplification of the rRNA 18S gene also has limitations inherent in the lack of universal *primers,* and that provides a consistent and impartial view of fungi in the plant microbiome [15].

Another challenge of research on plant microbiome is to characterize the wide variety of molecules responsible for the network communication between plants and micro-organisms with the technology currently available [15,20]. Thus, the increased sensitivity of existing tools, such as spectroscopies, and the use of molecular approaches, including metatranscriptomics and metabolomics, are necessary for the characterization of microbiome signaling molecules [15].

Although molecular tools are more used in studies on microbiomes, culture-dependent methods, including the isolation of micro-organisms, contribute to the consolidation of knowledge obtained from modern techniques in innovative biotechnological products, such as bioinoculants [15]. Thus, the creation of multidisciplinary approaches, which integrate new technologies and traditional tools, is of paramount importance for advancing research on plant microbiomes. These approaches may identify, for example, the microbiota that inhabits the different niches of the plant.

4. Microbial niches in plants

Plants have highly distinct microenvironments that harbor complex and diverse communities of fungi and bacteria, the so-called "plant microbiomes" [8]. These microbiomes represent a continuum of microbial communities associated with different niches on the surface (epiphytic interactions - Fig. 1) and inside plant tissues (endophytic interactions - Fig. 2). Studies on microbiomes explore different vegetative/reproductive organs and various compartments of the plant where micro-organisms can establish and colonize. In the plant, habitats such as the antosphere, carposphere, and spermosphere are less investigated [31–33]. So far, much research has focused on the study of the microbiota associated with the rhizosphere, seed, root, and aerial part of plants.

4.1. Rhizosphere microbiome

The soil is the largest reservoir of micro-organisms that interacts with plants [34] and houses, in its rhizospheric portion, almost 1011 microbial cells/gram of root, responsible for more than 30,000 prokaryotic species [35]. The rhizospheric part of the soil is defined as a region that is influenced by exudates and root secretions and is home to a microbial community that is crucial for plant growth and health [23]. This fraction of microbial diversity present in the soil is responsive to a variety of chemical compounds and other physicochemical changes in the rhizospheric compartment, favoring the selective enrichment of specific microbial communities over others [36]. In the rhizodeposition process, rhizoderm cells release a wide range of compounds, including organic acids, inorganic acids, siderophores, sugars, vitamins, amino acids, purines, nucleosides, and polysaccharide mucilage [22,37]. Because it is a carbon-rich environment, the rhizosphere has 10–100 times more micro-organisms than "bulk" soil [37].

The rhizodeposition process also refers to the release of specialized cells from the root tip, called "border cells," which influence the microbial colonization of the rhizosphere [38]. This increase in microbial activity in the rhizosphere is accompanied by a reduction in microbial diversity related to the adjacent soil [39].

As several studies have shown the role of root exudates in bacterial chemotaxis and, consequently, in the colonization of the host plant, it is believed that rhizodeposition contributes, at least in part, to the structuring of plant bacterial communities [40,41]. According to Bakker et al. [42], it is necessary to use the exudation characteristics of plants to select and improve beneficial microbial activities.

Root exudates contribute to shaping the rhizospheric microbial community. Nevertheless, its quantity and composition vary from one plant to another. The assembly of the microbiome also tends to be different among plant species. Turner et al. [2,43], when comparing the rhizosphere of wheat, oats, and peas, found similar microbial groups belonging to the phyla Proteobacteria, Actinobacteria, Firmicutes, Acidobacteria, Planctomycetes, and Bacteroidetes. However, they also observed that peas (legumes) had a more substantial effect on the rhizosphere than wheat and oats (cereals). Legume plants, due to symbiotic internship with nitrogen-fixing rhizobia, present a root exudation that differs, in quantity and quality, from non-leguminous plants, which generates the distinctive assembly of the rhizospheric microbiome. The exudation of the roots is essential in the recruitment and modulation of the rhizosphere microbiome; however, it is worth mentioning that other biotic and abiotic factors can affect this assembly [44].

On the other hand, some microbial taxa are always detected in a host plant or a condition, the so-called "central microbiome" or "microbial core" [45]. Sequencing the rhizospheric microbial community of 19 herbaceous plants showed that each species shared 8–111 select operational taxonomic units (OTUs) [44]. The existence of a central microbiome was also confirmed by identifying members of Rhizobiales, Sphingomonadales, Burkholderiales, and Xanthomonadales in the rhizosphere of six different plant species (*Artemisia argyi*, *Ageratum conyzoides*, *Erigeron annuus*, *Bidens biternate*, *Euphorbia hirta* and *Viola japônica*) as reported by Lei et al. [46].

In arid regions, the species *Adenium obesum*, *Aloe dhufarensis* and *Cleome austroarabica* present microbial taxa and rhizosphere-specific enzymatic activity, but share fungi from the phylum Ascomycota and Basidiomycota, in addition to bacteria from the groups' Actinobacteria, Proteobacteria, Bacteroidetes, Planctomycetes, Acidobacteria and, Verrucomicrobiae [47]. In wheat, a survey in the rhizosphere pointed out the bacterial phyla Proteobacteria, Actinobacteria, and Acidobacteria and the fungi Ascomycota, Chytridiomycota, and Basidiomycota as the dominant groups [48]. Currently, many works have been dedicated to the mapping of central microbiomes, in an attempt to use them in different plant species and contexts. However, these abundance-defined nuclei may miss non-dominant members of the microbiota, but essential in many processes. Moreover, perhaps the source of crucial microbes is not just the soil, as most studies point out, but the seed.

4.2. Seed microbiome

Soil is considered the primary source of microbial diversity in the rhizosphere. However, recent studies indicate that part of its microbial composition may result from the release of resident epiphytic and endophytic micro-organisms associated with plant tissues during the process of germination [33,49]. Seeds harbor beneficial and pathogenic microbiota on their surface and inside the tissue [50]. Beneficial seed micro-organisms act on the initial development of the plant, affecting germination and seedling survival [51,52].

The micro-organisms that inhabit the seeds can be acquired directly from the mother plant (vertical transfer) or the environment (horizontal transmission). In the vertical transfer, it is believed that micro-organisms associated with the flower, fruits, and leaves of the plant come into contact with the seed and become part of its microbiome. This same seed, upon reaching the soil and undergoing imbibition, initiates a germination process. In this process, several metabolites, such as organic acids, amino acids, fatty acids, and carbohydrates, are released around the seed - a region called the spermosphere - creating an attractive area for soil micro-organisms [37]. At that moment, the soil microbiota can start to compose, via horizontal transfer, the seed microbiome [53]. For that, it will have to compete against the microbiota already established in the seed. Kumar et al. [37] listed studies in which several bacterial taxa, such as *Bacillus*, *Pseudomonas* e *Rhizobium*, exhibited positive chemotaxis for seed exudates. They also showed that these exudates induce the expression of several metabolic genes in bacteria [37].

Based on the transmission pathways (vertical vs. horizontal), micro-organisms will occupy distinct niches in the seed, such as its coating

(pericarp), the embryo (embryonic and cotyledon axis), and storage tissue (endosperm or perisperm) [53]. According to Barret et al. [54], micro-organisms associated with the embryo and endosperm are more likely to be transmitted vertically, while those associated with the coating are transmitted horizontally.

Advances in sequencing and microscopy technologies have allowed the characterization of micro-organisms that live outside and inside seeds, with a more significant number of studies aimed at bacterial communities. In several plants, bacteria are often associated with the phyla Proteobacteria, Actinobacteria, Firmicutes, and Bacteroidetes [40,49,50], which can be explained, in part, due to the predominance of these phyla in the soil [55]. Of course, biotic and abiotic factors influence the recruitment of specific micro-organisms to the plant, but native bacterial species of the seed can be conserved in some vegetables [40,56]. However, some plant species have a highly specific microbial signature and with a small central microbiome, which was observed when comparing the seed microbiome of eight species of alpine plants [57].

The correlation between the seed and soil microbiome is still unclear, but it is known that seed bacteria can colonize different parts of the plant [58,59], and may even come out from the root to the rhizosphere and reach the ground [56]. On the other hand, soil microbes appear later and need to compete against the already established microbiota in the seed [51]. Micro-organisms selected in the rhizosphere will be able to move to other parts of the plant.

4.3. Root and shoot microbiome

The area of a leaf can harbor an average of 10^6-10^7 cm^{-2} bacteria and fungi ranging from 10^2-10^8 CFU g^{-1}, a number comparatively less than the rhizosphere diversity [60]. Even though the rhizosphere houses a rich and diverse microbiota, part of this community has developed mechanisms for physically interacting with plants. Through chemotaxis processes, followed by microbial adhesion mechanisms, bacteria, and fungi attach epidermal-cell wall root surface (Fig. 1). These epiphytic interactions in the rhizoplane may involve the participation of anchoring structures and adhesive proteins that result in the firm adhesion of micro-organisms to the plant cell wall, with subsequent formation of aggregates and biofilms [61]. Epiphytic interactions in the phylloplane can result from systemic spreading through the xylem vessel elements by rising water flux in the transpiratory processes of the plant or by direct access of micro-organisms to the surface of the stem and leaves [1].

Part of the epiphytic microbial community developed strategies to infect and colonize the interior of the plant's tissues, thus establishing an endophytic interaction (Fig. 2). Endophytic micro-organisms are those capable of colonizing at least in part of their life cycle, the interior of plant tissues without inciting symptoms of the disease [62]. In these interactions, micro-organisms can modulate the plant's innate immune response and live inside the planta, mainly in apoplastic compartments [63]. This compartment is also known, by some authors, as the endosphere [8].

According to life strategy, endophytes can be classified as mandatory or optional. Mandatory endophytes are dependent on the host plant for growth and survival and inherited through vertical transmission via seeds or vectors [26,64]. Optional endophytes have a biphasic life cycle, alternating between the host plant and the environment. These micro-organisms come from the soil, infect the host plant, and colonize the plant tissue [26,64]. Most endophytic plant-growth promoters are optional.

The structure of microbial communities in the different compartments that make up plant microbiomes can be influenced by the micro-organism's ability to colonize and allocate plant resources [18]. For efficient colonization, it is observed that root endophytes often adhere to the surface of the roots and multiply. Then, they invade the intercellular environment through natural openings caused mainly by the emission of lateral roots [61]. After initial colonization, some endophytes can move to the shoot of the plants [61]. The allocation of resources in different plant compartments also modulates the endophytic community distribution pattern on the tissues [18]. The persistence of the microbiota in the plant compartments also depends on its ability to compete with other micro-organisms and the impact of adverse conditions of the environment [65].

Phyllosphere microbiota is distributed in distinct leaf-niches microenvironments. It needs to adapt to a more hostile environment with frequent changes in temperatures, light, UV radiation levels, low availability of water, and nutrients [65]. These are the factors that alter the composition and microbial diversity in the phyllosphere. In this region, bacteria are predominant, and the phyla Firmicutes, Acidobacteria, Actinobacteria, and Cyanobacteria are frequently found. Yeasts of the genera *Cryptococcus*, *Sporobolomyces, Rhodotorula*, and filamentous fungi belonging to the genera *Cladosporium*, *Alternaria, Penicillium*, *Acremonium*, *Mucor,* and *Aspergillus* are also part of the leaf microbiome community [65].

Microbiome composition similarities between the root and its rhizosphere have been observed with the dominance of bacteria belonging to the phyla Proteobacteria, Actinobacteria, and Bacteroidetes. However, the bacterial diversity is higher in the rhizosphere compartment and lower in the interior of the root [66]. The endophytic microbial community associated with olive roots (*Olea europaea*) was less diverse than the rhizospheric, being represented by the bacteria *Actinophytocola*, *Streptomyces,* and *Pseudonocardia* and by the fungi *Canalisporium*, *Aspergillus*, *Minimelanolocus* and *Macrophomina* [67]. Probably, plant physical and biochemical barrier exerts selective pressure over the root-epiphytic community. As a result of this selective pressure to gain entrance into the plant tissue, the endophytic microbiota is less diversity and composition tends to remain stable inside the plant and vary significantly in the rhizosphere [68].

Recognizing the plant body as a complex interconnect niches that harbor the microbiota in the rhizosphere, rhizoplane/phylloplane, and endosphere domains represents a step forward to understand what are the modulatory factors that shape plant microbiome composition.

5. Factors that alter the structure of microbiomes (composition, wealth and abundance)

Although several types of micro-organisms form the microbiome, different groups respond similarly to biotic and abiotic factors that impose strong selection in the composition of their community [8]. Among these factors are the plant genotype, the interaction of microbial communities, the use of specific agricultural practices, and environmental conditions.

5.1. Effect of plant genotype

Plants benefit from several functions performed by their microbiome, which is why they invest part of their carbon sources in the formation and maintenance of the microbiota. In this sense, plants with different genotypes behave differently concerning root metabolism, the composition of root exudates, recognition systems, and innate immune response [25,69,70]. Studies indicate that variations of this nature can alter the structure and activity of the plant microbiome, which, in turn, acts on growth, nutrition, and resistance to biotic and abiotic stresses [71].

The contribution of the plant genotype to the structure of the root microbiome was observed in a study by Curlango-Rivera et al. [72], where the production of border cells varied between different cotton cultivars. These cells are formed from meristematic cells and were initially considered "dead" and responsible for the mechanical protection of the root tip. Today, it is known that border cells are related to colonization of the root by micro-organisms. Similarly, plant roots deposit high and low molecular weight compounds at the root-soil

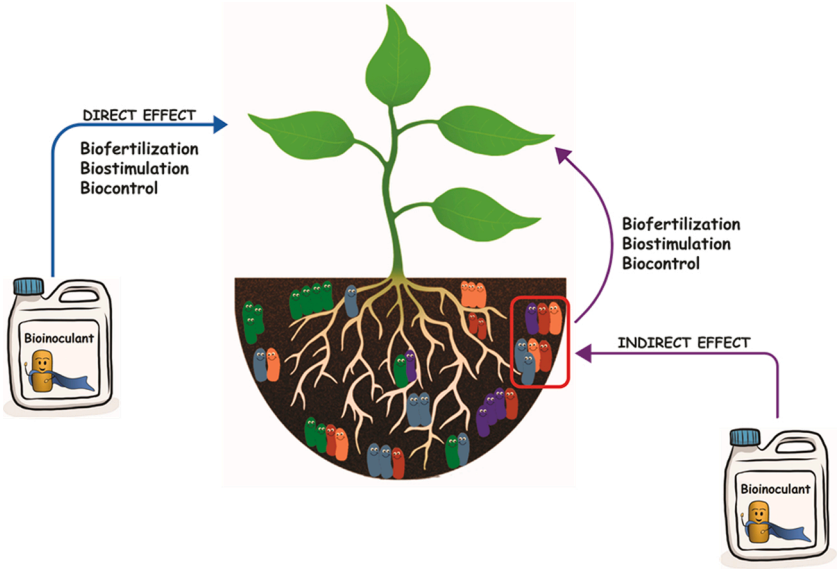

Fig. 3. Direct (blue arrow) and indirect (purple arrow) effects of the bioinoculant application on the plant host. Plant-growth promotion characterized by the release of phytohormones (biostimulation effect), the supply of nutrients (biofertilization effect), and protection against pathogens (biocontrol) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

interface, the rhizodeposition, which may vary according to their genotype [22]. Thus, plants with different genotypes can release compounds with different composition, promoting specific microbial signaling and colonization [73].

When analyzing the seed microbiome, different authors identified the impact of the plant genotype on the microbial composition of the seed [50,74]. In the *Arabidopsis* phyllosphere, a study revealed that four of the nine genotypes tested had a different microbial composition, which indicates that the genetic factors of the host plant may shape the associated microbiota [29]. Similar results were found by Morella et al. [75] in the tomato phyllosphere. In the rhizosphere, Mendes et al. [76] observed that bacteria from the families Pseudomonadaceae, Bacillaceae, Solibacteraceae, and Cytophagaceae were more abundant in *Fusarium*-resistant bean cultivars. *Fusarium* resistance shaped the microbial assembly of the rhizosphere and selected bacterial taxa with biocontrol activity [77]. In watermelon, bacteria and actinomycetes were more abundant in the rhizosphere of the cultivar resistant to *Fusarium oxysporum* than in the susceptible variety, while the population of the fungi *Penicillium, Fusarium* and *Aspergillus* were smaller in the resistant cultivar [78]. Rhizospheric soil transplantation from tomato plant resistant to *Ralstonia solanacearum* to a susceptible plant genotype suppressed the symptoms of the disease [79]. It was noted that the transplanted soil was abundant in flavobacteria and capable of suppressing the pathogen [79].

In this sense, genetic improvement should start to consider the microbiome associated with the plant, to design or select cultures capable of attracting the beneficial microbiota and of agricultural interest, as antagonists and growth promoters [71]. The practice of selecting and improving plants due to their association with the beneficial microbiota is highly promising. Nevertheless, the progress depends on understanding the functioning properties of the microbiome and its interaction with the plant [71].

5.2. Effect of bioinoculants (beneficial interactions)

Bioinoculant is a sustainable method to increase crop productivity while reducing the use of chemical fertilizers [61]. However, knowledge of its impact on the plant microbiome is very scarce [80]. Thus, understanding the interaction between native and inoculated microbial communities in plants will contribute to the improvement of biological products [80].

Microbial inoculants efficiency depends on the inoculum traits and its ability the overpass native microbial competition and establishes in the rhizosphere. Rhizospheric competence is a multigenic heritage, poorly understood but crucial for successful, positive plant-response [61]. The interaction between native micro-organisms of the plant and micro-organisms called “transients” can be parasitic, predatory, competitive, or mutualistic [81]. This interaction can be mediated by the production of specific molecules, with antimicrobial or probiotic activity, which selectively inhibits or stimulate microbial growth [70]. In this way, the native microbial community of the host plant is crucial for the success of its colonization by transient micro-organisms [81].

The establishment of micro-organisms in the root region involves different plant-microorganism signaling pathways and between microbes. For the interaction between micro-organisms, the *quorum sensing* system, which regulates population density by producing low molecular weight molecules, is a form of communication. The mechanism is underlined by acylated homoserine lactones (AHLs) between gram-negative bacteria and cyclic peptides between gram-positive bacteria [82]. Examples of plant-microorganism interaction include the release of effector proteins by symbionts to suppress, activate, or alter host defense [83] and reorganize plant metabolism [84]. Thus, bioinoculants with higher invasive capacity can establish themselves in the soil of the host plant, alter the structure of their native microbial community, and the response of the plant to the inoculum [85]. From this, one can classify the effects of bioinoculants on plant-growth promotion as indirect or direct.

The effect of the bioinoculant is considered indirect when it causes changes in the structure of the microbiome (Fig. 3). The first reports related to these changes were made by Andreote and colleagues [8] and Conn and Franco [86], where the inoculant modified the structure of endophytic populations. In a study by Ardanov et al. [85], changes in the structure of the endophytic potato community were observed in response to the inoculation of *Methylobacterium* sp. In this work, bacterial inoculants increased the relative abundance of *Massilia* sp., *Acinetobacter* sp., *Entyloma* sp., and *Phoma* sp., known for their biocontrol properties.

The influence of a microbial inoculant on the seed microbiome was reported by Mitter and colleagues [26]. In this study, *Paraburkholderia phytofirmans* (PsJN) inoculation in spring wheat seeds reduced the

population of Proteobacteria and enriched with Flavobacteria.

Certain fungi can also modify the composition of the microbiome, which was observed when inoculating arbuscular mycorrhizal fungi in salvia (*Salvia officinalis*), French lavender (*Lavandula dentata*), thyme (*Thymus vulgaris*), and santolina (*Santolina chamaecyparissus*), o that modified the composition of the bacterial community associated with the rhizosphere [87]. Likewise, inoculation of the fungus *Metarhizium* in beans increased the diversity of micro-organisms that promote plant growth, such as *Bradyrhizobium*, *Flavobacterium*, *Chaetomium,* and *Trichoderma*, in addition to suppressing the activity of the *Fusarium solani* pathogen [88].

Pyrosequencing the 16S rRNA gene, changes in the bacterial community of the chamomile rhizosphere were observed from the application of different bioinoculants, where bacteria belonging to the phylum Verrucomicrobia were only present in the treatment with *Stenotrophomonas rhizophila* [80]. Actinobacteria were only observed in samples treated with *Bacillus subtilis*, *Stenotrophomonas rhizophila,* and *Serratia plymuthica* [80]. However, dominant taxonomic groups were common to all treatments and included the phyla Proteobacteria, Bacteroides, Firmicutes and Actinobacteria; and the genera *Rhizobium* (phylum Proteobacteria), *Pseudoxanthomonas* (phylum Proteobacteria), *Pseudomonas* (phylum Proteobacteria), *Flavobacterium* (phylum Bacteroidetes) and *Arthrobacter* (phylum Actinobacteria) [80]. Gu and colleagues [89] had shown that microbial inoculants isolated from forest soils, soybeans, and tomatoes showed apparent effects on the bacterial structure of the rhizospheric community, where the genera *Streptomyces*, *Luteimonas,* and *Enterobacter* were identified as responsible for the growth of plants.

The effects of bioinoculants are considered direct when the inoculum can act on plant metabolism and improve, for example, the distribution of roots in the soil, increasing the colonization sites by the microbiota [61] or when the inoculation changes the profile of root exudates, which, in turn, modulate the structure and function of the microbial community (Fig. 3). In this sense, Da Silva Lima et al. [90] observed that root maize seedlings inoculated with *Herbaspirillum seropedicae* and the bacteria combined with humic acid exuded a diversity of nitrogen compounds, differing from the exudation profile of control or humic acid-treated plants. Changes in the secondary metabolism of chamomile (*Chamomilla recutita*) were also observed by the increase in the flavonoid apigenin-7-O-glycoside when this plant was inoculated with *Bacillus subtilis* and *Paenibacillus polymyxa* [80].

Biocompatibility studies between inoculated and native micro-organisms can solve several failures obtained in this interaction [91]. Molecular technologies allow characterizing the microbiome of different plants, comparing with the microbiome of plants treated with bioinoculants [91]. From this understanding, it will be possible to isolate new micro-organisms of agricultural relevance from the microbiome, and design new bioinoculants, or shape it according to the need of the culture [91].

5.3. Effect of a pathogen

The endophytic plant-community can present beneficial and harmful micro-organisms in a latent state. These dormant micro-organisms can be "reactivated" by external factors, such as the attack of pathogens, and promote the resistance of plants to diseases. The endophytic community can induce plant defense thought: 1) the endophytic community must contain micro-organisms that act in resistance; 2) native endophytic populations must be reactivated by an entry micro-organism, as a biological control agent, or by abiotic factors [92]. However, reactivation of latent micro-organisms is more efficient after attack by pathogens [92].

Little is known about the impact of pathogens on the plant microbiome structure, but some studies have devoted themselves to investigating this hypothesis. In *Arabidopsis thaliana*, the presence of phytopathogenic fungi altered the epiphytic and endophytic bacterial colonization of the phyllosphere [93]. Erlacher et al. [94] showed that the inoculation of the phytopathogenic fungus *Rhizoctonia solani* increased the diversity of Gammaproteobacteria in lettuce. In this study, the authors identified an increase in enterobacteria in the phyllosphere of lettuce with root rot, while the genera *Acinetobacter* and *Alkanindiges* were identified as indicators of healthy plants. Similar results were found by Köberl et al. [95], when reporting that banana trees infested by *Fusarium oxysporum* were colonized, preferably, by bacteria from the Enterobacteriaceae family, while healthy plants were associated with an increase in *Pseudomonas* and *Stenotrophomonas*. In kiwifruit, the bacterial canker disease, caused by *Pseudomonas syringae*, affected the microbiome of the *Actinidia delicious* phyllosphere, where it promoted a drastic reduction in microbial diversity [96]. The pathogen *Plasmodiophora brassicae* was responsible for reducing the abundance of *Flavisolibacter* and *Streptomyces* in the rhizosphere and *Bacillus* in the root of Chinese cabbage [97]. Also, in the rhizosphere, the abundance of *Ralstonia solanacearum* reduced the alpha diversity of the bacterial community, where *Bacillus* and *Chitinophaga* were negatively affected [41].

On the other hand, changes in microbial composition and diversity after infection of pathogens can be based on chemical changes in the plant, such as changes in the root exudation profile [98,99]. This change was observed in the *Arabidopsis* plant, which, when infected with the pathogen *Pseudomonas syringae*, had increased malate secretion, which attracted *Bacillus subtillis* to the roots, a bacterium active in biocontrol [100].

Since the microbiome can help plants withstand pathogen infections, it is conceivable that they can affect the host's microbiota. The works cited here sought to understand the complex phytopathogen-microbiome interaction in order to seek new alternatives for disease control.

5.4. Effect of agricultural practices (fertilizers, green manure, compost, vermicompost, agrochemicals, crop rotation, monoculture)

Agricultural practices are often related to changes in the plant microbiome. This influence occurs due to changes in soil properties, mainly nutritional and can affect the microbiome directly, by stimulating or inhibiting its activity according to their nutritional preferences, or indirectly, interfering in the way plants select their micro-organisms [23].

Sustainable technologies have been gaining ground in global agriculture and are identified as the most "committed" to preserving plant microbiomes. In this sense, several studies are being carried out in order to understand the effect of sustainable practices on the structure of the plant's microbial community. As an example, we can mention the use of native trees and residues of vegetable and animal origin. According to Bergottini et al. [24], the use of green manure and agroforestry system alters the composition of the yerba mate bacterial microbiome, and its cultivation in monoculture favors the development of an abundant fungal microbiome. Further evidence of the effect of the organic system on the microbiome is presented in work by Lupatini et al. [101], where the use of organic compost in soil with crop rotation increased the phylogenetic richness, diversity and bacterial heterogeneity of the soil when compared to the conventional cultivation system. In the potato rhizosphere, the use of mulch increased the diversity of fungi, with Ascomycota being the dominant phylum. On the other hand, this practice inhibited the reproduction of the *Fusarium* pathogen [102].

In a long-term field experiment (more than two decades), Hartmann et al. [103] compared soils managed in conventional and organic systems and concluded that these soils housed different microbiomes. The organic system was composed of specific microbiota, but known to degrade organic compounds, while the system that did not receive manure housed oligotrophic micro-organisms, typical of environments with few nutrients. In another study, Hartman et al. [104] identified that cultivation practices altered the microbial community of the soil and winter wheat roots, where no-tillage was determinant for soil bacteria and root fungi. On the other hand, the type of management influenced

soil fungi and root bacteria. In this case, about 10 % of the variations in the structure of the microbiomes are explained by the management practices. Campisano et al. [105], when studying the influence of agricultural practices on the endophytic bacterial community of vines, observed that the genera *Mesorhizobium*, *Caulobacter,* and *Staphylococcus* were more abundant in organic vines. At the same time, *Ralstonia*, *Burkholderia,* and *Stenotrophomonas* were abundant in vines subjected to integrated pest management (IPM).

Understanding the effect of nitrogen fertilization on the plant microbiome is of great importance for the sustainable management of this fertilizer in agriculture. The use of increasing doses of nitrogen, for example, increases the exudation of sugars, sugar alcohols and phenolic compounds in maize, which, in turn, can alter the structure and abundance of bacteria in the rhizosphere. In this case, bacteria of the orders Bacillales, Rhodocyclales, and Nitrosomonadales are more abundant because they are related to nitrogen cycling [106]. Cai et al. [23] also correlated the differential selection of the rhizospheric microbiome of tomatoes and cucumbers to nitrogen fertilization of crops.

In a study by Li et al. [39], the results found suggest that the monoculture of black pepper, in the long term (12, 18, 28, and 38 years), alters the composition of the soil microbial community and its physicochemical properties. In this study, the authors observed that monoculture increased the abundance of bacterial phyla in the rhizosphere, but reduced it in non-rhizospheric soil, with a significant decline in Firmicutes. An increase in soil/rhizosphere *Fusarium* population was observed accomplished by a decrease in *Pseudomonas* and *Bacillus*, beneficial bacteria for black pepper. On the other hand, in a crop rotation system with pepper and banana, a reduction in the *Fusarium oxysporum* pathogen was observed concerning the monoculture system. The soil submitted to the rotation was more abundant than monoculture cropping for *Gemmatimonas*, *Pseudomonas*, *Sphingob*ium, *Sphingomonas*, *Penicillium*, *Mortierella,* and *Chaetomium* [107].

5.5. Effects of abiotic factors (drought, salinity, high temperatures, low temperatures, pH)

Under natural conditions, plants and their microbial community are exposed to various environmental conditions, such as changes in temperature, humidity, pH, and UV rays, which directly or indirectly modify the composition of the microbiome [108]. A comparison of soil micro-organisms from different climatic conditions revealed distinct communities taxonomically and functionally, where functional diversity was less in arid environments [108]. This study has important implications for understanding the impacts of climate change on different microbiomes. When comparing the rhizosphere microbiome between eight soil types collected in different countries, Simonin et al. [109] found significant differences in the richness of taxa and the structure of the community (variation of 57 %). They attributed this variation to the pH of the soil.

Drought is one of the abiotic factors that most affect microorganisms. In addition to osmotic stress, the mobility of nutrients, and access to oxygen in the soil are limited [110]. In a study by Santos--Medellín et al. [111], rice plants subjected to water stress had their root microbiome restructured, which can contribute to the survival of plants in these extreme environmental conditions. In this work, the authors observed an increase in the phyla Actinobacteria and Chloroflexi in rice roots and a reduction in the phyla Acidobacteria and Deltaproteobacteria [111]. Similarly, Fitzpatrick et al. [112] concluded that drought altered the composition of root microbiomes, increasing the abundance of Actinobacteria.

Soils subjected to thermal treatments (50–80 °C) respond to heat disturbances by changing their bacterial community and reducing their ability to suppress diseases [113], a fact that can be attributed to the decrease in the relative abundance of Streptomycetaaeae, Micrococcaceae and Mycobacteriaceae, bacteria that are known for their antagonistic effect [113]. Plants are also regularly exposed to UV radiation, which leads to the stratification of the phyllosphere microbiome. By damaging microbial DNA, radiation selects bacterial populations of the leaf and its tolerance to radiation [37]. However, some bacteria withstand high UV exposure due to the presence of pigments (such as carotenoids, xanthomonadine, and melanin), extracellular polysaccharides (EPS), and the formation of spores [37].

Mark Ibekwe et al. [114] observed when evaluating the effect of salinity on the soil microbiome and the spinach rhizosphere that stress was more pronounced in the rhizospheric microbiome, which can be attributed to the increase in water absorption by plants due to transpiration. Besides, the authors noted that a second factor, temperature, also changed the diversity of the community. In March and May, the samples of the spinach rhizosphere showed a high relative abundance of Flavobacteriaceae, while in June, the samples contained more Halomonadaceae. For the soil microbiome, the months of May and June differed in the relative abundance of the Cytophagaceae, Comamonadaceae, Hyphomicrobiaceae, and Planctomycetaceae families.

The studies mentioned above showed that biotic and abiotic factors could alter the structure of plant microbiomes; it remains for us to understand if this change brings benefits to the plant.

6. Benefits of microbiomes for plants

Many micro-organisms, saprophytic, or necrotrophic, are harmful to plants and their native microbial diversity [37]. Examples of pathogens include *Agrobacterium*, *Pseudomonas*, *Xanthomonas*, *Ralstonia,* and *Erwinia* bacteria, and the fungi *Phytophthora*, *Mangnaporthe*, *Puccinia*, *Pythium*, *Rhizoctonia*, *Fusarium*, *Ustilago,* and *Alternaria* [37]. However, among the several benefits of the microbiome for plants, disease control stands out, also called biocontrol [115]. There are several examples of biocontrol by the plant-associated microbiota [116] through the production of siderophores (chelating agents capable of sequestering insoluble ferric ions), volatile compounds, enzymes, and antibiotics, in addition to modulation in phytohormone levels [71,117]. The plant microbiome also inhibits the growth and activity of pathogens through competition for nutrients and microenvironments, parasitism, antibiosis [115], and the resistance they confer to the plants' immune system [1]. Some soil bacteria capable of protecting the plant from pathogens belong to the genera *Pseudomonas*, *Streptomyces*, *Bacillus*, *Paenibacillus*, *Enterobacter*, *Pantoea*, *Burkholderia,* and *Paraburkholderia* [71]. In the soil microbiome, the phyla Acidobacteria, Actinobacteria, and Firmicutes were able to control the wilt caused by *Fusarium oxysporum* [118], while the endophytes *Serratia* and *Enterobacter* were able to control the pathogen *Gaeumannomyces graminis*, which caused take-all of wheat [119].

In addition to promoting plant growth through the biocontrol mechanism, the microbiome also stimulates growth through biofertilization, that is, by controlling the availability and acquisition of nutrients by plants [18]. Symbiotic associations between nitrogen-fixing bacteria, mainly rhizobia, arbuscular mycorrhizal fungi, and phosphate solubilizing bacteria, are typical examples of biofertilization and how plants obtain nitrogen and phosphorus, respectively [120]. The interaction between arbuscular mycorrhizal fungi and phosphate solubilizing bacteria increases the availability of phosphorus for plants since the bacteria solubilize phosphorus ions while the fungi translocate them to the plant [37]. *Azotobacter*, *Microbacterium*, *Erwinia*, *Bacillus*, *Beijerinckia*, *Serratia*, *Burkholderia*, *Enterobacter*, *Flavobacterium*, *Pseudomonas,* and *Rhizobium* bacteria are known as phosphate solubilizers [37]. Rhizobia, on the other hand, evolved together with vegetables, mainly legumes, to fix nitrogen directly from the air and help plants to establish themselves in limiting soils [1]. In addition to containing rhizobia, the legume nodule microbiome consists of other endophytic bacteria, both of which are responsible for direct and indirect growth promotion mechanisms in plants [121]. Some nitrogen-fixing endophytes are cyanobacteria *(Anabaena*, *Nostoc*, *Calothrix*), *Azotobacter*, *Azospirillum,* and *Gluconacetobacter* [37]. However, fixators can also be free-living and

Table 1
Description of work with essential contributions to the study of the plant microbiome.

Plant	Methodologies used	Key findings	Reference
Watermelon (*Citrullus lanatus*)	PCR-DGGE	Bacteria and actinomycetes are more abundant in the rhizosphere of the watermelon cultivar resistant to *Fusarium oxysporum* than in the susceptible variety, while populations of fungi are smaller in the resistant cultivar.	An et al. (2011)
Maize (*Zea mays*)	Terminal restriction fragment length polymorphism (TRFLP) Microscopy	Maize seeds have a conserved central microbiome. Seed isolates manage to leave the root and colonize the rhizosphere.	Johnston-Monje e Raizada (2011)
–	Sequencing	The diversity and function of the soil microbiome vary between terrestrial biomes.	Fierer et al. (2012)
Maize (*Zea mays*)	Sequencing	Endophytic bacterial communities of the seed vary between maize hybrids with different genotypes.	Liu et al. (2012)
Maize (*Zea mays*)	Sequencing	Variation of endophytic bacteria, in number and species, in maize seeds with different genotypes.	Liu e Xu (2012)
Cotton (*Gossypium hirsutum* and *Gossypium barbadense*)	Extracellular trap	Variation in the production of border cells between cotton cultivars.	Curlango-Rivera et al. (2013)
Arabidopsis thaliana	Sequencing Microscopy Catalyzed reporter deposition-fluorescent in situ hybridization (CARD-FISH)	Differences in the bacterial community, according to *Arabidopsis* genotypes.	Lundberg et al. (2013)
Spinach (*Spinacia oleracea*)	Sequencing	Bacterial communities of spinach seeds and cotyledons are similar in richness and diversity.	Lopez-Velasco et al. (2013)
Chamomile (*Chamomilla recutita*)	Sequencing Real-time PCR Polymerase chain reaction-single-strand conformation polymorphism (PCR-SSCP) High-performance liquid chromatography-mass spectrometry (HPLC-MS) Microscopy	Bacterial inoculants interact with the plant's microbiome and influence its secondary metabolism.	Schmidt et al. (2014)
Lettuce (*Lactuca sativa*)	Sequencing	Inoculation of the phytopathogenic fungus *Rhizoctonia solani* increases the diversity of Gammaproteobacteria (Enterobacteriaceae family) in the phyllosphere.	Erlacher et al. (2014)
Vine (*Vitis vinifera*)	Sequencing Automated ribosomal intergenic spacer analysis (ARISA)	The composition of endophytic bacterial communities differed between plants subjected to organic production and integrated pest management (IPM).	Campisano et al. (2014)
Arabidopsis thaliana	ARISA Real-time PCR Cultivation and plate counting	Differences in the phyllosphere microbiome of different *Arabidopsis* genotypes.	Bodenhausen et al. (2014)
Arabidopsis thaliana	Sequencing	Modulation of bacterial colonization of the rhizosphere by salicylic acid.	Lebeis et al. (2015)
Willow (*Salix purpúrea*)	Sequencing	Changes in willow growth in areas contaminated by hydrocarbons after the manipulation of their rhizospheric microbiome.	Yergeau et al. (2015)
Winter wheat (*Triticum aestivum*) Grass-clover	Sequencing	Agricultural soils under organic and conventional systems are home to distinct microbiomes.	Hartmann et al. (2015)
Tomato (*Lycopersicon esculentum*) Cucumber (*Cucumis sativus*)	Sequencing	Influence of soil nutrition on the composition of the rhizospheric microbiome.	Cai et al. (2016)
Maize (*Zea mays*)	Cultivation TRFLP Sequencing	Transmission of epiphytic and endophytic microorganisms from seeds to the rhizosphere.	Johnston-Monje et al. (2016)
Willow (*Salix purpúrea* and *Salix miyabeana*)	Sequencing	Influence of oil concentration from contaminated soils on the willow microbiome.	Tardif et al. (2016)
Maize (*Zea mays*)	Sequencing Real-time PCR Spectrophotometry	Increased root exudation and abundance of rhizospheric bacteria with increasing doses of nitrogen fertilization.	Zhu et al. (2016)
Potato (*Solanum Tuberosum*)	TRFLP Sequencing	Alteration in the structure of the potato endophytic microbial community after the inoculation of *Methylobacterium* sp.	Ardanov et al. (2016)
Black pepper (*Piper nigrum*)	Sequencing	Changes in the structure of the bacterial and fungal community of the soil and rhizosphere of black pepper.	Li et al. (2016)
Beet (*Beta vulgaris*)	PhyloChip	The soil temperature alters the bacterial diversity of the rhizosphere microbiome, which can lead to disease suppression.	Van der Voort et al. (2016)
White birch (*Betula pendula*) Rape (*Brassica napus*) Rye (*Secale cereal*) Autumn Crocus (*Colchicum autumnale*)	Cultivation Sequencing Microscopy Fluorescence in Situ Hybridization-Based Confocal Laser Scanning Microscopy (FISH-CLSM)	The high degree of diversity and specificity of the bacterial microbiome associated with flower pollen and its alteration concerning the type of pollination.	Manirajan et al. (2016)
Arabidopsis thaliana	Sequencing	Changes in the epiphytic and endophytic bacterial colonization of the phyllosphere after inoculation of the fungal pathogens *Albugo* and *Dioszegia*.	Agler et al. (2016)
Tomato (*Solanum lycopersicum* cv. Micro-Tom)	Sequencing High-performance liquid chromatography (HPLC)	*Ralstonia solanacearum* alters the root exudation profile of tomatoes and the composition of the bacterial community.	Gu et al. (2016)
Soy (*Glycine max*) Wheat (*Triticum aestivum*) Bean (*Phaseolus vulgaris*)	Sequencing Biochemical assays	Of 2.007 bacterial isolates from rhizospheres, 55 % showed characteristics of growth promotion.	Rascovan et al. (2016) Kumar et al. (2016)

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Table 1 (*continued*)

Plant	Methodologies used	Key findings	Reference
	Sequencing Biochemical assays	*Rhizobium leguminosarum* has an activity for ACC deaminase, phosphate solubilization, and biocontrol.	
Red clover (*Trifolium pratense*)	Sequencing	Neutralization of harmful effects of microorganisms by the root microbiome.	Hartman et al. (2017)
Spring wheat (*Triticum aestivum*)	Microscopy Real-time PCR Sequencing	Alteration of the seed microbiome due to the inoculation of endophytic microorganisms.	Mitter et al. (2017)
Yerba mate (*Ilex paraguariensis*)	Sequencing	Dynamic response of the plant microbiome to different agricultural practices.	Bergottini et al. (2017)
Chickpeas (*Cicer arietinum*)	Sequencing Biochemical assays	Correlation between microbiome and exudates released by plant roots.	Shcherbakova et al. (2017)
–	Sequencing	Impact of climate on the soil microbiome.	Tripathiet al. (2017)
Wheat (*Triticum* sp.) Potato (*Solanum tuberosum*) Lily (*Lilium* sp.) Carrot (*Dacus carota*) Maize (*Zea mays*)	Sequencing	The difference in soil microbiome promoted by type of production system (organic/conventional).	Lupatini et al. (2017)
Rice (*Oryza sativa*)	Microscopy Biochemical assays	Increased tolerance of plants to salt stress from the inoculation of endophytic bacteria.	Shahzad et al. (2017)
Potato (*Solanum tuberosum*) Mustard (*Brassica juncea*)	MicroResp™	Increased microbial activity in the rhizosphere concerning the soil by assessing substrate-induced respiration (SIR).	Brolsma et al. (2017)
Sage (*Salvia officinalis*) French lavender (*Lavandula dentata*) Thyme (*Thymus vulgaris*) Santolina (*Santolina chamaecyparissus*)	Sequencing	Changes in the structure of the rhizospheric bacterial community after the inoculation of arbuscular mycorrhizal fungi.	Rodríguez-Caballero et al. (2017)
Maize (*Zea mays*)	Nuclear magnetic resonance (NMR) Gas chromatography-mass spectrometry (GC/MS)	Change in root exudation of maize after inoculation of *Herbaspirillum seropedicae* and humic acid.	Da Silva Lima et al. (2017)
Spinach (*Spinacia oleracea*)	Sequencing	Temperature and salinity altered the bacterial diversity of the spinach and soil rhizosphere.	Mark Ibekwe et al. (2017)
Tomato (*Solanum lycopersicum*)	Sequencing Biochemical assays	IAA hormone production by bacteria from the tomato root endophytic microbiome.	Tian et al. (2017)
Rice (*Oryza sativa* and *Oryza glaberrima*)	Sequencing	Restructuring of the rice root microbiome under water stress.	Santos-Medellín et al. (2017)
Banana (*Musa acuminata*)	Sequencing	Banana trees infested with *Fusarium oxysporum* are colonized, preferably, by bacteria from the Enterobacteriaceae family.	Köberl et al. (2017)
Potato (*Solanum tuberosum*)	Sequencing	The use of mulch inhibited *Fusarium* reproduction.	Qin et al. (2017)
Wheat (*Triticum* sp.)	Sequencing	Endophytes *Serratia* and *Enterobacter* can control the pathogen *Gaeumannomyces graminis*.	Durán et al. (2018)
Arabidopsis thaliana	Sequencing GC-MS	*Pseudomonas syringae* alters the root exudation profile of *Arabidopsis* and the composition of the soil and rhizosphere bacterial community.	Yuan et al. (2018)
Kiwifruit (*Actinidia chinensis* and *Actinidia deliciosa*)	Sequencing	*Pseudomonas syringae* affects the structure of the phyllosphere microbiome and reduces its diversity.	Purahong et al. (2018)
Bean (*Phaseolus vulgaris*)	Sequencing	Plants resistant to the *Fusarium oxysporum* pathogen have different rhizobacteria (in composition and metabolic potential) than non-resistant plants.	Mendes et al. (2018)
Tomato (*Solanum lycopersicum*)	Sequencing	The rhizosphere microbiota of resistant plants suppresses the pathogen *Ralstonia solanacearum* from susceptible plants.	Kwak et al. (2018)
Thirty species of gymnosperms	Sequencing	Drought alters the composition of root microbiomes, increasing the abundance of Actinobacteria.	Fitzpatrick et al. (2018)
Wheat (*Triticum aestivum*)	Sequencing	Agricultural practices (no-tillage and type of management) alter the microbial composition of the soil and wheat roots.	Hartman et al. (2018)
Wheat (*Triticum aestivum*)	Sequencing Biochemical assays	Rhizobacteria promote wheat germination and growth, produce siderophores, and inhibit the *Fusarium solani* pathogen.	Kumar et al. (2018)
Chinese cabbage (*Brassica rapa*)	Sequencing	Changes in the bacterial and fungal community of Chinese cabbage infected with *Plasmodiophora brassicae* concerning healthy plants.	Lebreton et al. (2019)
Olive (*Olea europaea*)	Sequencing	The root's endophytic microbial community is less diverse concerning the rhizosphere and abundant in *Actinophytocola*, *Streptomyces,* and *Pseudonocardia*.	Fernández-González et al. (2019)
Bean (*Phaseolus vulgaris*)	Metatranscriptome	Bacterial microbiome of *Fusarium* resistant bean rhizosphere is physiologically actuated to biocontrol.	Mendes et al. (2019)
Hibiscus hamabo	Isolation Sequencing Greenhouse test	Rhizosphere microbiome promotes germination and plant growth under salinity conditions.	Yuan et al. (2019)
Artemisia argyi *Ageratum conyzoides* *Erigeron annuus* *Bidens biternate* *Euphorbia hirta* *Viola japônica*	Sequencing	Existence of a central microbiome in the rhizosphere of different plant species.	Lei et al. (2019)
Eight species of alpine plants	Sequencing	Plants have a highly specific seed microbiome, with a low microbial "core."	Wassermann et al. (2019)
Wheat (*Triticum* sp.)	Sequencing	The seed microbiome defines the structure of the root's endophytic bacterial community.	Kavamura et al. (2019)

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Table 1 (*continued*)

Plant	Methodologies used	Key findings	Reference
Tomato (*Solanum* sp.)	Sequencing	Plant genotype shapes the microbial composition of the phyllosphere.	Morella et al. (2020)
–	Sequencing	Soils from different countries differ in the richness of taxa and the structure of the microbial community. Nuclear microbiome consists of 177 taxa (archaea, bacteria, fungi, and protists).	Simonin et al. (2020)
Tomato (*Solanum* sp.)	Sequencing GC-MS	The abundance of the pathogen *Ralstonia solanacearum* reduces the bacterial diversity of the rhizosphere and the sugar content (alteration of the metabolome).	Wen et al. (2020)
Banana (*Musa* sp.) Chilli pepper (*Capsicum frutescens*)	Sequencing Real-time PCR	The rotation of pepper-banana culture reduces the abundance of the *Fusarium oxysporum* pathogen.	Hong et al. (2020)
Bean (*Phaseolus vulgaris*)	Sequencing Real-time PCR	Inoculation of *Metarhizium* in beans increases the diversity of plant growth promoters, such as *Bradyrhizobium*, *Flavobacterium*, *Chaetomium,* and *Trichoderma*, and suppresses the activity of the *Fusarium solani* pathogen.	Barelli et al. (2020)
Tomato (*Solanum lycopersicum*)	Sequencing	Microbial inoculants have effects on the bacterial structure of the rhizosphere, with the genera *Streptomyces*, *Luteimonas,* and *Enterobacter* responsible for promoting growth.	Gu et al. (2020)
Japanese knotweed (*Polygonum cuspidatum*)	Sequencing	The microbial composition of the root endosphere is stable and rich in *Stenotrophomonas*.	Zhang et al. (2020)
Wheat (*Triticum aestivum*)	Sequencing	The wheat rhizosphere is dominated by the phyla Proteobacteria, Actinobacteria, Acidobacteria, Ascomycota, Chytridiomycota, and Basidiomycota.	Rossmann et al. (2020)
Adenium obesum *Aloe dhufarensis* *Cleome austroarabica*	Sequencing Biochemical assays	Microbial diversity and extracellular enzymes differ between the rhizosphere of three plants in the arid region. However, they also share some taxa.	Khan et al. (2020)
Maize (*Zea mays*)	Sequencing Real-time PCR Microscopy Cultivation e isolation Biochemical assays	Maize seed-borne bacteria positively modulate germination and initial growth of the host plant.	Dos Santos et al. (2020)

establish themselves in non-leguminous plants, as is the case, for example, with the genera *Beijerinckia*, *Klebsiella,* and *Bacillus* [37].

Phytostimulation or biostimulation is another process causally related to plant growth and consists of the production of phytohormones by the microbiome [18], such as indole-3-acetic acid (IAA) or auxin-simulating molecules [122]. Other microbial phytohormones or molecules similar to phytohormones, such as gibberellins, cytokinins, salicylic acid (SA) and jasmonic acid, are also produced [122]. Besides, some bacteria can secrete an enzyme, 1-carboxylic acid-1-aminocyclopropane (ACC) deaminase, which reduces the level of ethylene in the plant [71,123]. In a study by Tian et al. [124], bacteria from the tomato root endophytic microbiome produced the hormone IAA from tryptophan and promoted plant growth. Rascovan et al. [125] found, in the roots of wheat and soybeans, bacteria capable of producing IAA and ACC deaminase, being them *Pseudomonas* spp., *Paraburkholderia* spp. and *Pantoea* spp.

The microbiome also plays an essential role in plant tolerance to extreme conditions, such as salinity, drought, and exposure to heavy metals [27]. Soil salinity has hindered the growth rates of plants and reduced their yield. However, the negative impact of high levels of salt in the soil can be minimized through the production of phytohormones by the microbiome, with a consequent increase in plant resistance to these extreme environments [27]. In a study by Yuan et al. [126], the rhizosphere microbiome was able to promote germination and growth of *Hibiscus hamabo* under salinity conditions.

In this sense, the excessive use of pesticides in agriculture has contaminated the soil with the release of xenobiotic compounds [127]. To eliminate the harmful effects of these contaminants, the use of plants in remediation processes has become common, and its effectiveness is attributed to the micro-organisms associated with it. These micro-organisms are capable of degrading and stabilizing contaminants [52,127]. Recently, Thijs et al. [128] proposed a model to explain the establishment and maintenance of the beneficial and degrading microbiome in the rhizosphere of contaminated soil plants. Four strategies were identified, including plant selection based on the microbiome, interference from root exudates, disturbance and feeding of supply lines, in order to ensure that the microbial community is kept under control in polluted environments.

Plants that live in oil-contaminated soils depend on their microbiome for survival, growth optimization, and biomass production [129]. At the same time, as the contamination of these areas increases, there are changes in the composition of the microbiome, favoring hydrocarbon-degrading micro-organisms associated with plant growth [130]. The plant-microbiome interaction will not always be efficient for phytoremediation; therefore, human interventions are necessary to optimize this interaction and promote the degradation of pollutants [128].

Understanding the potential of the microbiome for agriculture can lead to its use as an inoculant or its manipulation, in order to select more efficient microbial groups for plant development [11]. Besides that, reducing the use of pesticides and chemical fertilizers based on an understanding of the potential of the plant microbiome is of paramount importance for advancing sustainable agricultural practices.

7. State of the art

Several studies have been dedicated to investigating the structure and function of the microbiome for plants and have found that plants provide niches and nutrients to the microbiota, while the microbiota promotes plant growth, nutrition, and protection against pathogens. In Table 1, it is possible to observe different studies involving these themes. The main findings of these researches prove the beneficial effect of the microbiome for various plant cultures, as well as list biotic and abiotic factors that influence its structure (composition/diversity).

Among the works described in Table 1, almost all used sequencing as the central methodology; this shows the importance of new generation sequencing techniques for the study of microbiomes. These independent cultivation techniques allow us to capture a greater diversity of micro-organisms than traditional cultivation techniques. However, these two tools can be combined to structurally characterize microbiomes and analyze displacements from the community (after exposure to factors of different nature), as well as isolating and identifying their micro-organisms. After being isolated and identified, the micro-organisms can have their effects validated in different plant species and, if

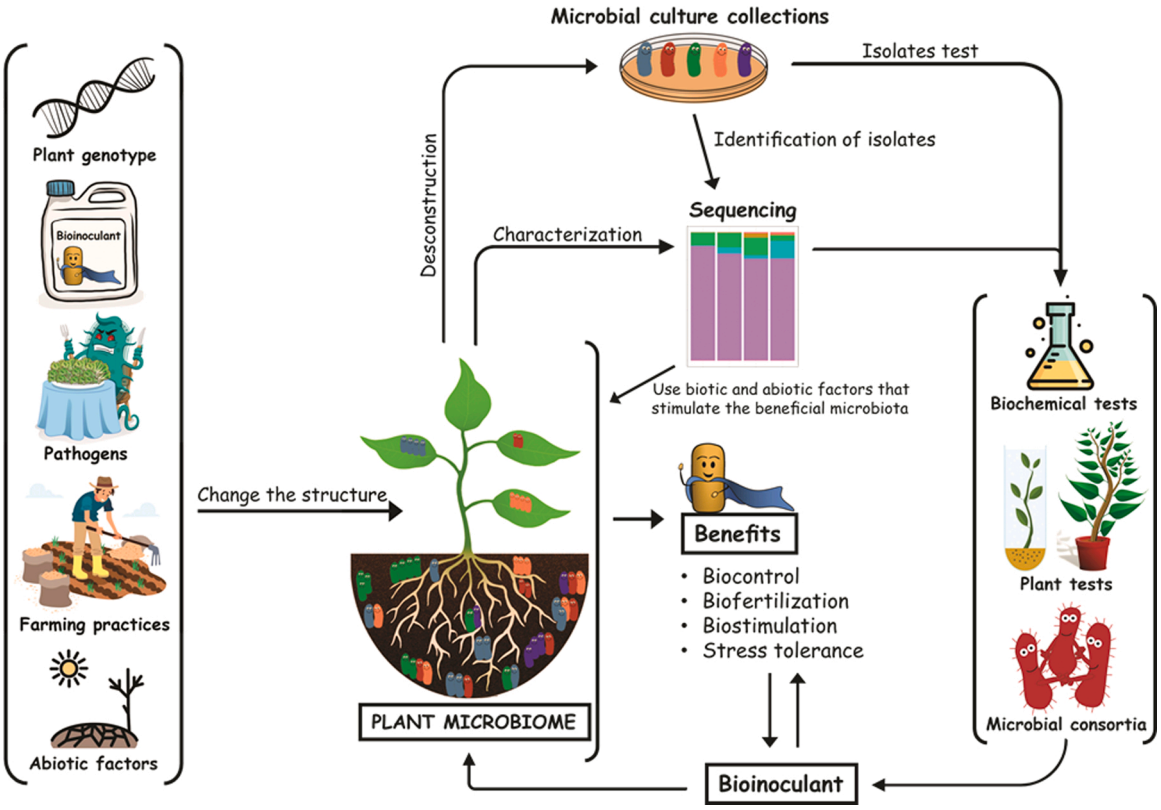

Fig. 4. Approaches that convert knowledge about microbiomes into a biotechnological product. Above and below ground, plants are colonized by microbiomes, which can be shaped by biotic and abiotic factors. The plant microbiome (molded or not) can be deconstructed and isolated by cultivation-dependent techniques, while sequencing techniques can trace the profile of the entire microbial communities. These two approaches must be combined to identify and isolate the principal members of the microbiome, who will have their functional skills tested in inoculation experiments (gnotobiotic system and greenhouse). These micro-organisms, if beneficial, may form new bioinoculants. Besides, factors that stimulate the beneficial microbiota of plants must be maintained or enhanced.

beneficial, will compose new bioinoculants. Within this logic, we propose in this review article a model that converts the knowledge acquired about microbiomes into a biotechnological product (Fig. 4).

8. Future perspectives

The global demand for food will increase by 70 % in 2050, at the same time, the farmer will have to face adverse climatic conditions, nutritionally depleted, contaminated soils and water scarcity [15]. In this scenario, using available natural resources, such as plant microbiomes, is the most sustainable alternative.

Despite knowledge accumulated on microbiomes in the scientific literature, advances are needed, such as the expansion of molecular approaches, including sampling, extraction, and amplification of DNA; the reduction of sequencing costs; the development of bioinformatics, among others. Also, the complementation of these factors with traditional techniques is necessary for a more detailed understanding of the behavior and functioning of plant microbiomes.

Once the structure, functioning, and factors that modulate the structure of microbiomes have been elucidated, it will be possible to use them in agriculture to optimize plant development. More precisely, it will be possible to shape or isolate the microbiome. When we talk about shaping the microbiome, we are referring to the use of approaches that alter the structure of its bacterial and fungal community, in order to select or attract micro-organisms with beneficial functions. Several factors that alter the structure of the microbiome were mentioned in this review, such as the selection of agricultural management practices that favor the microbiota with beneficial functions, plant breeding based on the associated beneficial microbiota, and the use of bioinoculants that positively interact with the microbiome. On the other hand, it is possible to identify, by independent cultivation techniques, members of the microbiome that are functionally important for plants and to isolate them by cultivation-dependent techniques. From these micro-organisms, new mixed bioinoculants can be assembled in order to enhance their functions in the plant.

Although knowledge about plant microbiomes has increased a lot in recent years, we still need to understand how the microbiome is structured, what its benefits are for plants, and how bioinoculants interact with the resident microbiota.

Declaration of Competing Interest

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

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References

[1] P. Hunter, Plant microbiomes and sustainable agriculture, EMBO Rep. (2016) 1–4.
[2] T.R. Turner, E.K. James, P.S. Poole, The plant microbiome, Genome Biol. 14 (2013) 209, https://doi.org/10.1186/gb-2013-14-6-209.
[3] C. Toft, S.G.E. Andersson, Evolutionary microbial genomics: insights into bacterial host adaptation, Nat. Rev. Genet. 11 (2010) 465–475, https://doi.org/10.1038/nrg2798.

[4] K. Schlaeppi, D. Bulgarelli, The plant microbiome at work, Mol. Plant-Microbe Interact. MPMI. 212 (2015) 212–217, https://doi.org/10.1094/MPMI-10-14-0334-FI.
[5] J.L. Sachs, R.G. Skophammer, J.U. Regus, Colloquium Paper: evolutionary transitions in bacterial symbiosis, Proc. Natl. Acad. Sci. 108 (Suppl) (2011) 10800–10807, https://doi.org/10.1073/pnas.1100304108.
[6] E. Boon, C.J. Meehan, C. Whidden, D.H.J. Wong, M.G.I. Langille, R.G. Beiko, Interactions in the microbiome: communities of organisms and communities of genes, FEMS Microbiol. Rev. 38 (2014) 90–118, https://doi.org/10.1111/1574-6976.12035.
[7] J.L. Foo, H. Ling, Y.S. Lee, M.W. Chang, Microbiome engineering: current applications and its future, Biotechnol. J. (2017) 1600099, https://doi.org/10.1002/biot.201600099.
[8] F.D. Andreote, T. Gumiere, A. Durrer, Exploring interactions of plant microbiomes, Sci. Agric. 71 (2014) 528–539, https://doi.org/10.1590/0103-9016-2014-0195.
[9] Brasil. Decreto n° 4.954, de 14 de janeiro de 2004. Aprova o regulamento da Lei no 6.894, de 16 de dezembro de 1980, que dispõe sobre a inspeção e fiscalização da produção e do comércio de fertilizantes, corretivos, inoculantes ou biofertilizantes destinados à agricultura, e dá outras providências. Diário Oficial [da] República Federativa do Brasil, Brasília, DF, 15 jan. 2004.
[10] P.E. Busby, C. Soman, M.R. Wagner, M.L. Friesen, J. Kremer, A. Bennett, M. Morsy, J. a Eisen, J.E. Leach, J.L. Dangl, Research priorities for harnessing plant microbiomes in sustainable agriculture, PLoS Biol. (2017) 1–14, https://doi.org/10.1371/journal.pbio.2001793.
[11] N.I. Johns, T. Blazejewski, A.L.C. Gomes, H.H. Wang, Principles for designing synthetic microbial communities, Curr. Opin. Microbiol. 31 (2016) 146–153, https://doi.org/10.1016/j.mib.2016.03.010.
[12] V. Torsvik, L. Ovreas, T.F. Thingstad, Prokaryotic diversity - magnitude, dynamics, and controlling factors, Science 296 (2002) 1064–1066, https://doi.org/10.1126/science.1071698.
[13] B.J. Lederberg, A.T. McCray, 'Ome sweet' omics - a genealogical treasury of words, Science 15 (2001) 8.
[14] H.M.P. Consortium, Structure, function and diversity of the healthy human microbiome, Nature 486 (2012) 207–214, https://doi.org/10.1038/nature11234.
[15] B.K. Singh, P. Trivedi, Microbiome and the future for food and nutrient security, Microb. Biotechnol. 10 (2017) 50–53, https://doi.org/10.1111/1751-7915.12592.
[16] V.S. Pylro, L.F.W. Roesch, J.M. Ortega, A.M. do Amaral, M.R. Tótola, P.R. Hirsch, A.S. Rosado, A. Góes-Neto, et al., Brazilian microbiome project: revealing the unexplored microbial diversity-challenges and prospects, Microb. Ecol. 67 (2014) 237–241, https://doi.org/10.1007/s00248-013-0302-4.
[17] K. Hartman, M.G. vander Heijden, V. Roussely-Provent, J.-C. Walser, K. Schlaeppi, Deciphering composition and function of the root microbiome of a legume plant, Microbiome. 5 (2017) 2, https://doi.org/10.1186/s40168-016-0220-z.
[18] J.R. Gaiero, Ca. McCall, Ka. Thompson, N.J. Day, A.S. Best, K.E. Dunfield, Inside the root microbiome: bacterial root endophytes and plant growth promotion, Am. J. Bot. 100 (2013) 1738–1750, https://doi.org/10.3732/ajb.1200572.
[19] V. Aguiar-Pulido, W. Huang, V. Suarez-Ulloa, T. Cickovski, K. Mathee, G. Narasimhan, Metagenomics, metatranscriptomics, and metabolomics approaches for microbiome analysis, Evol. Bioinforma. 12 (2016) 5–16, https://doi.org/10.4137/EBO.S36436.
[20] J. Nesme, W. Achouak, S.N. Agathos, M. Bailey, P. Baldrian, D. Brunel, A. Frostegard, T. Heulin, J.K. Jansson, E. Jurkevitch, K.L. Kruus, et al., Back to the future of soil metagenomics, Front. Microbiol. 7 (2016) 1–5, https://doi.org/10.3389/fmicb.2016.00073.
[21] J. Handelsman, M.R. Rondon, S.F. Brady, J. Clardy, R.M. Goodman, Molecular biological access to the chemistry of unknown soil microbes: a new frontier for natural products, Chem. Biol. 5 (1998) R245–R249, https://doi.org/10.1016/S1074-5521(98)90108-9.
[22] E.J.B.N. Cardoso, F.D. Andreote, Microbiologia Do Solo, 2016.
[23] F. Cai, G. Pang, Y. Miao, R. Li, R. Li, Q. Shen, W. Chen, The nutrient preference of plants in fl uences their rhizosphere microbiome, Agric., Ecosyst. Environ., Appl. Soil Ecol. (2016) 4–8, https://doi.org/10.1016/j.apsoil.2016.11.006.
[24] V.M. Bergottini, V. Herv, Da. Sosa, M.B. Otegui, P.D. Zapata, P. Junier, Exploring the diversity of the root-associated microbiome of Ilex paraguariensis St. Hil. (Yerba Mate), Agric., Ecosyst. Environ., Appl. Soil Ecol. 109 (2017) 23–31, https://doi.org/10.1016/j.apsoil.2016.09.013.
[25] E.N. Shcherbakova, V. Shcherbakov, E.E. Andronov, L.N. Gonchar, S. M. Kalenskaya, V.K. Chebotar, Combined pre-seed treatment with microbial inoculants and Mo nanoparticles changes composition of root exudates and rhizosphere microbiome structure of chickpea (*Cicer arietinum* L.) plants, Symbiosis (2017), https://doi.org/10.1007/s13199-016-0472-1.
[26] B. Mitter, N. Pfaffenbichler, R. Flavell, S. Compant, L. Antonielli, A. Petric, T. Berninger, M. Naveed, R. Sheibani-Tezerji, G. von Maltzahn, A. Sessitsch, A new approach to modify plant microbiomes and traits by introducing beneficial bacteria at flowering into progeny seeds, Front. Microbiol. 8 (2017) 11, https://doi.org/10.3389/fmicb.2017.00011.
[27] R. Shahzad, A.L. Khan, S. Bilal, M. Waqas, S.-M. Kang, I.-J. Lee, Inoculation of abscisic acid-producing endophytic bacteria enhances salinity stress tolerance in Oryza sativa, Environ. Exp. Bot. 136 (2017) 68–77, https://doi.org/10.1016/j.envexpbot.2017.01.010.
[28] F.L. Olivares, V.L.D. Baldani, V.M. Reis, J.I. Baldani, J. Döbereiner, Occurrence of the endophytic diazotrophs *Herbaspirillum* spp. In roots, stems, and leaves, predominantly of Gramineae, Biol. Fertil. Soils 21 (1996) 197–200, https://doi.org/10.1007/s003740050049.
[29] N. Bodenhausen, M. Bortfeld-Miller, M. Ackermann, Ja. Vorholt, A synthetic community approach reveals plant genotypes affecting the phyllosphere microbiota, PLoS Genet. 10 (2014), https://doi.org/10.1371/journal.pgen.1004283.
[30] R.K. Dubey, V. Tripathi, R. Prabha, R. Chaurasia, D.P. Singh, C.S. Rao, A. El-Keblawy, P.C. Abhilash, Methods for exploring soil microbial diversity. Unravelling Soil Microbiome Perspect. Environ. Sustain., Springer International Publishing, Cham, 2020, pp. 23–32, https://doi.org/10.1007/978-3-030-15516-2_3.
[31] B.A. Manirajan, S. Ratering, V. Rusch, A. Schwiertz, R. Geissler-Plaum, M. Cardinale, S. Schnell, Bacterial microbiota associated with flower pollen is influenced by pollination type, and shows a high degree of diversity and species-specificity, Environ. Microbiol. 18 (2016) 5161–5174, https://doi.org/10.1111/1462-2920.13524.
[32] M.M. Zasloff, Antimicrobial peptides of multicellular organisms, Environ. Microbiol. 19 (2017) 1–2, https://doi.org/10.1038/415389a.
[33] E.A.-N. Nelson EricB, The seed microbiome: origins, interactions, and impacts, Plant Soil 422 (1–2) (2018) 28–34, https://doi.org/10.1007/s11104-017-3289-7.
[34] T.M. Vogel, P. Simonet, J.K. Jansson, P.R. Hirsch, J.M. Tiedje, V. Elsas, J. Dirk, M. J. Bailey, R. Nalin, L. Philippot, TerraGenome: a consortium for the sequencing of a soil metagenome, Nat. Rev. Microbiol. 7 (2009) (2009), https://doi.org/10.1038/nrmicro2119.
[35] R.L. Berendsen, C.M.J. Pieterse, P.A.H.M. Bakker, The rhizosphere microbiome and plant health, Trends Plant Sci. 17 (2012) 478–486, https://doi.org/10.1016/j.tplants.2012.04.001.
[36] K.M. Brolsma, J.A. Vonk, L. Mommer, J. Van Ruijven, E. Hoffland, R.G.M. De Goede, Microbial catabolic diversity in and beyond the rhizosphere of plant species and plant genotypes, Pedobiologia (Jena). 61 (2017) 43–49, https://doi.org/10.1016/j.pedobi.2017.01.006.
[37] I. Kumar, M. Mondal, R. Gurusamy, S. Balakrishnan, S. Natarajan, Plant-microbiome interaction and the effects of biotic and abiotic components in agroecosystem, Microb. Interv. Agric. Environ. Vol. 2 Rhizosphere, Microbiome Agro-Ecology (2019) 517–546, https://doi.org/10.1007/978-981-13-8383-0_18.
[38] L.P. Canellas, F.L. Olivares, Production of border cells and colonization of maize root tips by *Herbaspirillum seropedicae* are modulated by humic acid, Plant Soil (2017), https://doi.org/10.1007/s11104-017-3267-0.
[39] Z. Li, C. Zu, C. Wang, J. Yang, H. Yu, H. Wu, Different responses of rhizosphere and non-rhizosphere soil microbial communities to consecutive *Piper nigrum* L. monoculture, Sci. Rep. 6 (2016) 35825, https://doi.org/10.1038/srep35825.
[40] D. Bulgarelli, K. Schlaeppi, S. Spaepen, E. van Themaat, P. Schulze-Lefert, Structure and functions of the bacterial microbiota of plants, Annu. Rev. Plant Biol. 64 (2013) 807–838, https://doi.org/10.1146/annurev-arplant-050312-120106.
[41] T. Wen, M. Zhao, T. Liu, Q. Huang, J. Yuan, Q. Shen, High abundance of Ralstonia solanacearum changed tomato rhizosphere microbiome and metabolome, BMC Plant Biol. 20 (2020) 1–11, https://doi.org/10.1186/s12870-020-02365-9.
[42] M.G. Bakker, D.K. Manter, A.M. Sheflin, T.L. Weir, J.M. Vivanco, Harnessing the rhizosphere microbiome through plant breeding and agricultural management, Plant Soil 360 (2012) 1–13, https://doi.org/10.1007/s11104-012-1361-x.
[43] T.R. Turner, K. Ramakrishnan, J. Walshaw, D. Heavens, M. Alston, D. Swarbreck, A. Osbourn, A. Grant, P.S. Poole, Comparative metatranscriptomics reveals kingdom level changes in the rhizosphere microbiome of plants, ISME J. 7 (2013) 2248–2258, https://doi.org/10.1038/ismej.2013.119.
[44] Q. Qu, Z. Zhang, W.J.G.M. Peijnenburg, W. Liu, T. Lu, B. Hu, J. Chen, J. Chen, Z. Lin, H. Qian, Rhizosphere microbiome assembly and its impact on plant growth, J. Agric. Food Chem. 68 (2020) 5024–5038, https://doi.org/10.1021/acs.jafc.0c00073.
[45] A. Shade, N. Stopnisek, Abundance-occupancy distributions to prioritize plant core microbiome membership, Curr. Opin. Microbiol. 49 (2019) 50–58, https://doi.org/10.1016/j.mib.2019.09.008.
[46] S. Lei, X. Xu, Z. Cheng, J. Xiong, R. Ma, L. Zhang, X. Yang, Y. Zhu, B. Zhang, B. Tian, Analysis of the community composition and bacterial diversity of the rhizosphere microbiome across different plant taxa, Microbiologyopen. 8 (2019) 1–10, https://doi.org/10.1002/mbo3.762.
[47] A.L. Khan, S. Asaf, R.M.M. Abed, Y.N. Chai, A.N. Al-Rawahi, T.K. Mohanta, A. Al-Rawahi, D.P. Schachtman, A. Al-Harrasi, Rhizosphere microbiome of arid land medicinal plants and extra cellular enzymes contribute to their abundance, Microorganisms. 8 (2020), https://doi.org/10.3390/microorganisms8020213.
[48] M. Rossmann, J.E. Pérez-Jaramillo, V.N. Kavamura, J.B. Chiaramonte, K. Dumack, A.M. Fiore-Donno, L.W. Mendes, M.M.C. Ferreira, M. Bonkowski, J. M. Raaijmakers, T.H. Mauchline, R. Mendes, Multitrophic interactions in the rhizosphere microbiome of wheat: from bacteria and fungi to protists, FEMS Microbiol. Ecol. 96 (2020), https://doi.org/10.1093/femsec/fiaa032.
[49] D. Johnston-Monje, D.S. Lundberg, G. Lazarovits, V.M. Reis, M.N. Raizada, Bacterial populations in juvenile maize rhizospheres originate from both seed and soil, Plant Soil 405 (2016) 337–355, https://doi.org/10.1007/s11104-016-2826-0.
[50] Y. Liu, S. Zuo, L. Xu, Y. Zou, W. Song, Study on diversity of endophytic bacterial communities in seeds of hybrid maize and their parental lines, Arch. Microbiol. 194 (2012) 1001–1012, https://doi.org/10.1007/s00203-012-0836-8.
[51] C. Sánchez-Cañizares, B. Jorrín, P.S. Poole, A. Tkacz, Understanding the holobiont: the interdependence of plants and their microbiome, Curr. Opin. Microbiol. 38 (2017) 188–196, https://doi.org/10.1016/j.mib.2017.07.001.

[52] L.F. Dos Santos, M.M. Cazotti, sobre Os diferentes micro-organismos do solo I mpacto do glifosato, Duc Altum - Cad. Direito 15 (2016) 27–38.
[53] A. Shade, M.A. Jacques, M. Barret, Ecological patterns of seed microbiome diversity, transmission, and assembly, Curr. Opin. Microbiol. 37 (2017) 15–22, https://doi.org/10.1016/j.mib.2017.03.010.
[54] M. Barret, J.F. Guimbaud, A. Darrasse, M.A. Jacques, Plant microbiota affects seed transmission of phytopathogenic micro-organisms, Mol. Plant Pathol. 17 (2016) 791–795, https://doi.org/10.1111/mpp.12382.
[55] N. Fierer, J.W. Leff, B.J. Adams, U.N. Nielsen, S.T. Bates, C.L. Lauber, S. Owens, J. A. Gilbert, D.H. Wall, J.G. Caporaso, Cross-biome metagenomic analyses of soil microbial communities and their functional attributes, Proc. Natl. Acad. Sci. U. S. A. 109 (2012) 21390–21395, https://doi.org/10.1073/pnas.1215210110.
[56] D. Johnston-Monje, M.N. Raizada, Conservation and diversity of seed associated endophytes in Zea across boundaries of evolution, ethnography and ecology, PLoS One 6 (2011), https://doi.org/10.1371/journal.pone.0020396.
[57] B. Wassermann, T. Cernava, H. Müller, C. Berg, G. Berg, Seeds of native alpine plants host unique microbial communities embedded in cross-kingdom networks, Microbiome. 7 (2019) 108, https://doi.org/10.1186/s40168-019-0723-5.
[58] G. Lopez-Velasco, P.A. Carder, G.E. Welbaum, M.A. Ponder, Diversity of the spinach (*Spinacia oleracea*) spermosphere and phyllosphere bacterial communities, FEMS Microbiol. Lett. 346 (2013) 146–154, https://doi.org/10.1111/1574-6968.12216.
[59] V.N. Kavamura, R.J. Robinson, R. Hayat, I.M. Clark, D. Hughes, M. Rossmann, P. R. Hirsch, R. Mendes, T.H. Mauchline, Land management and microbial seed load effect on rhizosphere and endosphere bacterial community assembly in wheat, Front. Microbiol. 10 (2019) 1–11, https://doi.org/10.3389/fmicb.2019.02625.
[60] G. Berg, D. Rybakova, M. Grube, M. Köberl, The plant microbiome explored: implications for experimental botany, J. Exp. Bot. 67 (2015) 995–1002, https://doi.org/10.1093/jxb/erv466.
[61] A. Ambrosini, R. de Souza, L.M.P. Passaglia, Ecological role of bacterial inoculants and their potential impact on soil microbial diversity, Plant Soil 400 (2016) 193–207, https://doi.org/10.1007/s11104-015-2727-7.
[62] D. Wilson, Endophyte: the evolution of a term, and clarification of its use and definition, Oikos 73 (1995) 274–276, https://doi.org/10.2307/3545919.
[63] D.S. Lundberg, S.L. Lebeis, S.H. Paredes, S. Yourstone, J. Gehring, S. Malfatti, J. Tremblay, A. Engelbrektson, V. Kunin, T.G. del Rio, R.C. Edgar, T. Eickhorst, R. E. Ley, P. Hugenholtz, S.G. Tringe, J.L. Dangl, Defining the core *Arabidopsis thaliana* root microbiome, Nature. 488 (2013) 86–90, https://doi.org/10.1038/nature11237.
[64] P.R. Hardoim, L.S. van Overbeek, J.D. Van Elsas, Properties of bacterial endophytes and their proposed role in plant growth, Trends Microbiol. 16 (2008) 463–471, https://doi.org/10.1016/j.tim.2008.07.008.
[65] N. Sivakumar, R. Sathishkumar, G. Selvakumar, R. Shyamkumar, K. Arjunekumar, Phyllospheric microbiomes: diversity, ecological significance, and biotechnological applications, Plant Microbiomes Sustain. Agric. 25 (2020) 113–172, https://doi.org/10.1007/978-3-030-38453-1_5.
[66] S. Hacquard, R. Garrido-Oter, A. González, S. Spaepen, G. Ackermann, S. Lebeis, A.C. McHardy, J.L. Dangl, R. Knight, R. Ley, P. Schulze-Lefert, Microbiota and host nutrition across plant and animal kingdoms, Cell Host Microbe 17 (2015) 603–616, https://doi.org/10.1016/j.chom.2015.04.009.
[67] A.J. Fernández-González, P.J. Villadas, C. Gómez-Lama Cabanás, A. Valverde-Corredor, A. Belaj, J. Mercado-Blanco, M. Fernández-López, Defining the root endosphere and rhizosphere microbiomes from the World Olive Germplasm Collection, Sci. Rep. 9 (2019) 1–13, https://doi.org/10.1038/s41598-019-56977-9.
[68] Y. Zhang, L. Zheng, Y. Zheng, S. Xue, J. Zhang, P. Huang, Y. Zhao, X. Hao, Z. He, Z. Hu, C. Zhou, Q. Chen, J. Liu, G. Wang, M. Sang, X. Sun, X. Wang, X. Xiao, C. Li, Insight into the assembly of root-associated microbiome in the medicinal plant *Polygonum cuspidatum*, Ind. Crops Prod. 145 (2020) 112163, https://doi.org/10.1016/j.indcrop.2020.112163.
[69] S.L. Lebeis, S.H. Paredes, D.S. Lundberg, N. Breakfield, J. Gehring, M. McDonald, S. Malfatti, T.G. del Rio, C.D. Jones, S.G. Tringe, J.L. Dangl, Salicylic acid modulates colonization of the root microbiome by specific bacterial taxa, Science (80-.) 349 (2015) 860–864, https://doi.org/10.1126/science.aaa8764.
[70] C.M.J. Pieterse, R. de Jonge, R.L. Berendsen, The soil-borne supremacy, Trends Plant Sci. 21 (2016) 171–173, https://doi.org/10.1016/j.tplants.2016.01.018.
[71] S. Compant, A. Samad, H. Faist, A. Sessitsch, A review on the plant microbiome: ecology, functions, and emerging trends in microbial application, J. Adv. Res. 19 (2019) 29–37, https://doi.org/10.1016/j.jare.2019.03.004.
[72] G. Curlango-Rivera, Da. Huskey, A. Mostafa, J.O. Kessler, Z. Xiong, M.C. Hawes, Intraspecies variation in cotton border cell production: rhizosphere microbiome implications, Am. J. Bot. 100 (2013) 1706–1712, https://doi.org/10.3732/ajb.1200607.
[73] La. Moe, Amino acids in the rhizosphere: from plants to microbes, Am. J. Bot. 100 (2013) 1692–1705, https://doi.org/10.3732/ajb.1300033.
[74] E. Adam, M. Bernhart, H. Müller, J. Winkler, G. Berg, The *Cucurbita pepo* seed microbiome: genotype-specific composition and implications for breeding, Plant Soil 422 (2018) 35–49, https://doi.org/10.1007/s11104-016-3113-9.
[75] N.M. Morella, F.C.H. Weng, P.M. Joubert, C. Jessica, S. Lindow, B. Koskella, Successive passaging of a plant-associated microbiome reveals robust habitat and host genotype-dependent selection, Proc. Natl. Acad. Sci. U. S. A. 117 (2020) 1148–1159, https://doi.org/10.1073/pnas.1908600116.
[76] L.W. Mendes, J.M. Raaijmakers, M. De Hollander, R. Mendes, S.M. Tsai, Influence of resistance breeding in common bean on rhizosphere microbiome composition and function, ISME J. 12 (2018) 212–224, https://doi.org/10.1038/ismej.2017.158.
[77] L.W. Mendes, M.G. de Chaves, M. de C. Fonseca, R. Mendes, J.M. Raaijmakers, S. M. Tsai, Resistance breeding of common bean shapes the physiology of the rhizosphere microbiome, Front. Microbiol. 10 (2019) 1–10, https://doi.org/10.3389/fmicb.2019.02252.
[78] M. An, X. Zhou, F. Wu, Y. Ma, P. Yang, Rhizosphere soil micro-organism populations and community structures of different watermelon cultivars with differing resistance to *Fusarium oxysporum* f. Sp. *Niveum*, Can. J. Microbiol. 57 (2011) 355–365, https://doi.org/10.1139/w11-015.
[79] M.J. Kwak, H.G. Kong, K. Choi, S.K. Kwon, J.Y. Song, J. Lee, P.A. Lee, S.Y. Choi, M. Seo, H.J. Lee, E.J. Jung, H. Park, N. Roy, H. Kim, M.M. Lee, E.M. Rubin, S. W. Lee, J.F. Kim, Rhizosphere microbiome structure alters to enable wilt resistance in tomato, Nat. Biotechnol. 36 (2018) 1100–1116, https://doi.org/10.1038/nbt.4232.
[80] R. Schmidt, M. Köberl, A. Mostafa, E.M. Ramadan, M. Monschein, K.B. Jensen, R. Bauer, G. Berg, Effects of bacterial inoculants on the indigenous microbiome and secondary metabolites of chamomile plants, Front. Microbiol. 5 (2014) 1–11, https://doi.org/10.3389/fmicb.2014.00064.
[81] K.L. Adair, A.E. Douglas, Making a microbiome: the many determinants of host-associated microbial community composition, Curr. Opin. Microbiol. 35 (2017) 23–29, https://doi.org/10.1016/j.mib.2016.11.002.
[82] A. Hartmann, M. Rothballer, B.A. Hense, P. Schröder, Bacterial quorum sensing compounds are important modulators of microbe-plant interactions, Front. Plant Sci. 5 (2014) 1–4, https://doi.org/10.3389/fpls.2014.00131.
[83] C.M.J. Pieterse, C. Zamioudis, R.L. Berendsen, D.M. Weller, S.C.M. Van Wees, Pa H.M. Bakker, Induced systemic resistance by beneficial microbes, Annu. Rev. Phytopathol. 52 (2014) 347–375, https://doi.org/10.1146/annurev-phyto-082712-102340.
[84] H.M. Chou, N. Bundock, S. a Rolfe, J.D. Scholes, Infection of *Arabidopsis thaliana* leaves with *Albugo candida* (white blister rust) causes a reprogramming of host metabolism, Mol. Plant Pathol. 1 (2000) 99–113, https://doi.org/10.1046/j.1364-3703.2000.00013.x.
[85] P. Ardanov, S. Lyastchenko, K. Karppinen, H. Haggman, N. Kozyrovska, A. M. Pirttila, Effects of *Methylobacterium* sp. On emergence, yield, and disease prevalence in three cultivars of potato (*Solanum tuberosum* L.) were associated with the shift in endophytic microbial community, Plant Soil 405 (2016) 299–310, https://doi.org/10.1007/s11104-015-2500-y.
[86] V.M. Conn, C.M.M. Franco, Effect of microbial inoculants on the indigenous actinobacterial endophyte population in the roots of wheat as determined by terminal restriction fragment length polymorphism, Appl. Environ. Microbiol. 70 (2004) 6407–6413, https://doi.org/10.1128/AEM.70.11.6407-6413.2004.
[87] G. Rodríguez-Caballero, F. Caravaca, A.J. Fernández-González, M.M. Alguacil, M. Fernández-López, A. Roldán, Arbuscular mycorrhizal fungi inoculation mediated changes in rhizosphere bacterial community structure while promoting revegetation in a semiarid ecosystem, Sci. Total Environ. (2017) 1–11, https://doi.org/10.1016/j.scitotenv.2017.01.128.
[88] L. Barelli, A.S. Waller, S.W. Behie, M.J. Bidochka, Plant microbiome analysis after *Metarhizium amendment* reveals increases in abundance of plant growth-promoting organisms and maintenance of disease-suppressive soil, PLoS One 15 (2020) 1–23, https://doi.org/10.1371/journal.pone.0231150.
[89] Y. Gu, K. Dong, S. Geisen, W. Yang, Y. Yan, D. Gu, N. Liu, N. Borisjuk, Y. Luo, V. P. Friman, The effect of microbial inoculant origin on the rhizosphere bacterial community composition and plant growth-promotion, Plant Soil (2020), https://doi.org/10.1007/s11104-020-04545-w.
[90] L. da Silva Lima, F.L. Olivares, R. Rodrigues de Oliveira, M.R.G. Vega, N. O. Aguiar, L.P. Canellas, Root exudate profiling of maize seedlings inoculated with *Herbaspirillum seropedicae* and humic acids, Chem. Biol. Technol. Agric. 1 (2014) 1–18, https://doi.org/10.1186/s40538-014-0023-z.
[91] C.B. Sruthilaxmi, S. Babu, Microbial bio-inoculants in Indian agricultureEcological perspectives for a more optimized use, Agric. Ecosyst. Environ. 242 (2017) 23–25, https://doi.org/10.1016/j.agee.2017.03.019.
[92] O. Podolich, P. Ardanov, I. Zaets, A.M. Pirttilä, N. Kozyrovska, Reviving of the endophytic bacterial community as a putative mechanism of plant resistance, Plant Soil (2014) 367–377, https://doi.org/10.1007/s11104-014-2235-1.
[93] M.T. Agler, J. Ruhe, S. Kroll, C. Morhenn, S.T. Kim, D. Weigel, E.M. Kemen, Microbial hub taxa link host and abiotic factors to plant microbiome variation, PLoS Biol. 14 (2016) 1–31, https://doi.org/10.1371/journal.pbio.1002352.
[94] A. Erlacher, M. Cardinale, R. Grosch, M. Grube, G. Berg, The impact of the pathogen Rhizoctonia solani and its beneficial counterpart *Bacillus amyloliquefaciens* on the indigenous lettuce microbiome, Front. Microbiol. 5 (2014) 1–8, https://doi.org/10.3389/fmicb.2014.00175.
[95] M. Köberl, M. Dita, A. Martinuz, C. Staver, G. Berg, Members of Gammaproteobacteria as indicator species of healthy banana plants on Fusarium wilt-infested fields in Central America, Sci. Rep. 7 (2017) 1–9, https://doi.org/10.1038/srep45318.
[96] W. Purahong, L. Orru, I. Donati, G. Perpetuini, A. Cellini, A. Lamontanara, V. Michelotti, G. Tacconi, F. Spinelli, Plant microbiome and its link to plant health: host species, organs and *Pseudomonas syringae* pv. *Actinidiae* infection shaping bacterial phyllosphere communities of kiwifruit plants, Front. Plant Sci. 871 (2018) 1–16, https://doi.org/10.3389/fpls.2018.01563.
[97] L. Lebreton, A.Y. Guillerm-Erckelboudt, K. Gazengel, J. Linglin, M. Ourry, P. Glory, A. Sarniguet, S. Daval, M.J. Manzanares-Dauleux, C. Mougel, Temporal dynamics of bacterial and fungal communities during the infection of *Brassica rapa* roots by the protist *Plasmodiophora brassicae*, PLoS One 14 (2019) 1–26, https://doi.org/10.1371/journal.pone.0204195.
[98] Y. Gu, Z. Wei, X. Wang, V.P. Friman, J. Huang, X. Wang, X. Mei, Y. Xu, Q. Shen, A. Jousset, Pathogen invasion indirectly changes the composition of soil

microbiome via shifts in root exudation profile, Biol. Fertil. Soils 52 (2016) 997–1005, https://doi.org/10.1007/s00374-016-1136-2.

[99] J. Yuan, J. Zhao, T. Wen, M. Zhao, R. Li, P. Goossens, Q. Huang, Y. Bai, J. M. Vivanco, G.A. Kowalchuk, R.L. Berendsen, Q. Shen, Root exudates drive the soil-borne legacy of aboveground pathogen infection, Microbiome 6 (2018) 1–12, https://doi.org/10.1186/s40168-018-0537-x.

[100] T. Rudrappa, K.J. Czymmek, P.W. Paré, H.P. Bais, Root-secreted malic acid recruits beneficial soil bacteria, Plant Physiol. 148 (2008) 1547–1556, https://doi.org/10.1104/pp.108.127613.

[101] M. Lupatini, G.W. Korthals, M. de Hollander, T.K.S. Janssens, E.E. Kuramae, Soil Microbiome is more heterogeneous in organic than in conventional farming system, Front. Microbiol. 7 (2017) 1–13, https://doi.org/10.3389/fmicb.2016.02064.

[102] S. Qin, S. Yeboah, X. Xu, Y. Liu, B. Yu, Analysis on fungal diversity in rhizosphere soil of continuous cropping potato subjected to different furrow-ridge mulching managements, Front. Microbiol. 8 (2017) 845, https://doi.org/10.3389/fmicb.2017.00845.

[103] M. Hartmann, B. Frey, J. Mayer, P. Mäder, F. Widmer, Distinct soil microbial diversity under long-term organic and conventional farming, ISME J. 9 (2015) 1177–1194, https://doi.org/10.1038/ismej.2014.210.

[104] K. Hartman, M.G.A. van der Heijden, R.A. Wittwer, S. Banerjee, J.C. Walser, K. Schlaeppi, Cropping practices manipulate abundance patterns of root and soil microbiome members paving the way to smart farming, Microbiome. 6 (2018) 1–14, https://doi.org/10.1186/s40168-017-0389-9.

[105] A. Campisano, L. Antonielli, M. Pancher, S. Yousaf, M. Pindo, I. Pertot, Bacterial endophytic communities in the grapevine depend on pest management, PLoS One 9 (2014) 1–10, https://doi.org/10.1371/journal.pone.0112763.

[106] S. Zhu, J.M. Vivanco, D.K. Manter, Nitrogen fertilizer rate affects root exudation, the rhizosphere microbiome and nitrogen-use-efficiency of maize, Agric., Ecosyst. Environ., Appl. Soil Ecol. 107 (2016) 324–333, https://doi.org/10.1016/j.apsoil.2016.07.009.

[107] S. Hong, H. Jv, M. Lu, B. Wang, Y. Zhao, Y. Ruan, Significant decline in banana *Fusarium* wilt disease is associated with soil microbiome reconstruction under chilli pepper-banana rotation, Eur. J. Soil Biol. 97 (2020) 103154, https://doi.org/10.1016/j.ejsobi.2020.103154.

[108] B.M. Tripathi, I. Moroenyane, C. Sherman, Y.K. Lee, J.M. Adams, Y. Steinberger, Trends in taxonomic and functional composition of soil microbiome along a precipitation gradient in Israel, Microb. Ecol. 74 (2017) 168–176, https://doi.org/10.1007/s00248-017-0931-0.

[109] M. Simonin, C. Dasilva, V. Terzi, E.L.M. Ngonkeu, Di. DIouf, A. Kane, G. Béna, L. Moulin, Influence of plant genotype and soil on the wheat rhizosphere microbiome: evidences for a core microbiome across eight African and European soils, FEMS Microbiol. Ecol. 96 (2020), https://doi.org/10.1093/femsec/fiaa067.

[110] F.T. De Vries, R.I. Griffiths, C.G. Knight, O. Nicolitch, A. Williams, Harnessing rhizosphere microbiomes for drought-resilient crop production, Science (80-.) 368 (2020) 270–274, https://doi.org/10.1126/science.aaz5192.

[111] C. Santos-Medellín, J. Edwards, Z. Liechty, B. Nguyen, V. Sundaresan, Drought stress results in a compartment-specific restructuring of the rice root-associated microbiomes, MBio 8 (2017) e00764–17.

[112] C.R. Fitzpatrick, J. Copeland, P.W. Wang, D.S. Guttman, P.M. Kotanen, M.T. J. Johnson, Assembly and ecological function of the root microbiome across angiosperm plant species, Proc. Natl. Acad. Sci. U. S. A. 115 (2018) E1157–E1165, https://doi.org/10.1073/pnas.1717617115.

[113] M. van der Voort, M. Kempenaar, M. van Driel, J.M. Raaijmakers, R. Mendes, Impact of soil heat on reassembly of bacterial communities in the rhizosphere microbiome and plant disease suppression, Ecol. Lett. 19 (2016) 375–382, https://doi.org/10.1111/ele.12567.

[114] A. Mark Ibekwe, S. Ors, J.F.S. Ferreira, X. Liu, D.L. Suarez, Seasonal induced changes in spinach rhizosphere microbial community structure with varying salinity and drought, Sci. Total Environ. 579 (2017) 1485–1495, https://doi.org/10.1016/j.scitotenv.2016.11.151.

[115] J.G. Ellis, Can plant microbiome studies lead to effective biocontrol of plant diseases? Mol. Plant Microbe Interact. 30 (2017) 190–193.

[116] S.R. Hopkins, J.M. Wojdak, L.K. Belden, Defensive symbionts mediate host–Parasite interactions at multiple scales, Trends Parasitol. 33 (2017) 53–64, https://doi.org/10.1016/j.pt.2016.10.003.

[117] P. Kumar, S. Thakur, G.K. Dhingra, A. Singh, M.K. Pal, K. Harshvardhan, R. C. Dubey, D.K. Maheshwari, Inoculation of siderophore producing rhizobacteria and their consortium for growth enhancement of wheat plant, Biocatal. Agric. Biotechnol. 15 (2018) 264–269, https://doi.org/10.1016/j.bcab.2018.06.019.

[118] P. Trivedi, M. Delgado-Baquerizo, C. Trivedi, K. Hamonts, I.C. Anderson, B. K. Singh, Keystone microbial taxa regulate the invasion of a fungal pathogen in agro-ecosystems, Soil Biol. Biochem. 111 (2017) 10–14, https://doi.org/10.1016/j.soilbio.2017.03.013.

[119] P. Durán, G. Tortella, S. Viscardi, P.J. Barra, V.J. Carrión, M. De La Luz Mora, M. J. Pozo, Microbial community composition in take-All suppressive soils, Front. Microbiol. 9 (2018) 1–15, https://doi.org/10.3389/fmicb.2018.02198.

[120] D.B. Müller, C. Vogel, Y. Bai, Ja. Vorholt, The Plant Microbiota: Systems-Level Insights and Perspectives, Annu. Rev. Genet. 50 (2016) 211–234, https://doi.org/10.1146/annurev-genet-120215-034952.

[121] E. Velázquez, L. Carro, J.D. Flores-Félix, et al., The legume nodule microbiome: a source of plant growth-promoting Bacteria, Probiotics Plant Heal. (2017) 41–70, https://doi.org/10.1007/978-981-10-3473-2.

[122] I.A. Stringlis, H. Zhang, C.M.J. Pieterse, M.D. Bolton, R. De Jonge, Microbial small molecules-weapons of plant subversion, Nat. Prod. Rep. 35 (2018) 410–433, https://doi.org/10.1039/c7np00062f.

[123] P. Kumar, R.C. Dubey, D. Maheshwari, V.K. Bajpai, ACC Deaminase producing *Rhizobium leguminosarum* RPN5 isolated from root nodules of *Phaseolus vulgaris* L, Bangladesh J. Bot. 45 (2016) 477–484.

[124] B. Tian, C. Zhang, Y. Ye, J. Wen, Y. Wu, H. Wang, H. Li, S. Cai, W. Cai, Z. Cheng, S. Lei, R. Ma, C. Lu, Y. Cao, X. Xu, K. Zhang, Beneficial traits of bacterial endophytes belonging to the core communities of the tomato root microbiome, Agric. Ecosyst. Environ. 247 (2017) 149–156, https://doi.org/10.1016/j.agee.2017.06.041.

[125] N. Rascovan, B. Carbonetto, D. Perrig, M. Díaz, W. Canciani, M. Abalo, J. Alloati, G. González-Anta, M.P. Vazquez, Integrated analysis of root microbiomes of soybean and wheat from agricultural fields, Sci. Rep. 6 (2016) 1–12, https://doi.org/10.1038/srep28084.

[126] Y. Yuan, C. Brunel, M. van Kleunen, J. Li, Z. Jin, Salinity-induced changes in the rhizosphere microbiome improve salt tolerance of Hibiscus hamabo, Plant Soil 443 (2019) 525–537, https://doi.org/10.1007/s11104-019-04258-9.

[127] S. Thijs, W. Sillen, F. Rineau, N. Weyens, J. Vangronsveld, Towards an enhanced understanding of plant-microbiome interactions to improve phytoremediation: engineering the metaorganism, Front. Microbiol. 7 (2016) 1–15, https://doi.org/10.3389/fmicb.2016.00341.

[128] S. Thijs, W. Sillen, N. Weyens, J. Vangronsveld, Phytoremediation: state-of-the-art and a key role for the plant microbiome in future trends and research prospects, Int. J. Phytoremediation 6514 (2016), https://doi.org/10.1080/15226514.2016.1216076, 00–00.

[129] E. Yergeau, T.H. Bell, J. Champagne, C. Maynard, S. Tardif, J. Tremblay, C. W. Greer, Transplanting soil microbiomes leads to lasting effects on willow growth, but not on the rhizosphere microbiome, Front. Microbiol. 6 (2015) 1–14, https://doi.org/10.3389/fmicb.2015.01436.

[130] S. Tardif, Et. Yergeau, J. Tremblay, P. Legendre, L.G. Whyte, C.W. Greer, The willow microbiome is influenced by soil petroleum-hydrocarbon concentration with plant compartment-specific effects, Front. Microbiol. 7 (2016) 1–14, https://doi.org/10.3389/fmicb.2016.01363.

CHAPTER 2:

Insights into the structure and role of seed-borne bacteriome during maize germination

Chapter 2: Manuscript accepted

Insights into the structure and role of seed-borne bacteriome during maize germination

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One sentence summary: Partial removal of the seed-borne microbiota negatively affects maize seedlings growth performance and altered bacterial community structure associated with emerged roots. Partial microbial rebuilding mainly with *Burkholderia*-related isolates partially restores the germination phenotype of disinfected seeds.

RESUMO

A germinação das sementes modula a composição da comunidade microbiana, que em última análise, influencia o crescimento das plântulas. Neste estudo, avaliamos a comunidade bacteriana da raiz de milho (variedade SHS 5050) germinado de sementes desinfestadas (DS) e de sementes não desinfestadas (NDS). Utilizando um sistema gnotobiótico, sementes tratadas com hipoclorito de sódio (1,25%, 30 min) apresentaram redução no tamanho da população bacteriana e aparente aumento na diversidade da comunidade associada a uma redução seletiva de *Burkholderia*. Mudanças na composição da comunidade de bactérias em DS afetou negativamente a velocidade de germinação do milho, o crescimento das plântulas e a mobilização de reservas em comparação com NDS. Uma comunidade bacteriana sintética (syncom), formada por doze isolados (9 *Burkholderia* spp.; 2 *Bacillus* spp. e 1 *Staphylococcus* sp.) obtidos da microbiota natural de sementes de milho, foram capazes de recuperar a germinação e o crescimento das plântulas quando reintroduzidas em DS. Os resultados gerais mostraram que as mudanças na composição da comunidade bacteriana e a redução seletiva da dominância de membros relacionados a *Burkholderia* interferem nos eventos de germinação e no crescimento inicial das plântulas de milho. Por meio de abordagens independentes e dependentes de cultivo, decifamos a estrutura do microbioma semente-milho, a localização dos nichos bacterianos e os táxons bacterianos com papéis relevantes no crescimento das plântulas. Uma relação entre a sucessão da comunidade microbiana das sementes e a germinação abre oportunidades em tecnologias de sementes para construir comunidades microbianas para impulsionar o crescimento e a saúde das plantas.

Palavras-chave: microbioma de sementes; sementes desinfestadas; bactérias endofíticas; colonização das raízes; sucessão microbiana; tecnologias microbianas.

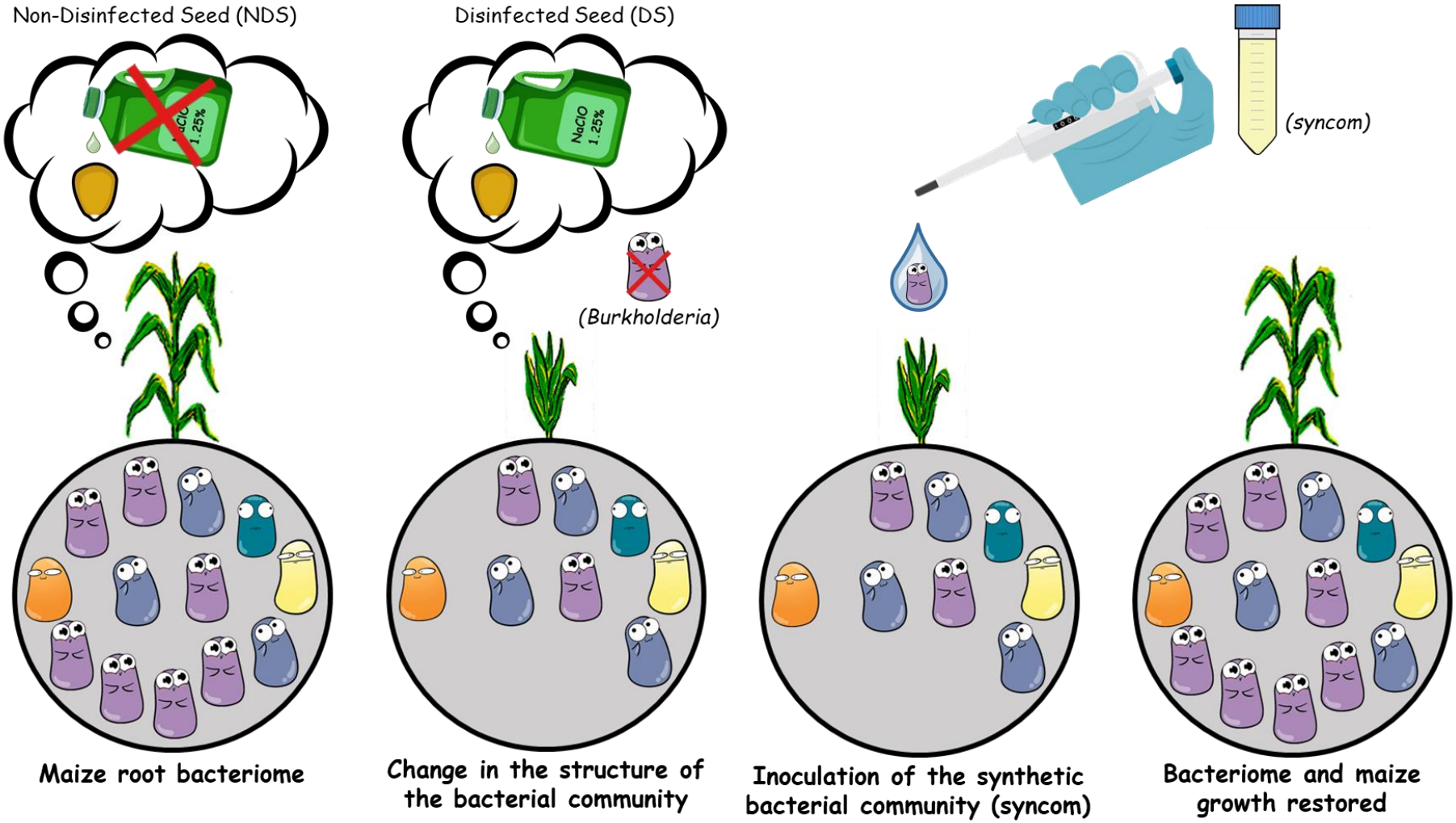
ABSTRACT

Seed germination events modulate microbial community composition, which ultimately influences seed to seedling growth performance. Here we evaluate the germinated maize (variety SHS 5050) root bacterial community of disinfected seed (DS) and non-disinfected seed (NDS). Using a gnotobiotic system, sodium hypochlorite (1.25%, 30 min) treated seeds showed a reduction of bacterial population size and an apparent increase of bacterial community diversity associated with a significant selective reduction of *Burkholderia*. The shift in the bacteria community composition in DS negatively affects germination speed, seedling growth, and reserve mobilization rates in comparison with NDS. A synthetic bacterial community (syncom) formed by twelve isolates (9 *Burkholderia* spp.; 2 *Bacillus* spp. and 1 *Staphylococcus* sp.) obtained from natural microbiota of maize seeds herein were capable of recovering germination and seedling growth when reintroduced in DS. Overall results showed that changes in bacterial community composition and selective reduction of *Burkholderia* related members dominance interfere with germination events and initial growth of the maize plantlets. By cultivation-dependent and independent approaches, we deciphered seed-maize microbiome structure, bacterial niches location, and bacterial taxa with relevant roles in seedlings growth performance. A causal relationship between seed microbial community succession and germination performance open opportunities in seed technologies to build-up microbial communities to boost plant growth and health.

Keywords: seed microbiome; disinfected seeds; endophytic bacteria; root colonization; microbial succession; microbial technologies.

Graphical Abstract

(Resumo Gráfico)



INTRODUCTION

Soil microbial community represents the primary source of mutualistic microbes, which colonizes the soil rhizosphere of plants (Bakker *et al*. 2015; Hardoim *et al*. 2012). From the soil to the rhizosphere continuum, plant-root exudates availability and composition are the major selective forces that shape rhizospheric microbiota, stimulating proliferation of certain bacterial specific groups (Bakker *et al*. 2015; Baudoin *et al*. 2003). Presumably, such enriched bacterial populations have more chance to colonize root surface (epiphytically) and root inner tissue (endophytically), contributing to the significant fraction of the plant bacteriome composition.

Bacterial endophytes recruited from microbial diversity enriched by plant exudates have their soil-origin widely recognized (Bulgarelli *et al*. 2012). However, controversial reports have been published on the concern of the pivotal contribution of soil-borne bacteria for the plant bacteriome assembly (Johnston-Monje *et al*. 2014). Several studies pointed out the importance of seed-borne bacteria to build-up microbiome composition during plant growth and development (Nelson 2017).

Vertically transmitted bacteria through seeds or vegetative plant parts have been extensively reported for different plant species. The underlying mechanisms of bacterial community dynamics and colonization of seeds, their offspring transmission, and predominant taxa under the germination process have been considered (Truyens *et al*. 2015).

More comprehensive studies about seed-associated microbes have been leveraged by cultivation-independent approaches (Adam *et al*. 2016; Hardoim *et al*. 2012; Johnston-Monje *et al*. 2016). For instance, 16S rRNA gene amplicon sequencing allows broader assessment and comparison of the bacterial community of seeds, rhizosphere, and bulk soil. Thereby, the relative contribution of different factors (host genotypes, plant organ, and ontogeny, soil type, geography) that determine the assembly of seed endophytes can be adequately covered.

Among the plant species, maize seeds have received much attention as a niche for microbial communities (Truyens *et al*. 2015). Using culture-dependent techniques, Rosenblueth *et al*. (2010) isolated distinct bacteria endophytes from surface-disinfected maize kernels detached from cobs and germinated under gnotobiotic conditions. In the early stages of germination, the predominant genera were *Bacillus* and *Paenibacillus*. Already *Methylobacterium*, *Alcaligenes*, *Tsukamurella*, *Erwinia*, *Microbacterium*, and *Rhodococcus* were detected later. Furthermore, *Burkholderia* was detected inside seeds using PCR of the 16S rRNA gene. Bacterial endophytes were isolated from surface-sterilized maize kernels of

four different cultivars under aseptic conditions, and the bacterial isolates were identified by 16S rRNA gene sequencing as *Pantoea* sp., *Microbacterium* sp., *Frigoribacterium* sp., *Bacillus* sp., *Paenibacillus* sp., and *Sphingomonas* sp. (Rijavec *et al*. 2007). Among eight bacteria isolated from the surface of disinfected seeds of thirty maize genotypes, *Bacillus* spp. was the most predominant species, with few isolates from the genera *Staphylococcus* and *Corynebacterium* (Bodhankar *et al*. 2017).

Cultivation-independent methods were applied to decipher maize seed microbiome. Eight bacteria species were common in all six maize hybrids, representing a seed-inhabiting endophytic core. Among them, *Pantoea agglomerans*, *Enterobacter cloacae*, and *Aeribacillus palli* were the most representative taxa, accounting for 60% of relative abundance (Liu *et al*. 2017). The relative contribution from the soil and surrounding environment (horizontal transmission) and those inherited from seeds (vertical transmission) for endophytic assemblage in maize were accessed by Johnston-Monje *et al*. (2014). It was concluded that the bacterial community from juvenile maize plants resembled a more seed bacterial community profile than the soil bacterial community in which the plants were growing. Also, around one-fifth of the bacterial community profiles harboured by roots and sprouts of the plant were typical in all soil geographical origin used, emphasizing the selective role of maize root exudates. In another study, it was demonstrated that despite the contribution of the soil bacteria for maize rhizosphere richness, the most dominant bacterial groups in juvenile maize rhizosphere are seed transmitted (Johnston-Monje *et al*. 2016).

To gain insight into the role of seed microbiome, we used sodium hypochlorite as a seed disinfecting agent and further evaluated bacterial community structure, root-bacteria colonization and growth performance of maize seedlings under gnotobiotic conditions. To date, it is the first time that the bacterial community structure is assessed after seed chemical disinfection compared to non-disinfected seeds. We hypothesized that the chemical treatment changes the successional bacterial community structure during seed to seedling transition and modulate plantlets growth performance. We analyzed maize seed-borne bacteria by assessing the culture-dependent and culture-independent fraction of the bacterial community associated with germinated seedlings. We have also assessed the effect of the reintroduction of a synthetic bacterial community (syncom) formed by isolates obtained from the same maize seed-borne genotype herein. Ultimately, we can take advantage of the succession population changes in microbial seed technologies to boost plant growth and health.

MATERIAL AND METHODS

Disinfection and germination of maize seeds

Seeds of commercial maize (*Zea mays* L.) hybrid SHS 5050 (Santa Helena Sementes, Brazil) were washed five times in sterile distilled water and remained immersed in water for 5 h. After the imbibition period, the seeds were divided into two treatments: a) Non-disinfected seeds (NDS) and b) Disinfected seeds (DS). For DS, seeds were surface disinfected in 70% ethanol for 5 min and soaked in sodium hypochlorite (NaClO; Butterfly Ecologia, Audax Company) at 1.25% for 30 min with subsequent five rinses in sterile distilled water. NDS received the same sequential treatment substituting ethanol and NaClO for sterilized water. The experimental unit comprised twelve seeds from NDS or DS treatment aseptically placed in a Petri dish containing agar-water medium (0.5%) under axenic conditions. Five replicates were used for each treatment in a completely randomized design (DIC). NDS and DS seeds were germinated in a growth chamber at 30 ºC and photoperiod 12/12 h (light/dark) for 5 and 8 days, respectively. During this period, the number of germinated seeds (radicle $\geq$ 5 mm) was recorded daily to calculate different germination parameters, including germination percentage (%G), germination speed index (GSI), average germination time (AGT) and average germination speed (AGS) (Maguire 1962). Significance between treatments was calculated using an unpaired t-test ($p \leq 0.05$). The 30 min immersion time in sodium hypochlorite was defined in an assay that evaluated the effect of different disinfection times on seed bacteria's density (Supplementary data SD.1).

Counting and isolating maize seed-borne bacteria

The population size of total and diazotrophic bacteria were estimated with root samples obtained in the germination assay for DS and NDS of maize. For this, 1 g of root from each Petri dish was macerated in saline 99 mL of sodium chloride (NaCl, 8.5 g L^{-1}) and subjected to serial dilution from 10^{-1} to 10^{-7}. After that, 100 µL of each dilution was transferred to culture media. Plates were incubated in the growth chamber at 30 ºC for 5 to 7 days. Solid Nutrient Broth (NB) medium was used for counting total heterotrophic bacteria, and results were expressed in $\log_{10}$ cells per g (fresh weight root). For diazotrophic bacteria count, two media were used, the JNFb semisolid medium (malic acid as C-source) and LGI semisolid medium (sucrose as C-source). The media composition and procedure to determine Most Probable Number (MPN) per g of a fresh root followed the recommendation of Baldani *et al*. (2014).

Microscopy evaluation of the maize seed microbiota

Non-germinated and germinated maize seeds of DS and NDS were collected and processed for scanning electron microscopy (SEM) aiming to characterize the seed (Supplementary data SD.2) and root-associated bacteria. From the germination assay described herein, the whole primary root of the maize seedlings was obtained from each treatment. For SEM, root segments (≈ 1 cm) and transversal/longitudinal sections of whole seeds were fixed in a solution containing 2.5% glutaraldehyde and 4% paraformaldehyde in potassium phosphate buffer (0.05 mol L^{-1}, pH 7.0). Then, the samples were washed (3 times for 20 min for root; 30 min for seed) in the same buffer and dehydrated in a growing series of ethanol (15, 30, 50, 70, 90 and 2 x 100% at 15 min for each root; 30 min for seed). After dehydration, samples were dried in critical point drier apparatus (BAL-TEC CPD 030), mounted on Al-stubs and metalized with ionized platinum in a sputtering coat apparatus (BAL-TEC SCD 050); and visualized under an SEM Zeiss EVO 40. Macroscopic structures of the seeds were recorded using a magnifying glass (Zeiss Stemi SV 11) coupled to a digital camera and used as a reference for the SEM images.

Sequencing of the bacteriome

Samples of emerged roots from DS and NDS were macerated in liquid nitrogen and stored at -70 ºC until the extraction of the genetic material. The total genomic DNA was isolated from roots (~ 0.2 g) using two adapted extraction protocols: 1) Plant DNAzol® kit (Ausubel *et al*. 1990; Wilfinger *et al*. 1997); 2) Cetyltrimethylammonium bromide (CTAB) (Chen and Ronald 1999; Doyle and Doyle 1990). Then, the DNA was quantified by a NanoDrop 2000® spectrophotometer (Thermo Scientific) and Qubit® fluorometer (Invitrogen); and its quality evaluated on agarose gel (0.8%) electrophoresis (80 V, for 70 min). The total DNA was sent to the company "WEMSeq Biotecnologia" for sequencing of the 16S rRNA gene in Illumina MiSeq, with six replicates per treatment. Twenty nanograms of DNA were used as a template for 18 cycles of amplification of the V4 region of the 16S rRNA gene, using primers 515F and 806R (Caporaso *et al*. 2012) and GoTaq Master Mix (Promega). PCR products were quantified with the Qubit dsDNA HS kit (Invitrogen) and sequenced with the 300V2 Kit (Illumina) in Illumina MiSeq (Illumina), following the manufacturer's instructions.

The sequences obtained from MiSeq were analyzed using the QIIME (Quantitative Insights Into Microbial Ecology) version 1.9.0 (Caporaso *et al*. 2010), where they were

filtered, grouped into Operational Taxonomic Units (OTUs) with a 97% similarity cut and taxonomically classified (phylum, class, order, family, and genus) using the SILVA database as a reference (Quast *et al*. 2012). BIOM files were imported into the R environment using the phyloseq (McMurdie and Holmes 2013) and vegan (Oksanen *et al*. 2013) packages. Sampling quality was estimated using Good's coverage. To test the hypothesis that disinfection shapes the bacteriome, principal coordinate analysis (PCoA) graphs were constructed based on the Bray-Curtis dissimilarity matrix. Differences between treatments were obtained by Multivariate Analysis of Permutational Variance (PERMANOVA) (Anderson 2014). Alpha diversity was estimated by applying the Shannon diversity index. Also, the differential abundance at the family and genus level was calculated using the aldex function (Fernandes *et al*. 2014). Venn diagrams and genus-level heatmaps were built. The rarefied dataset was used for statistical analyses. The raw FASTQ files were submitted to NCBI public data under accession number study PRJNA669054.

Quantitative PCR (qPCR) for eubacteria

Pre-germinated seeds and emerged maize roots (NDS and DS) had their bacterial community quantified by qPCR from DNA extracted according to the methodology mentioned above (chosen method: CTAB). Each PCR reaction (15 µL) contained template DNA (100 ng for seed and 40 ng root); 7.5 µL of SYBR Green (Promega), 0.5 µL of each primer (10 µM; 926F: AAACTCAAAKGAATTGACGG; 1062R: CTCACRRCACGAGCTGAC) (De Gregoris *et al*. 2011) and water. The reactions were carried out in triplicate, with 5 min incubation at 95 °C, 40 cycles of 15 s at 95 °C and 1 min at 60 °C in Step-One-Plus Real-Time PCR System (Applied Biosystems). The proportion of bacteria in the microbiome was calculated based on the Ct (cycle threshold) values of the samples (three replicates per treatment) and using a standard curve generated from the model bacteria *Escherichia coli* ATCC 25922. *E. coli* was grown in NB liquid medium (180 rpm, at 30 ºC) and had its DNA extracted with Wizard Genomic DNA Purification Kit (Promega). The reference DNA was diluted in series of 10^{-2} to 10^{-8} (20 - 2 x 10^{9} ng of DNA), and its quantification in qPCR (expressed in Ct) plotted concerning the number of *E. coli* cells.

Partial recomposition of the bacterial maize microbiota (syncom)

Bacteria isolates obtained from maize NDS axenically were recovered from NB solid medium and JNFb or LGI semisolid medium herein. Based on distinct colony morphology, 12

out of 19 isolates were selected for partial recomposition microbiota (syncom) assays after a greenhouse inoculation screening for plant-growth promotion of maize (data not shown). Strain designation and origin of the isolates are quoted in Supplementary data SD.3. The taxonomic affiliation of the selected bacterial isolates can be seen in Supplementary data SD.4. Each bacterium of this consortium was grown in liquid NB medium, kept under agitation (180 rpm, for 24-48 h at 30 ºC). The bacterial cells were collected by centrifugation (2.000 x g, for 10 min (Eppendorf)) and resuspended in sterile distilled water. The values of OD_{595} (optical density at 595 nm) of the isolates were standardized on a spectrophotometer (OD_{595} = ~ 0.5 in NB medium), and these were mixed to form a synthetic bacterial community diluted until 10^{-7} concentration from an initial cell density of 2 x 10^8 CFU.g^{-1}.

For the inoculation of the synthetic bacterial community, disinfected maize seeds were immersed in the inoculum for 10 min, aiming at the partial rebuilding of the microbiota removed by seed disinfection with sodium hypochlorite. Then, the seeds that were disinfected and that received the mix of 12 bacteria (DS + MIX) were placed in Petri dishes with agar-water medium (0.5%). Ten seeds per plate constituted the experimental plot. The experimental design was completely randomized with the following treatments: 1) NDS; 2) DS; 3) DS + MIX and five repetitions for germination test evaluation and four repetitions for the plant-growth evaluation. The DS and NDS were used as controls. All plates were conditioned in the growth chamber at 30 ºC and photoperiod 12/12 h (light/dark) for five days. Germination rate was calculated, and the length and mass (fresh and dry) of the aerial part and root seedlings with the aid of a millimetre ruler and analytical balance. SEM was used for the structural characterization of reinoculated seeds and roots, respectively. The results were submitted to analysis of variance (ANOVA) and the means compared by the Tukey test ($p \leq 0.05$).

Biochemical analysis of seed reserves under seedling germination

All seeds were treated and germinated as described herein. Stored seed reserves were quantified from three compartments of maize: 1) whole seed: sampled after the soaking and disinfection phase; 2) embryonic axis: collected 24 (for NDS) and 48 h (for DS and DS + MIX) after radicle protrusion; 3) seedling root: obtained after five days of germination. The collected material was macerated in liquid nitrogen and analyzed for protein, glucose, triglyceride, reducing sugar, and alpha-amylase activity. Three replicates per treatment were used. For protein dosing, the bicinchoninic acid (BCA) method (Smith *et al*. 1985) was used,

characterized by the reduction of copper ions ($Cu^{+2} \rightarrow Cu^{+1}$) and formation of the violet BCA-Cu^{+1} complex. The protein concentration was measured at 562 nm in a spectrophotometer. The albumin curve was used as a standard. Reducing sugars were quantified using the 3,5-dinitrosalicylic acid (DNS) method (Miller 1959). In this method, the sugars react with the DNS (yellow), which is reduced to 3-amino-5-nitrosalicylic acid (dark red). Absorbance was measured at 540 nm. Colourimetric methods determined glucose, triglyceride levels, and alpha-amylase activity and according to the manufacturer's instructions (Bioclin® K082, K117, K003). Hydrogen peroxide in the presence of specific reagents forms cherry-coloured and cherry-red compounds, whose intensity (500-505 nm) is proportional to the concentration of triglyceride and glucose, respectively. The alpha-amylase activity in the samples was inversely proportional to the intensity of the blue colour, a product of the complexation between iodine and non- starch and calculated by comparing it to a control substrate (600 nm).

RESULTS

Disinfection assay showed that the increase of the immersion time with sodium hypochlorite (1.25%) proportionally reduced the seed-borne bacterial population associated with pre-germinated seeds (Supplementary data SD.1). The chosen time for disinfection (30 min) had shown significant microbial population reduction without compromise germination performance (Supplementary data SD.1). The seed disinfection affected the successional bacterial population size associated with emerged roots at 5-d of germination. Bacterial counts confirmed it in culture media with different carbon sources. Using LGI (C-sucrose) and JNFb (C-malic acid) N-free semisolid medium, the diazotrophic bacterial population levels in roots of NDS were, respectively 6.23 $\pm$ 0.8 and 5.05 $\pm$ 1.0 $\log_{10}$ bacteria cells per gram of fresh root (Fig. 1). For the roots of DS, the diazotrophic bacterial population level was dramatically reduced, being not detected (Fig. 1). The total heterotrophic bacterial population (NB solid medium) recovered from roots of NDS was 8.5 $\pm$ 0.5 $\log_{10}$ bacteria cells per gram of fresh root. Surprising, values of 7.21 $\pm$ 0.5 $\log_{10}$ bacteria cells per gram of fresh root were observed in DS (Fig. 1). Up to one hundred purified colonies were obtained from NB solid medium, JNFb and LGI semisolid medium in the present study. The bacteria were isolated from NDS and DS.

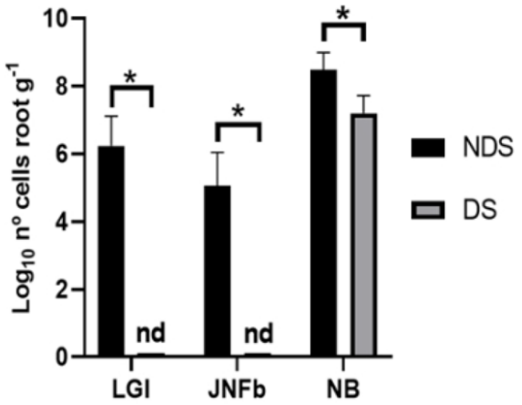


Figure 1. Estimation of bacterial population size associated with emerged roots of non-disinfected (NDS) and disinfected seed (DS). Count for diazotrophic bacteria was obtained in LGI and JNFb semisolid nitrogen-free media. Count for total heterotrophic bacteria in Nutrient Broth (NB) solid medium. Data expressed $\log_{10}$ cell number per g of fresh weight root with four replicates per treatment and statistical differences tested with Student t-test at 5 % probability (*). nd means not detected at 10^{-1} dilution.

SEM analysis of DS and NDS-treated maize seeds after imbibition (Supplementary data SD.2) and two days after germination (Fig. 2) were carried out aiming to characterize seed-borne microbiota structural interaction. The surface of the pericarp external layer of NDS non germinated seeds was colonized by bacteria aggregates in monolayer pattern (Supplementary data SD.2 A1). However, bacteria cells density (cell per unit area) associated with NDS was greater than DS (Supplementary data SD.2 B1). Cross-section of maize kernel also revealed the presence of bacteria in inner tissues colonizing the region of the endosperm (Supplementary data SD.2 A2-B2) and embryo (Supplementary data SD.2 A3-B3) with no apparent differences in bacteria density. In early germination stages, with the rupture of the integument by the primary root, more bacteria cells were visualized by SEM in both treatments. The bacterial aggregates were mainly localized between the pedicel (tip cap) and the bottom of the emerged radicle as well in the root hair formation zone of the radicle (Supplementary data SD.2 A4- B4). Disinfection of seeds remove most of the epiphytic bacteria from the pericarp surface, but the microscopy observation suggests that the endophytic niches colonization remains unchanged.

The influence of seed-disinfection on the microbial colonization pattern of the root axis in germinated seeds was confirmed by SEM (Fig. 2). It was observed in roots of NDS after five days of growth in axenic conditions, extensive colonization by the microbiota. In this case, bacterial biofilms were seen more frequently, mainly in lateral roots emergence sites

(Fig. 2A1-A2), the root hair zone (Fig. 2A3-A4), and root cap (Fig. 2A5-A6). Microbial colonization in roots of DS had shown the same colonization sites described for NDS, however, with visible reduced established population, where single cells and small aggregates were seen more frequently (Fig. 2B). The bacteria cell shape diversity of the bacterial microbiota was also affected by the disinfection of the seeds, which removed small bacteria cells, in ovoid or short rod shape, and selected long rod shape (Fig. 2A vs 2B4). Interestingly, fungus hyphae were frequently seen in roots that emerged from disinfected seeds (data not shown).

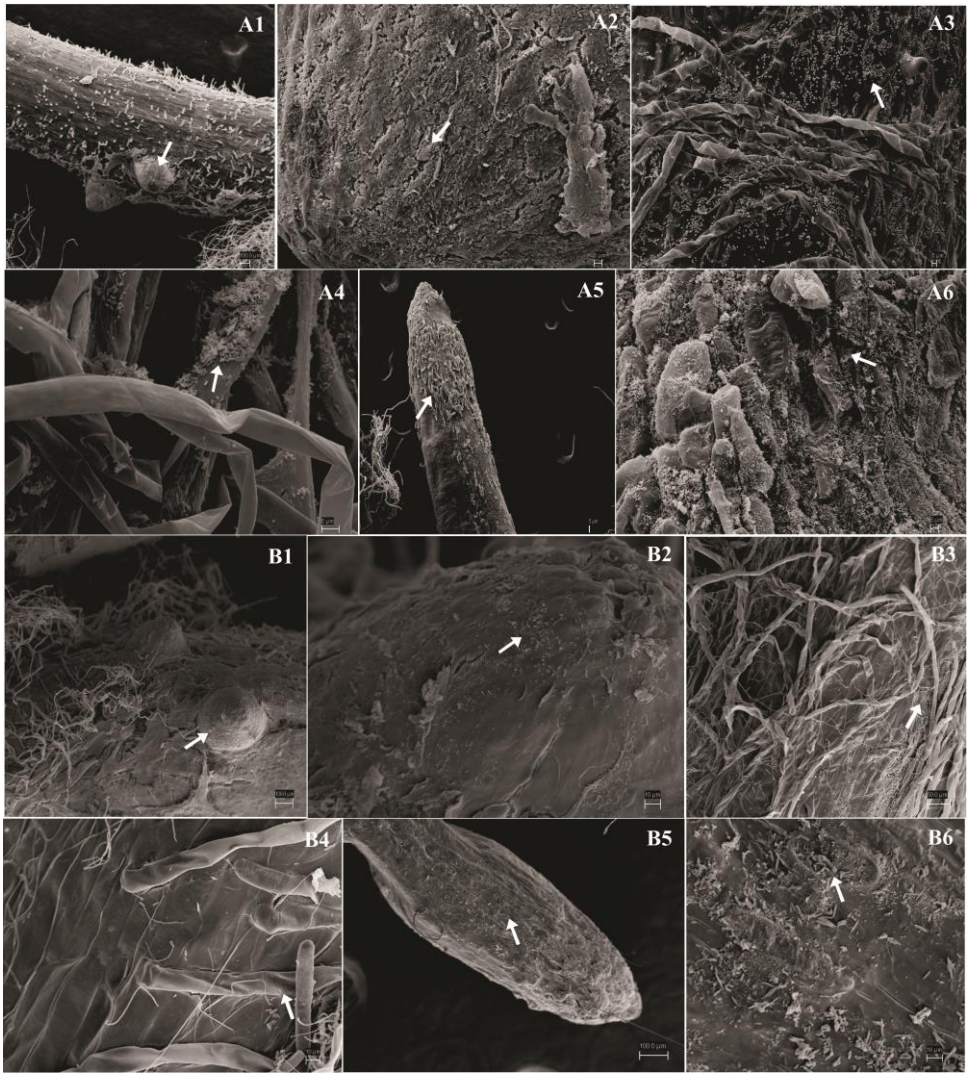


Figure 2. Colonization of maize roots by the microbiota. Bacterial cells were visualized by scanning electron microscopy (SEM). Maize seeds non-disinfected (A) and disinfected treatments (B). Root regions: mitotic sites (A1 and A2; B1 and B2), root hair (A3 and A4; B3 and B4) and root cap (A5 and A6; B5 and B6). White arrows indicate biofilms and small bacterial aggregates. Bars represent the following scales: panel A2, A3, A4, A5, and A6: 5 µm; B2, B4, and B6: 10 µm; B3: 50 µm; A1, B1, and B5: 100 µm.

Bacteriome community associated with the emerged root that succeeds the DS and NDS maize seed germination under gnotobiotic conditions was evaluated. Illumina MiSeq sequencing of the root bacteriome by 16S rRNA gene amplicons from 12 maize root samples, produced a total of 250,303 reads and 88% average sample coverage (Supplementary data SD.5). Principal coordinate analysis (PCoA) based on the Bray-Curtis dissimilarity matrix representing beta diversity shows that disinfection with hypochlorite shapes the maize root bacteriome (Fig. 3A). The structure of the bacterial community differs between roots from DS and NDS. Alpha diversity measures indicated significant differences between the roots analysed (p = 0.0051), with greater apparent diversity in the DS treatment whose bacteriome was removed by superficial disinfection of the seed (Fig. 3B). PCoA results were confirmed by Permanova, where the disinfection factor was significant (p = 0.006), contributing 36% of the variation (Supplementary data SD.6).

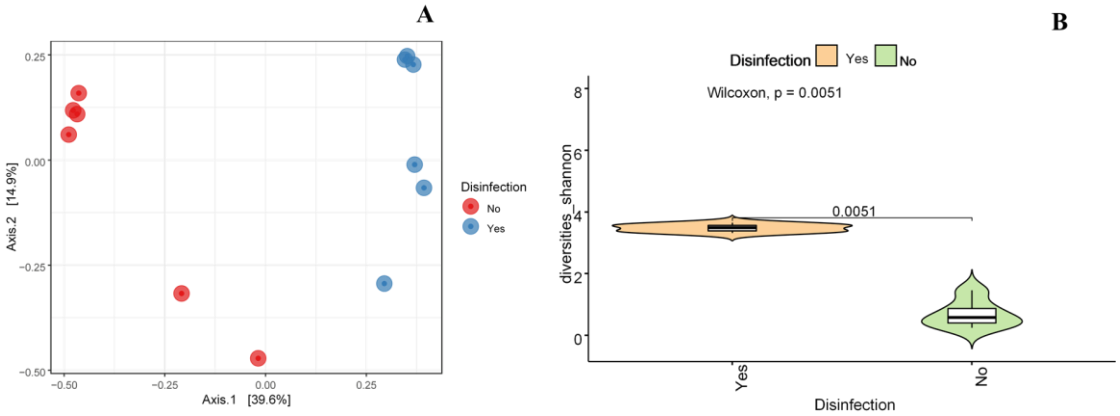


Figure 3. The principal coordinate graph (PCoA) based on the Bray-Curtis dissimilarity matrix (A) and measurements of the alpha diversity of root bacteriome from disinfected (Yes) and non-disinfected (No) seeds. Different colours indicate the treatments. Shannon = microbial diversity index.

Venn diagram shows a higher number of unique OTUs in roots of NDS treatment (51), followed by a lower number in the DS treatment (5). The intersection between treatments shared 4 OTUs (Fig. 4). The comparative relative abundance of bacteria assigned for the same taxon revealed that five genera were abundant in each treatment (Fig. 4). In the NDS-roots, the genus *Burkholderia* was the most abundant, followed by f_Enterobacteriaceae_922761 (unassigned genus), *Rubrobacter*, *Lactobacillus*, and *Azospirillum*. In the DS-roots, the most abundant bacteria belong to the genus *Pseudomonas*, *Bacillus*, *Acinetobacter*, f_Alcaligenaceae_575028 (unassigned genus) and *Corynebacterium*. Proteobacteria

dominated the maize root bacteriome in both conditions (7 out of 10 genera), followed by the phyla Actinobacteria, Firmicutes, and Bacteroidetes in equal proportion (1 out of 10 genera).

Differential abundance analysis was performed to filter bacterial genus removed from the maize root by the disinfection process (Supplementary data SD.7). Disinfection with hypochlorite significantly removed ($p \leq 0.05$) mainly bacteria taxon assigned as *Burkholderia* genus (Fig 4). Three groups of operational taxonomic units not assigned (NA) genus, *Corynebacterium*, *Staphylococcus* and *Cryocola* were more abundant in roots of DS compared to NDS. Independent of the disinfection, *Burkholderia* representatives were the most abundant taxon associated with the seedling's roots (Fig. 4 and SD 7).

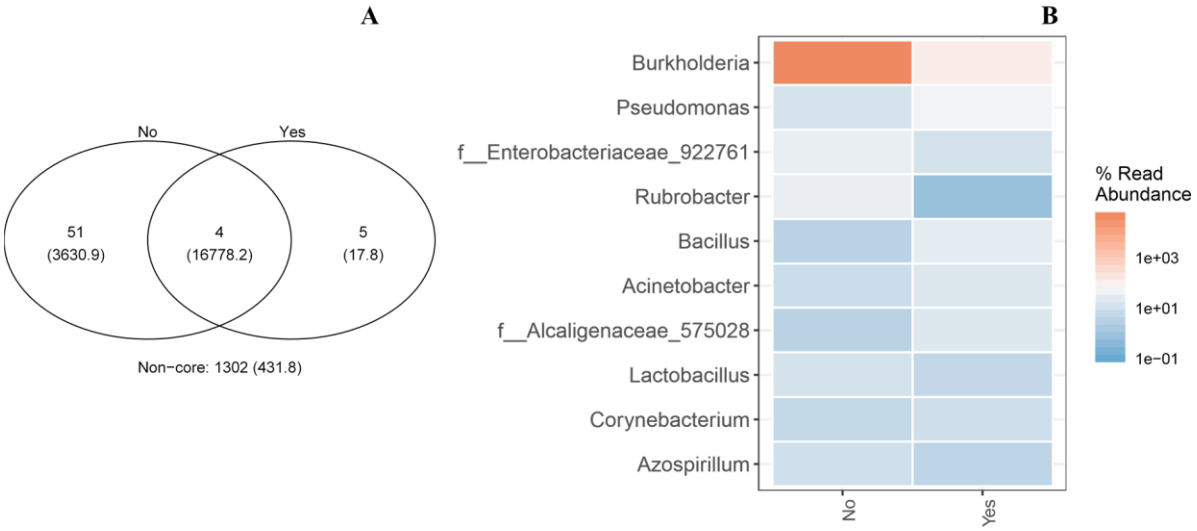


Figure 4. Venn diagram showing OTUs overlap (A) and the relative abundance between roots from disinfected (Yes) and non-disinfected (No) seeds (B). Colour intensity of heatmap indicated in the legend to the right of the figure, shows the relative values for the genus.

Quantification of the seed bacteriome by qPCR showed that disinfection reduced from 5.9 to 3.3 ($p \leq 0.05$) the number of bacteria/ng DNA (Fig. 5A). In the maize root, there were no significant differences between treatments (NDS: 6.9 bacteria/ng DNA; DS: 6.3 bacteria/ng DNA; $p \geq 0.05$) (Fig. 5B).

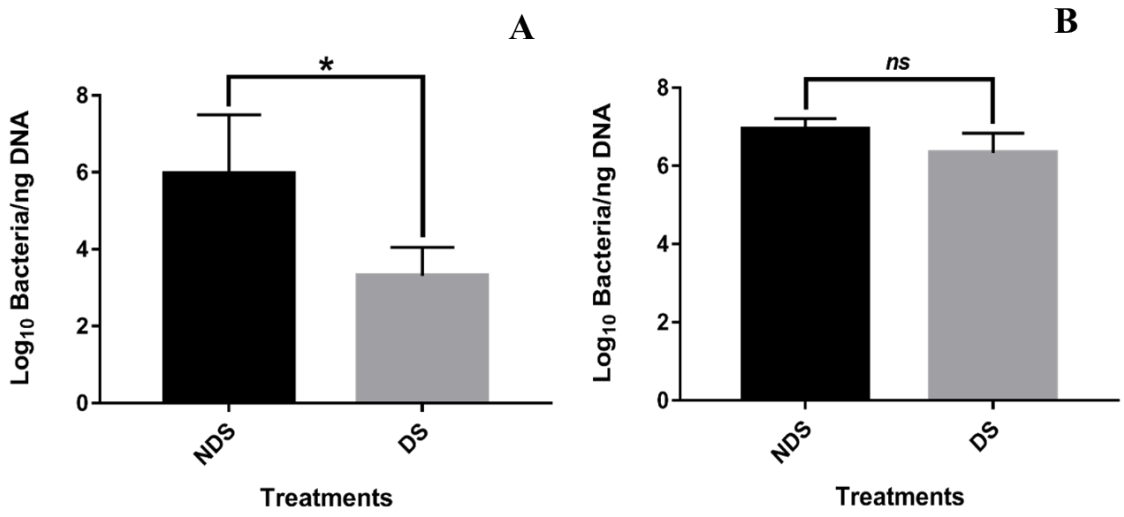


Figure 5. Quantification of bacteriome by qPCR in non-disinfected (NDS) and disinfected seeds (DS) before germination (A) and emerged roots after germination (B). *Significant difference between treatments according to the Tukey test ($p \leq 0.05$).

To evaluate the influence of the microbiota on the germination and growth of maize plantlets, non-germinated DS with a reduced bacterial population was subjected to a bacterial recomposition using twelve seed-borne isolates obtained in the present study. Members of this synthetic bacterial community (syncom) were identified by sequencing the 16S rRNA gene (Supplementary data SD.4). Nine isolates were attributed to the genus *Burkholderia* (*Burkholderia* sp. and *Burkholderia gladioli*); two to the genus *Bacillus* (*Bacillus drentensis* and *Bacillus camelliae*) and one identified as belonging to the genus *Staphylococcus* sp.

The seed inoculation with the synthetic bacterial community (DS +MIX) resulted in higher germination percentage and average germination time (82.5%; 2.57 days), when compared to non-inoculated DS (52.5%; 3.21 days) (Fig. 6). When comparing inoculated seeds (82.5%) and non-disinfected seeds (92.5%), the %G had shown the same trend with no statistical differences. However, for average germination time (AGT), the treatments differed ($p \leq 0.05$). For the germination speed index (GSI) and average germination speed AGS), no significant differences were observed between DS and DS + MIX, only when DS and DS+MIX were compared to NDS.

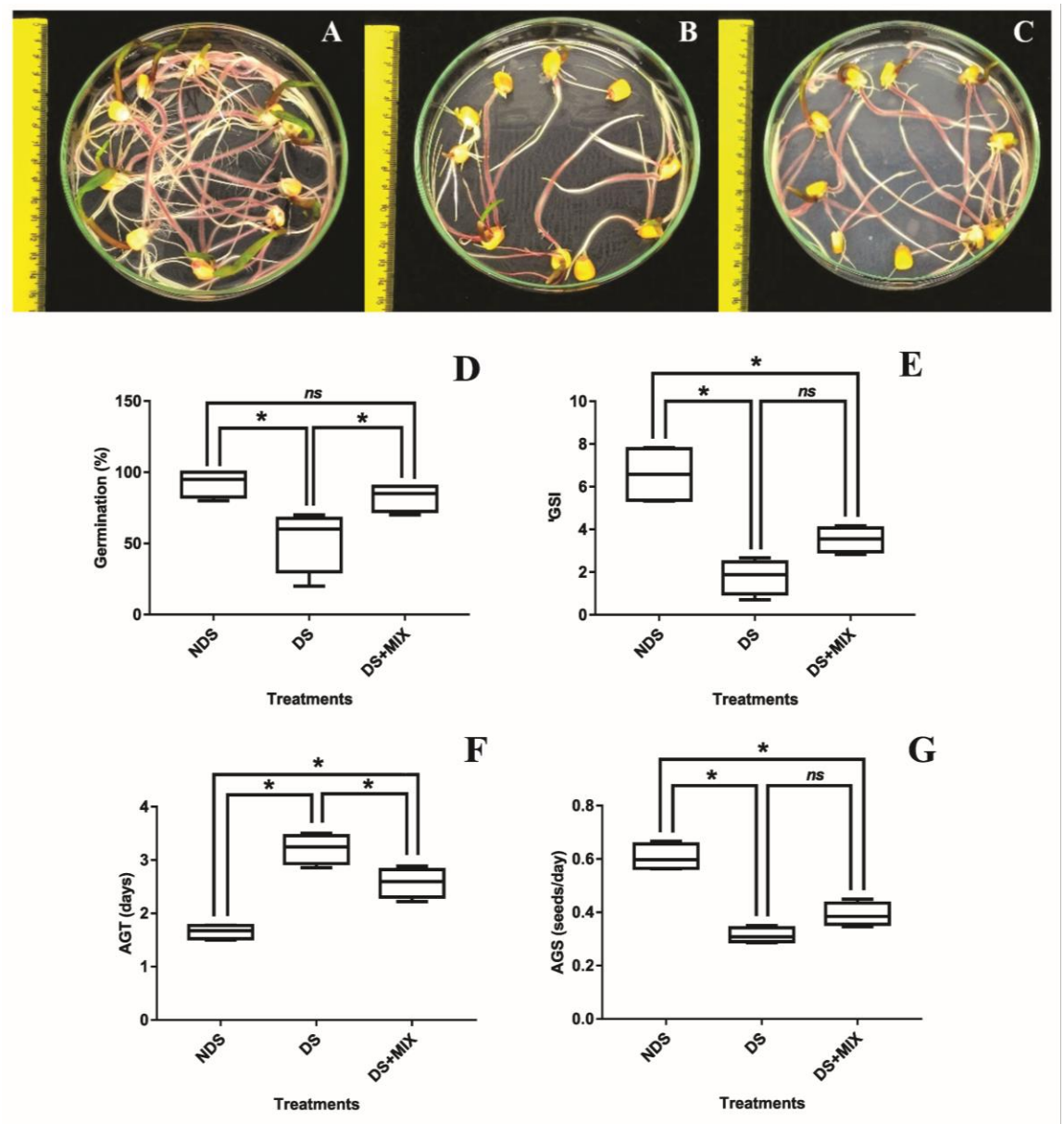


Figure 6. Maize non-disinfected seeds (A; NDS), disinfected seed (B; DS), and disinfected-syncom inoculated seeds (C; DS + MIX). Germination percentage (D), germination speed index (E), average germination time (F), and average germination speed (G). *Significant difference between treatments according to the Tukey test ($p \leq 0.05$).

Five days after germination, the length of the root and aerial part of the seedlings from NDS was significantly longer (17 cm; 6.25 cm) than DS (8.62 cm; 2.83 cm), but did not differ from DS + MIX (syncom inoculated) (14.79 cm; 4.95 cm) (Fig. 7). DS + MIX seedlings showed more significant growth and produced higher amounts of dry root biomass (0.109 g dry matter) than DS-treatment (0.063 g dry matter), demonstrating that the microbiota influence germination and plantlets growth. For the other parameters analysed, no differences were observed between the DS and DS + MIX.

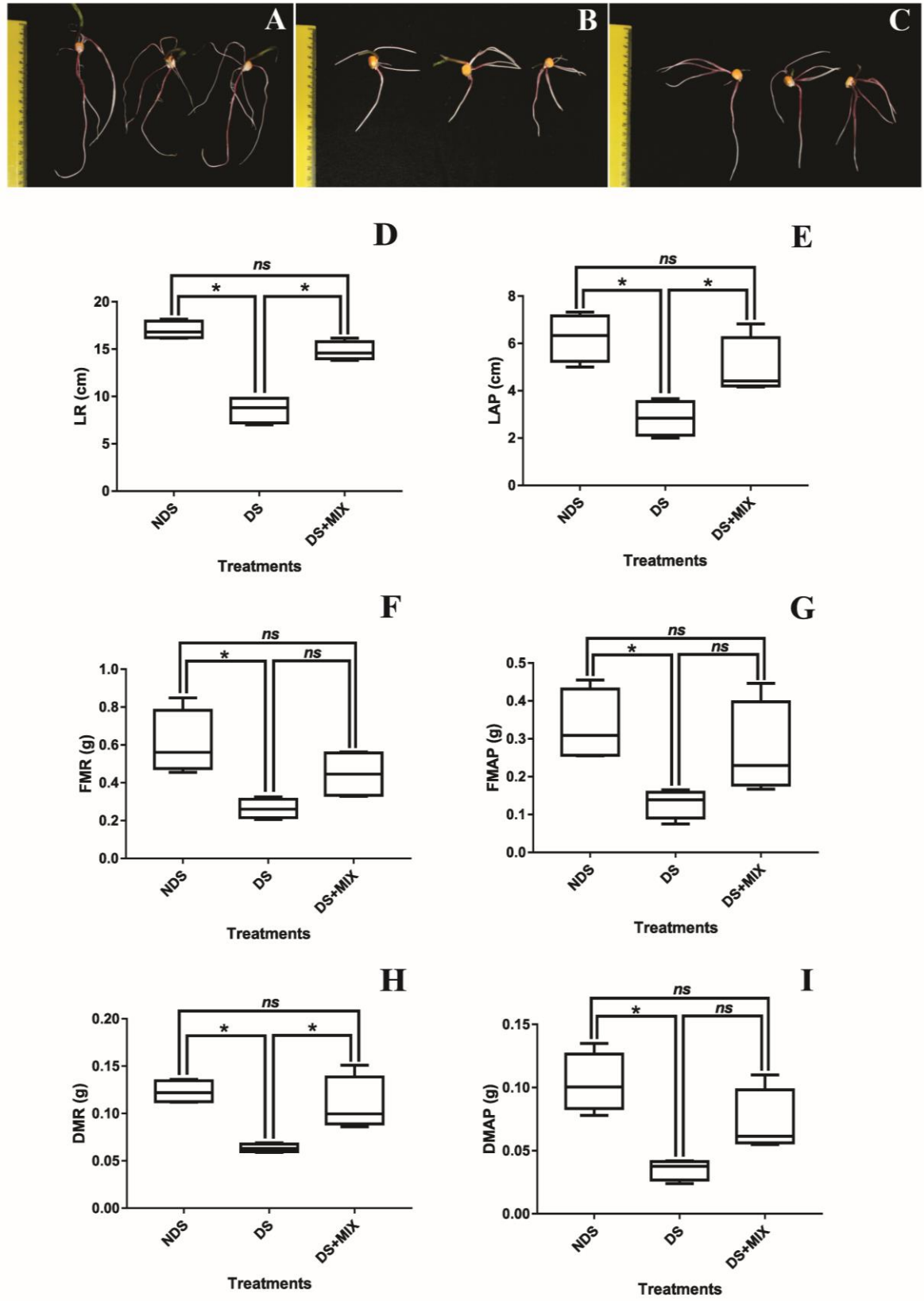


Figure 7. Maize seeds non-disinfected (A; NDS), disinfected (B; DS), and disinfected-inoculated (C; DS + MIX). Shoot length (D) and root (E), shoot fresh weight (F) and root (G), shoot dry weight (H), and root (I). *Significant difference between treatments according to the Tukey test ($p \leq 0.05$).

Structural microscopy analysis of disinfected seeds confirmed the bacterial colonization by syncom inoculation (Supplementary data SD.8). The pericarp cell wall surface was pronouncedly colonized by single cells and aggregates along the cell junctions (Supplementary data SD.8 A1-A2). In the previous observation of the disinfected seeds, epiphytic bacteria (from the pericarp) were removed by the hypochlorite treatment (Supplementary data SD.2 B1). In cross-sectioned inoculated-seeds, we visualized bacteria cells in low density at endosperm and embryo regions (Supplementary data SD.8 A3-A6). Interesting to quote that the site of radicle emergence was heavily colonized by bacteria aggregates and biofilms (Supplementary data SD.8 A7-A8). This region seems to be the preferential site ("hot spot") for bacterial growth on germinated seeds since seed-borne bacteria naturally colonized it in non-disinfected seeds, as previously showed in this study.

After seed germination, the colonization pattern of the syncom was evaluated. The SEM data od DS + MIX revealed that roots were densely colonized by bacteria (Fig. 8) compared with previous data obtained for DS (Fig, 2B1-B6). Massive bacteria colonization was observed in regions with lateral root emission and root cap (Fig. 8). More significant bacterial aggregation was identified at points of emergence of lateral root (Fig. 8A1-A2) and elongation zone (Fig. 8A5-A6) of the root axis growing zone. In the root-hair zone (Fig. 8A3-A4) and root cap (Fig. 8A7-A8), the cells were more dispersed. The bacterial syncom (DS + MIX) establishment was confirmed by estimation of total heterotrophic bacterial population (NB solid medium) recovered from roots where the numbers were 10,1 $\pm$ 1,2 $\log_{10}$ bacteria cells per gram of fresh root, higher count than NDS (8.5 ± 0.5 $\log_{10}$) and DS (7.21 ± 0.5 $\log_{10}$) treatments.

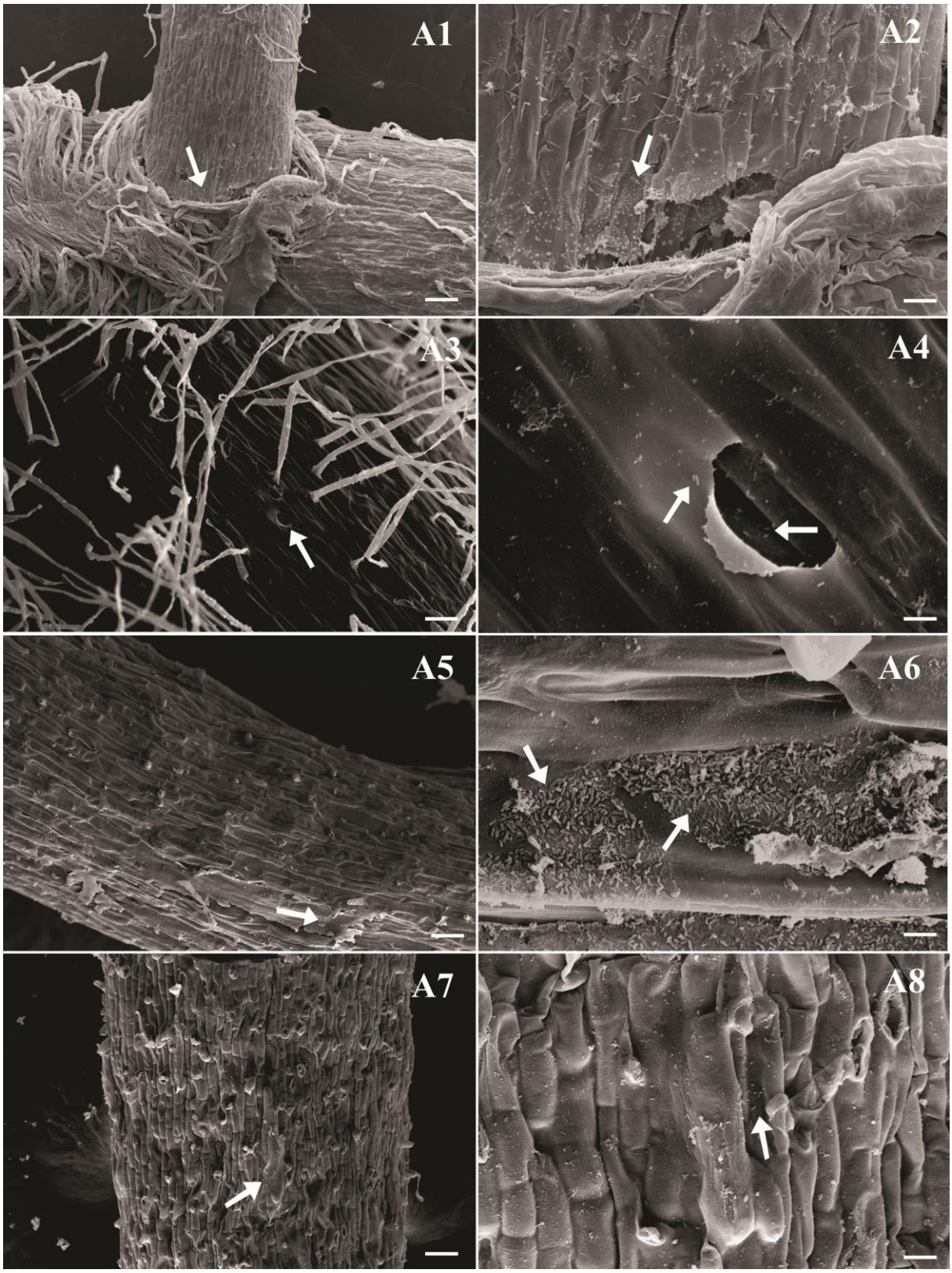


Figure 8. Roots colonization of disinfected maize seeds inoculated with syncom (A). Bacterial cells were visualized by SEM. Root regions: lateral root (A1 and A2), root hair (A3 and A4), zone of elongation (A5 and A6) and root cap (A7 and A8). Biofilms and small bacterial aggregates are indicated by white arrows. Bars represent the following scales: panel A4 and A6: 10 µm; A2 and A8: 20 µm; A3, A5 and A7: 100 µm; A1: 200 µm.

Analysis of the stored reserve mobilization in germinated seeds (Fig. 9) was accessed in three seed to seedling developmental stages. No germinated imbibed seeds (called Seed), an earlier stage of germination where embryonic axis emitted visible radicle protuberance (called Embryonic axis) and the later stage of germination where roots were emerged (called Roots). Generally, there is no effect of the disinfection (partial bacteriome remotion) in the early stages of reserve mobilization (seeds and embryonic axis). We notice a positive effect of the syncom (DS + MIX) on protein remobilization compared to DS. Also, the degradation of triglycerides was higher in the roots of the NDS treatment compared to DS + MIX for the embryo region.

Great accumulation of protein, glucose, triglycerides and reducing sugars contents was observed in the root tissue when the seed was previously disinfected concerning to NDS (Fig. 9 A-D). Such accumulation may suggest an altered pattern of catabolic energy conversion and decreased anabolic conversion of monomers units in polymeric structures to fulfil demanding cellular structures to accommodate the root growth. Supposedly, glucose was quickly driven to respiration or cell wall formation in the NDS-treatment.

When we inoculated the syncom (SD + MIX), a more efficient reserve mobilization for reducing sugars was noticed concerning to DS, but not so efficient than NDS-treatment. The rapid mobilization of reserves in NDS resulted in a low content of these sugar classes.

The activity of the alpha-amylase enzyme did not change between treatments in the seed compartment. In the region of the embryonic axis, the enzyme reached higher activity with 24/48 h of germination, with no activity modulation by disinfection and syncom inoculation. Removing part of the maize microbiota increased the activity of alpha-amylase in the roots. The same was observed for the DS + MIX (Fig. 9E).

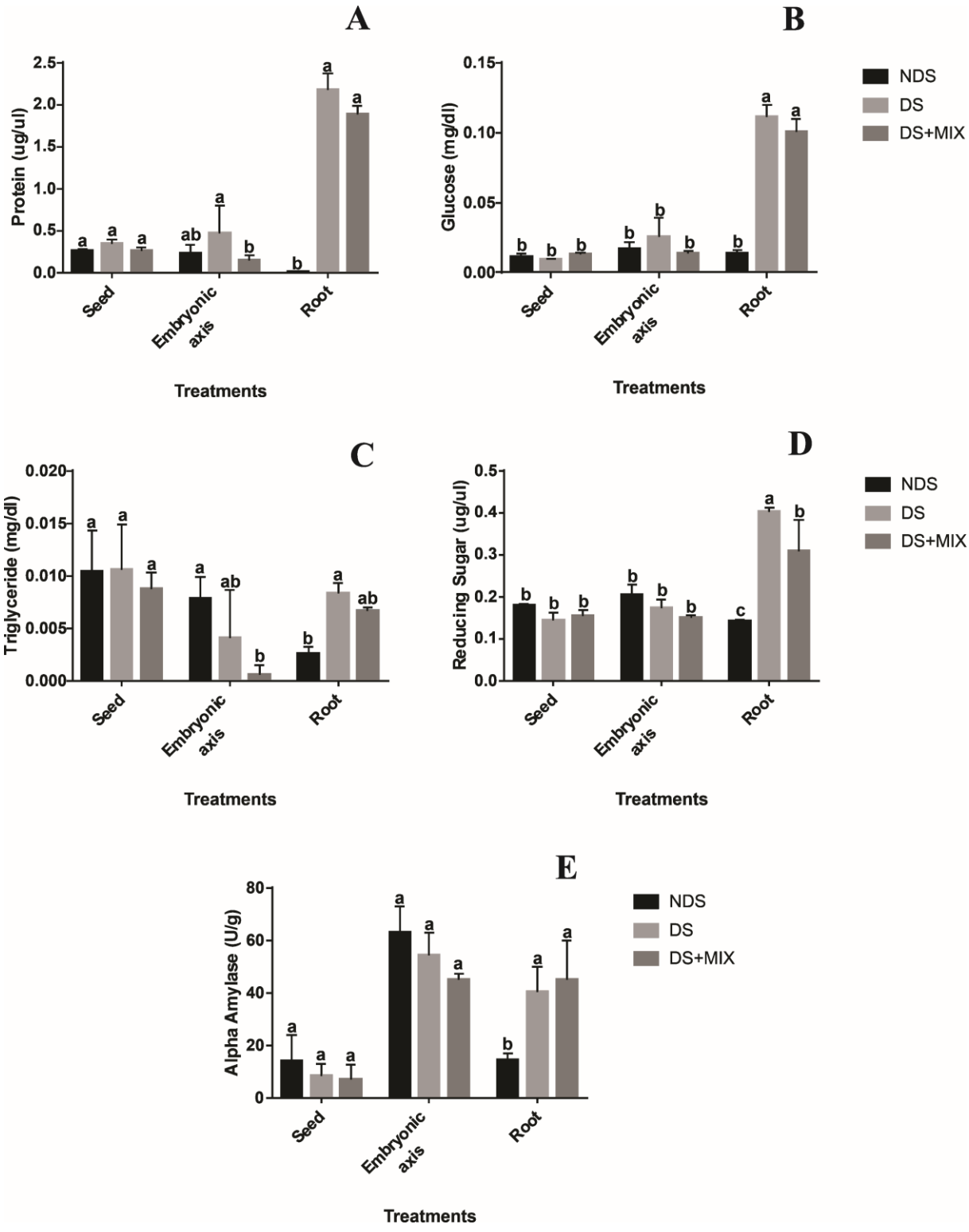


Figure 9. Quantification of protein (A), glucose (B), triglyceride (C), reducing sugar (D), and alpha-amylase activity (E) in different compartments of maize; disinfected or not. *Significant difference between treatments according to the Tukey test ($p \leq 0.05$).

DISCUSSION

The present work unravels the bacterial community structure from maize-root seedlings under the gnotobiotic system. Seed-disinfection with sodium hypochlorite was performed, aiming to reduce the natural seed microbial inhabitants to access the functional roles of the seed-bacteriome. The comparative analysis emphasizes the importance of the bacterial microbiome to seedlings growth rates and stored reserve dynamic mobilization.

The multiple events of germination begin after water is absorbed by the seed and is completed with the radicle emission, a structure that will give rise to a differentiated root (Bewley 1997). During this process, not only the seeds but also its microbiome, change from a quiescent state to an active physiological state. According to Frank *et al*. (2017), the seeds exude carbon in the form of sugars, proteins, and fatty acids, which act as an energy source for microorganisms and have the potential to shape the bacterial composition around the seed. In response to the availability of nutrients released from the seed or water, microbial cells leave their dormant state and are transferred to seedlings and various organs of mature plants (Nelson *et al*. 2018), where they can have direct consequences on their germination and growth.

The contribution of the microbiome to plant physiology is still not entirely understood. Herein, we demonstrated that the partial removal of seed-borne bacteria by rigorous disinfection of maize seeds in sodium hypochlorite significantly reduced the seed germination speed and seedling growth. This result can be justified by the hypochlorite's antimicrobial action, which killed relevant bacteria groups modulating germination and growth processes. Increased seed-disinfection time reduces bacterial population communities and eliminates certain species lowering seedlings germination. Similar results were found by Verma and White (2018) and Verma *et al*. (2018) when increasing the sterilization time in NaOCl from 20 to 40 min. In similar studies, seedling growth was also restricted by removing epiphytic and endophytic microorganisms from seeds through superficial sterilization with NaClO, use of antibiotics (Verma and White 2018; Verma *et al*. 2018; Verma *et al*. 2017) or heat treatment (Holland 2016; Holland 2019).

Our data revealed that the disinfection of seeds with hypochlorite (even for a long time as 90 minutes) does not guarantee the complete elimination of microorganisms, especially those living inside the seed. In roots emerged from untreated-seeds, bacterial cells were viewed in the microscope as aggregates and biofilms as observed by SEM technique. Since the cultivation system was axenic, the bacterial community described come from the seed

itself and can proliferate during seed germination and seedling development. Thus, it is claimed that part of the initial bacterial community of maize seedlings, detected by microscopy, originates from the seed itself, by vertical transfer (Frank *et al*. 2017; Johnston-Monje *et al*. 2016).

For disinfected seeds, only surface-living resistant microbes or inhabitant of inner tissues were able to colonize emerged primary roots. In this case, the colonization pattern was affected, with reduced bacteria density, mainly viewed as single cells or small groups with cell morphology distinct from the control (natural seed inhabitants). Surface disinfection methods usually carried out with ethanol and sodium hypochlorite, eliminate, or inactivate mainly epiphytic microorganisms from the seed, but not those occupying endophytic sites.

The antimicrobial effect of the hypochlorite was demonstrated by the absence of detectable diazotrophic bacterial population and a reduced number of total bacteria associated with emerged roots from disinfected seeds. In control, due to the absence of hypochlorite, the bacterial count was high for all the culture media used. On the other hand, the high quantification of bacteria at the root of the disinfected treatment by qPCR can be explained by the niche theory, which predicts that the microbial diversity of plants is determined by the number of available niches (Hardoim 2015). In this study, the disinfection of seeds “vacated” niches in the maize root, which started to be colonized by other bacteria (endophytic or resistant to hypochlorite).

Cultivation-dependent approach and cultivation-independent sequencing by Illumina MiSeq revealed similar trends into the bacteriome shift for roots from NDS and DS. The root sequencing analyses revealed high bacterial diversity by the Shannon index when the hypochlorite removed part of its bacteriome. Diversity values were lower in non-disinfected seeds. We have to look carefully for this result. Disinfection procedure could eliminate or substantially reduce the dominant taxon (high OTU abundance), mainly those occupying more superficial niches. Such effect allowed sampling and detection of low abundant OTU that harbour more protected seed niches. Thus, what we measure is an apparently increased diversity related to the experimental conditions.

Among the bacteria groups associated with emerged maize plantlets radicles, the genus *Burkholderia* was the most affected by the hypochlorite treatment. In contrast, roots that showed higher growth rates (NDS treatment) were abundantly colonized by *Burkholderia*. This genus was defined by Yabuuchi *et al*. (1992) and had inhabited broader ecological niches (Coenye and Vandamme 2003). *Burkholderia* species can colonize diverse

plant host, and thus promote their growth and protection against pathogens (Tagele *et al*. 2018; Tagele *et al*. 2019). *Burkholderia* abundance has been reported in maize and other plants (Johnston-Monje *et al*. 2016; Mashiane *et al*. 2017; Rosenblueth *et al*. 2010), being able to fix nitrogen, solubilizing phosphate, producing IAA (indole-acetic acid; auxin) and siderophores (Batista *et al*. 2018). Genomic studies had shown several genes related to the biosynthesis of IAA, ACC (1-aminocyclopropane-1-carboxylate) deaminase, dioxygenases, multiple efflux pumps, degradation of aromatic compounds, and diverse protein secretion systems (Dias *et al*. 2019). Some of these genetic traits may have contributed to the growth of seedlings with *Burkholderia* in its bacteriome.

Twelve bacteria isolated from the non-disinfected seeds were used to compose a synthetic bacterial community aiming to evaluate the microbiota recomposition phenotype. Nine from twelve bacterial isolates obtained were identified as belonging to the *Burkholderia* genus. After inoculation in disinfected seeds, the germination and growth of the maize seedlings were recovered, being comparable to the development rates of seeds that were not treated with hypochlorite. Bacteria counts and microscopy analysis confirmed the partial rebuilding of the microbiota. In similar studies, it has been found that removing the microbiota from seeds has reduced the germination and development of rice, soybeans, beans, and millet (Verma and White 2018; Verma *et al*. 2018). Holland (2019) used thermotherapy at 50 ºC for 48 h for seed disinfection on soybean seeds and demonstrated a population reduction of *Methylobacterium* by 3% associated with reduced germination and abnormal seedling growth. All of the growing and developmental plant traits were restored after the reintroduction of removed bacterial community part. Collectively, these studies suggest that the germination and growth rates of seedlings can be positively modulated, at least in part, by the bacteriome associated with seeds. The increased seedling fitness can be related to the production of phytohormones, synthesis of other metabolites and alleviation of damage caused by pathogens, through the production of antifungal and antibacterial compounds.

The plantlets growth performance can be partially associated with the bacteriome. The underlined mechanism can be, in part, related to the positive modulation of seed reserve mobilization during germination. Thus, for root from non-disinfected maize seeds, protein, glucose, and triglyceride reserve degradation is more efficient than when the associated microbiota was partially removed. The low content of reducing sugars and the increase of the activity of the alpha-amylase enzyme in the embryonic axis supports rapid plant growth in intact and restored microbiota. On the other hand, the disinfection of the seeds removed part

of the microbiota and promoted late mobilization of its biomolecule reserves, evidenced by the accumulation of protein, glucose, triglyceride, and reducing sugar in the root, in addition to high alpha-amylase activity in the post-germination phase. Late mobilization of reserves affected maize growth. Similar results were found by Zhu *et al*. (2017), where *Ammodendron bifolium* endophytic bacteria increased the efficiency of endosperm use, including more significant degradation of sucrose, protein, and triglyceride from the seed, in addition to the production of hydrolytic enzymes by these bacteria. For Verma *et al*. (2017), seed bacteria can express the enzyme amylase and increase the efficiency of endosperm use during germination and seedling growth. The idea that the microbiota can mobilize nutrients from the endosperm to the embryo is supported by the presence of bacteria in these regions, located by scanning microscopy.

Overall results point that even after the removal of part of seed-borne bacteria, similar population levels were detected by qPCR in roots from disinfected and non-disinfected germinated seeds. Therefore, the remaining bacteriome from disinfected-seeds exhibited selective removal of prevalent fast-grown groups and specific taxon. Then, under less competition, the remaining bacteria can move to the root and colonize it during germination when the seed releases several metabolites that shape the bacterial composition (Frank *et al*. 2017). This fact was proven by detecting *Enterobacteriaceae*, *Acinetobacter*, *Pseudomonas*, *Lactobacillus*, and *Burkholderia* in maize seeds and roots by amplicon sequencing. SEM also confirmed the migration of the remaining bacteria from seeds to radicle. However, the population size and diversity of these bacteria in the treatments are distinct. As quoted before, in disinfected treatment, the root bacteriome is more diverse, but this diversity does not include or contemplates a reduced number of bacteria that promote germination and growth, which negatively affected the development of these seedlings.

On the other hand, the natural microbiome of maize roots is composed of more dominant groups and less diverse bacterial taxa, which act positively in the efficient mobilization of seed reserves and processes of biostimulation and biofertilization. It can be inferred from the sequencing results that bacteria of the genus *Burkholderia* play a significant role in these processes since the partial rebuild of the microbiota with members of this genus has recovered maize germination and plant growth processes. Whatever the underlined mechanism displayed, it is evident that physiological processes during germination and seedling growth of maize SHS 5050 seeds were modulated by seed-borne bacteriome. However, the consequences of such change for different maize genotypes must be evaluated

case by case due to its effect over the seed-borne microbial structure. In this study, results from cultivable approaches (population counts and isolation) and non-cultivation methods suggest the following ascending order variation: seed from the same cob < cobs from same maize plant < seed from different maize plants (same genotypes) and higher variation when comparing different genotypes. The challenger is to gain accumulative insights in understanding the microbial community changes and take advantage of it to maximise plant growth and plant health.

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REFERENCES

Adam E, Bernhart M, Müller H *et al.* The Cucurbita pepo seed microbiome: genotype-specific composition and implications for breeding. *Plant and Soil* 2016;**422**: 35-49.

Anderson MJ. Permutational multivariate analysis of variance (PERMANOVA). *Wiley state ref: statistics reference online* 2014, DOI 10.1002/9781118445112.stat07841: 1-15.

Ausubel F, Brent R, Kingston R *et al.* Preparation of genomic DNA from bacteria. *Current protocols in molecular biology John Wiley Sons, Inc, New York, NY* 1990, DOI https://doi.org/10.1002/0471142727.mb0204s56.

Bakker MG, Chaparro JM, Manter DK *et al.* Impacts of bulk soil microbial community structure on rhizosphere microbiomes of *Zea mays*. *Plant and Soil* 2015;**392**: 115-26.

Baldani JI, Reis VM, Videira SS *et al.* The art of isolating nitrogen-fixing bacteria from non-leguminous plants using N-free semisolid media: a practical guide for microbiologists. *Plant and Soil* 2014;**384**: 413-31.

Batista BD, Lacava PT, Ferrari A *et al.* Screening of tropically derived, multi-trait plant growth-promoting rhizobacteria and evaluation of corn and soybean colonization ability. *Microbiological Research* 2018;**206**: 33-42.

Baudoin E, Benizri E, Guckert A. Impact of artificial root exudates on the bacterial community structure in bulk soil and maize rhizosphere. *Soil Biology and Biochemistry* 2003;**35**: 1183-92.

Bewley JD. Seed germination and dormancy. *The Plant Cell* 1997;**9**: 1055.

Bodhankar S, Grover M, Hemanth S *et al.* Maize seed endophytic bacteria: dominance of antagonistic, lytic enzyme-producing *Bacillus* spp. *3 Biotech* 2017;**7**: 232.

Bulgarelli D, Rott M, Schlaeppi K *et al.* Revealing structure and assembly cues for *Arabidopsis* root-inhabiting bacterial microbiota. *Nature* 2012;**488**: 91-5.

Caporaso JG, Kuczynski J, Stombaugh J *et al.* QIIME allows analysis of high-throughput community sequencing data. *Nature methods* 2010;**7**: 335.

Caporaso JG, Lauber CL, Walters WA *et al.* Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *The ISME journal* 2012;**6**: 1621-4.

Chen D-H, Ronald P. A rapid DNA mini preparation method suitable for AFLP and other PCR applications. *Plant Molecular Biology Reporter* 1999;**17**: 53-7.

Coenye T, Vandamme P. Diversity and significance of *Burkholderia* species occupying diverse ecological niches. *Environmental Microbiology Reports* 2003;**5**: 719-29.

De Gregoris TB, Aldred N, Clare AS *et al.* Improvement of phylum-and class-specific primers for real-time PCR quantification of bacterial taxa. *Journal of Microbiological Methods* 2011;**86**: 351-6.

Dias GM, de Sousa Pires A, Grilo VS *et al.* Comparative genomics of *Paraburkholderia kururiensis* and its potential in bioremediation, biofertilization, and biocontrol of plant pathogens. *Microbiology Open* 2019;**8**: e00801.

Doyle J, Doyle J. Isolation of plant DNA from fresh tissue. *Focus* 1990;**12**: 13-5.

Fernandes AD, Reid JN, Macklaim JM *et al.* Unifying the analysis of high-throughput sequencing datasets: characterizing RNA-seq, 16S rRNA gene sequencing and selective growth experiments by compositional data analysis. *Microbiome*. 2014;**2**: 15.

Frank AC, Saldierna Guzman JP, Shay JE. Transmission of Bacterial Endophytes. *Microorganisms* 2017;**5**.

Fernandes AD, Reid JN, Macklaim JM *et al.* Unifying the analysis of high-throughput sequencing datasets: characterizing RNA-seq, 16S rRNA gene sequencing and selective growth experiments by compositional data analysis. *Microbiome*. 2014;**2**: 15.

Hardoim PR, Hardoim CC, van Overbeek LS *et al.* Dynamics of seed-borne rice endophytes on early plant growth stages. *PloS one* 2012;**7**: e30438.

Holland MA. Probiotics for Plants? What the PPFMs told us and some ideas about how to use them. *Journal of the Washington Academy of Sciences* 2016;**102**: 31-42.

Holland MA. Thinking About PPFM Bacteria as a Model of Seed Endophytes: Who Are They? Where Did They Come from? What Are They Doing for the Plant? What Can They Do for Us? *Seed Endophytes*, DOI 10.1007/978-3-030-10504-4: Springer, 2019, 21-34.

Johnston-Monje D, Lundberg DS, Lazarovits G *et al.* Bacterial populations in juvenile maize rhizospheres originate from both seed and soil. *Plant and Soil* 2016;**405**: 337-55.

Johnston-Monje D, Mousa WK, Lazarovits G *et al.* Impact of swapping soils on the endophytic bacterial communities of pre-domesticated, ancient and modern maize. *BMC Plant Biology* 2014;**14**: 233.

Liu Y, Wang R, Li Y *et al.* High-throughput sequencing-based analysis of the composition and diversity of endophytic bacterial community in seeds of "Beijing" hybrid maize planted in China. *Plant Growth Regulation* 2017;**81**: 317-24.

Maguire J. Speed of germination aid in selection and evaluation for seedling emergence and vigor. *Crop Science* 1962;**2**: 176-7.

Mashiane RA, Ezeokoli OT, Adeleke RA *et al.* Metagenomic analyses of bacterial endophytes associated with the phyllosphere of a Bt maize cultivar and its isogenic parental line from South Africa. *World Journal of Microbiology* 2017;**33**: 80.

McMurdie PJ, Holmes S. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PloS one* 2013;**8**.

Nelson EB. Microbial dynamics and interactions in the spermosphere. *Annual review of phytopathology* 2004;**42**: 271-309.

Nelson EB. The seed microbiome: Origins, interactions, and impacts. *Plant and Soil* 2017;**422**: 7-34.

Nelson EB, Simoneau P, Barret M *et al.* Editorial special issue: the soil, the seed, the microbes and the plant. *Plant and Soil* 2018;**422**: 1-5.

Oksanen J, Blanchet FG, Kindt R *et al*. Vegan: Community Ecology Package. R Package Version 2.0-8, 2013. http://CRAN.R-project.org/package=vegan (access on 30 March 2020).

Quast C, Pruesse E, Yilmaz P *et al.* The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *J Nucleic Acids Research* 2012;**41**: D590-D6.

Rijavec T, Lapanje A, Dermastia M *et al.* Isolation of bacterial endophytes from germinated maize kernels. *Canadian Journal of Microbiology* 2007;**53**: 802-8.

Rosenblueth M, López-López A, Martínez J *et al.* Seed bacterial endophytes: common genera, seed-to-seed variability and their possible role in plants *XXVIII International Horticultural Congress on Science and Horticulture for People (IHC2010): International Symposium on 938*, 2010, 39-48.

Tagele SB, Kim SW, Lee HG *et al.* Effectiveness of multi-trait *Burkholderia contaminans* KNU17BI1 in growth promotion and management of banded leaf and sheath blight in maize seedling. *Microbiological Research* 2018;**214**: 8-18.

Tagele SB, Kim SW, Lee HG *et al.* Potential of novel sequence type of *Burkholderia* cenocepacia for biological control of root rot of maize (*Zea mays* L.) caused by fusarium temperatum. *International Journal of Molecular Sciences* 2019;**20**: 1005.

Truyens S, Weyens N, Cuypers A *et al.* Bacterial seed endophytes: genera, vertical transmission and interaction with plants. *Environmental Microbiology Reports* 2015;**7**: 40-50.

Verma S, White J. Indigenous endophytic seed bacteria promote seedling development and defend against fungal disease in browntop millet (*Urochloa ramosa* L.). *Journal of Applied Microbiology* 2018;**124**: 764-78.

Verma SK, Kingsley K, Bergen M *et al.* Bacterial endophytes from rice cut grass (*Leersia oryzoides* L.) increase growth, promote root gravitropic response, stimulate root hair formation, and protect rice seedlings from disease. *Plant and Soil* 2018;**422**: 223-38.

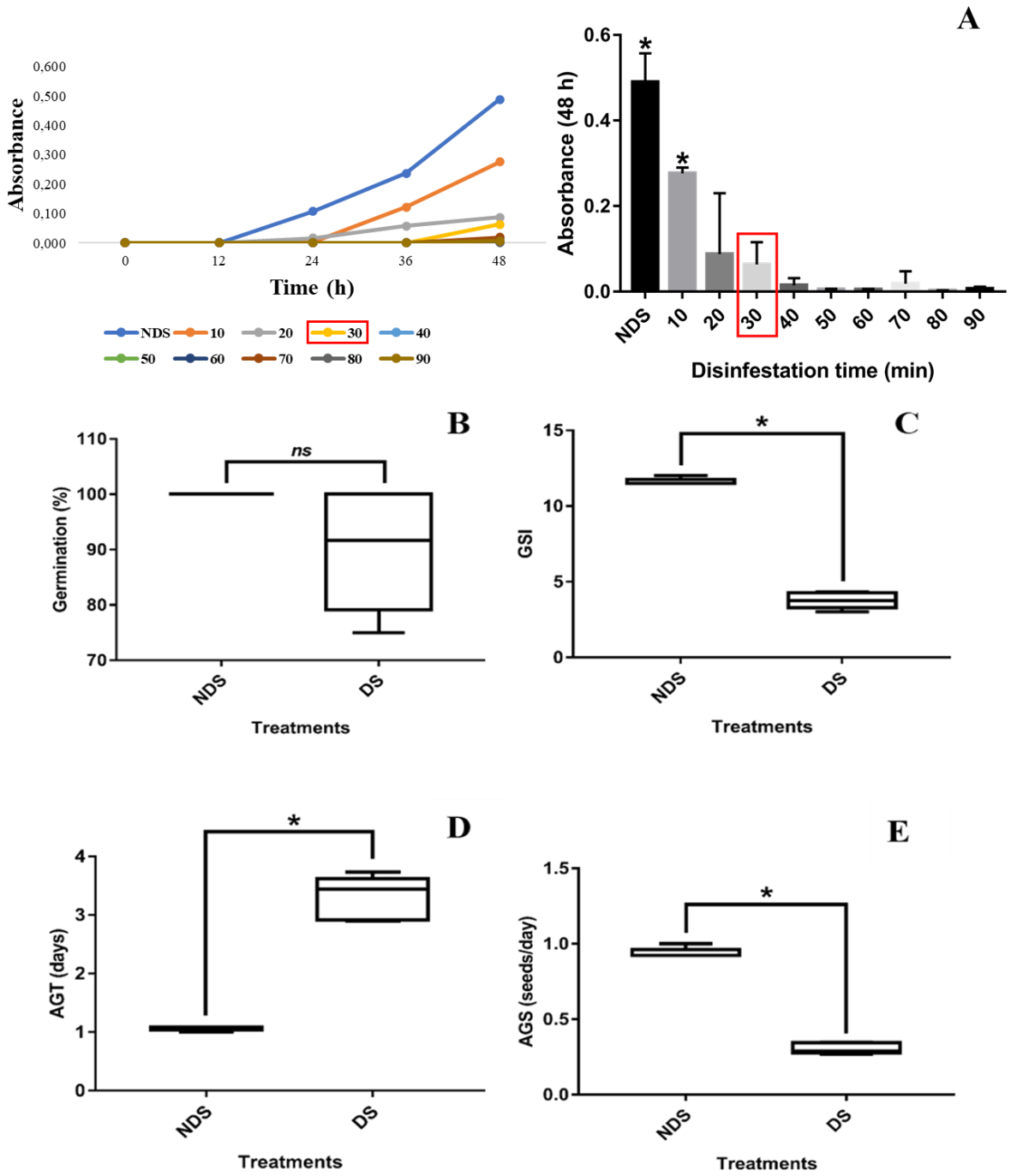
Verma SK, Kingsley K, Irizarry I *et al.* Seed vectored endophytic bacteria modulate development of rice seedlings. *Journal of Applied Microbiology* 2017;**122**: 1680-91. Wilfinger WW, Mackey K, Chomczynski P. Effect of pH and ionic strength on the spectrophotometric assessment of nucleic acid purity. *Biotechniques* 1997;**22**: 474-81.

Yabuuchi E, Kosako Y, Oyaizu H *et al.* Proposal of *Burkholderia* gen. nov. and transfer of seven species of the genus *Pseudomonas* homology group II to the new genus, with the type species *Burkholderia* cepacia (Palleroni and Holmes 1981) comb. nov. *Microbiology Open* 1992;**36**: 1251-75.

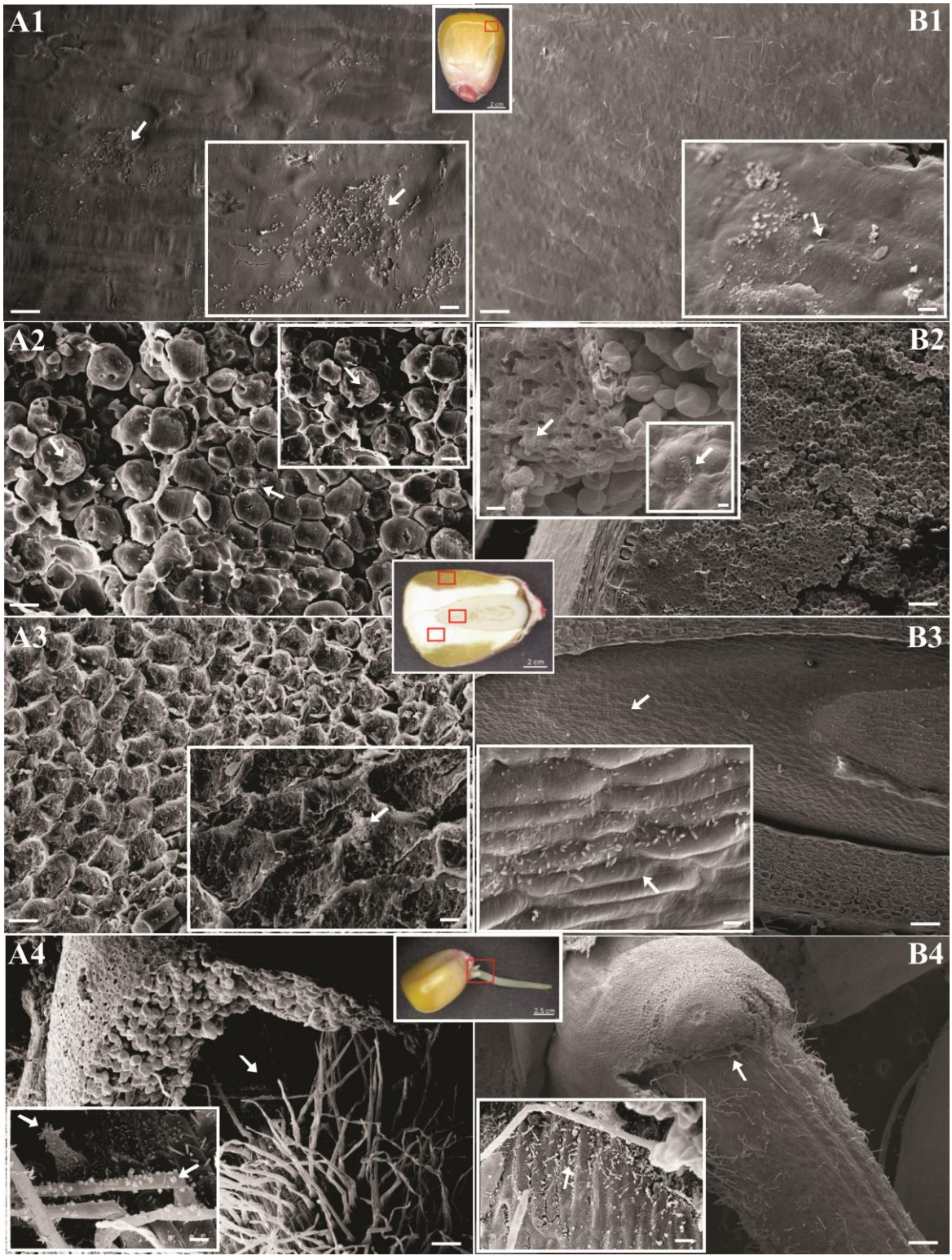
Zhu Y-L, She X-P, Wang J-S *et al.* Endophytic bacterial effects on seed germination and mobilization of reserves in *Ammodendron biofolium*. *Pakistan Journal of Botany* 2017;**49**: 2029-35.

SUPPLEMENTARY DATA

SD. 1. Effect of disinfection time (0, 10, 20, 30, 40, 50, 60, 70, 80, 90 minutes) with sodium hypochlorite 1.25% on seed-borne bacterial population size on non-germinated seeds (A) measured as optical density increase over the time, and germination parameters associated with maize seeds (B-E). The time of 30 minutes had shown significant bacterial population reduction without affect germination performance, and it was selected for further assays.



SD. 2. Colonization of maize seeds by its microbiota. Bacterial cells were visualized by scanning electron microscopy (SEM). Maize seeds non-disinfected (A) and disinfected (B). Seed regions: pericarp (A1 and B1), endosperm (A2 and B2), embryo (A3 and B3), and radicle (A4 and B4). White arrows indicate biofilms and small bacterial aggregates. Bars represent the following scales: panel A1 and A2: 20 µm; A3 and B1: 20 and 10 µm; A4 and B4: 200 and 20 µm; B2: 100 and 20 µm; B3: 100 and 2 µm.



SD. 3. Bacterial isolates obtained from seed and maize root microbiota (*Zea mays* L.) variety SHS 5050 that was used to compose the synthetic community (syncom) for further studies of the partial rebuilding of the seed-borne bacteria microbiota.

Bacteria strain designation	Origin
LMS 3b	NB NDS R2 -5
LMS 4	NB NDS R2 -6
LMS 5	NB NDS R2 -6
LMS 7	NB NDS R2 -6
LMS 8	NB NDS R2 -6
LMS 10	NB NDS R2 -6
LMS 23	NDS NB overnight R2 -4
LMS 25b	NDS NB overnight R2 -6
LMS 73b1	LGI NDS R2 -6 (-7)
LMS 77	LGI NDS R4 -5 (-7)
LMS 81	JNFb NDS R1 -4 (-7)
LMS 99	JNFb NDS R4 -6 (-7)

Culture media used for isolation: NB solid medium and JNFb and LGI semi-solid medium; Non-disinfected seed (NDS); the number of experimental unit replication and dilution obtained (RX - X).

SD 4. Identification by sequencing the 16S rRNA after BLAST in the NCBI database of the maize seed-borne bacteria isolates used to compose the synthetic community (syncom).

Isolate	Isolate identified by 16S rRNA gene sequence	Access number	Identity (%)	E value	Query cover (%)	Score
3b	*Staphylococcus sp.* strain Firmi-16	MH683105.1	99.59	0.00	91	1332
4	*Burkholderia sp.* strain Beta-30	MH698880.1	99.57	0.00	100	1279
5	*Burkholderia gladioli* strain E37CS3	MK474979.1	99.87	0.00	100	1413
7	*Burkholderia sp.* strain BIS1062	MN810235.1	99.87	0.00	100	1408
8	*Burkholderia gladioli* strain 16BC	MK474977.1	100	0.00	100	1232
10	*Burkholderia gladioli* strain T3.3.1	MT001453.1	99.85	0.00	100	1247
23	*Bacillus drentensis* strain CD3	MK216756.1	100	0.00	100	1330
25b	*Bacillus camelliae* strain 7578-1	NR_159341.1	99.72	0.00	98	1321
73b1	*Burkholderia gladioli* strain E37CS3	MK474979.1	100	0.00	100	1291
77	*Burkholderia gladioli* strain TWS (19)-Abf_48Ba	MN049500.1	100	0.00	99	1293
81	*Burkholderia sp.* strain Beta-30	MH698880.1	100	0.00	100	1291
99	*Burkholderia gladioli strain* TWS (19)-Abf_48Ba	MN049500.1	100	0.00	100	1293

The identification of the twelve bacteriome selected isolates (SD. 4) was carried out by sequencing the 16S rRNA gene. For this, the bacteria were cultured in NB liquid medium (180 rpm, for 24-48 h at 30 ºC) and resuspended by centrifugation (5.000 rpm, for 10 min). The total DNA was extracted as described previously (CTAB method) and quantified in a NanoDrop 2000® spectrophotometer (Thermo Scientific). Subsequently amplified by PCR using the following reaction: 1 µL of genomic DNA (100 ng); 5 µL of buffer (10x Buffer, Invitrogen), five µL of $MgCl_2$, one µL of dNTP (10 µM), one µL of each primer (10 µM; 27F: AGAGTTTGATCCTGGCTCAG; 1492R: GGTTACCTTGTTACGACTT), 0.5 µL of Taq polymerase enzyme and water (35.5 µL), totalling 50 µL of reaction. The mixture was incubated in a thermocycler with the following conditions: initial

denaturation of 5 min at 95 ºC, 35 cycles of 95 ºC for 30 s, 55 ºC for 2:15 min and 72 ºC for 1:15 min. The PCR product was quantified, and the amplification confirmed on an agarose gel (1.5%) submitted to electrophoresis (90 V for 90 min).

The PCR products were purified with the Exo-SAP kit according to the manufacturer's description and sequenced on the ABI PRISM 3500 Genetic Automatic analyser sequencer (Applied Biosystems) using the commercial cycle Big Dye® Terminator Sequencing Kit v3.1 from Thermo Fisher Scientific (Cat. No 4333456). The amplicons were sequenced at both ends, using the same pair of PCR primers. The sequences of the 16S rRNA genes were analysed and assembled using the program BioEdit version 7.1.9 (Hall, 1999). The sequences were then aligned and compared to those deposited in the GenBank database using the BLAST program (Altschul et al., 1997). The sequences were deposited in the NCBI database under submission code SUB8322664.

SD 5. Distribution of reads by root sample of germinated disinfected (yes) and no disinfected seeds (no) of maize variety SHS 5050.

X.Sample ID	Genotype	Extraction	Disinfection	Treatment	Total Reads	Coverage
O12	SHS5050	CTAB	No	R_SHS5050_ND_CTAB	64695	0.9944354
O11	SHS5050	CTAB	No	R_SHS5050_ND_CTAB	23212	0.9912545
O4	SHS5050	DNAZol	No	R_SHS5050_ND_DNAZol	49737	0.9941291
O2	SHS5050	DNAZol	Yes	R_SHS5050_D_DNAZol	1248	0.9551282
O8	SHS5050	CTAB	Yes	R_SHS5050_D_CTAB	118	0.6949153
O9	SHS5050	CTAB	Yes	R_SHS5050_D_CTAB	144	0.5555556
O5	SHS5050	DNAZol	No	R_SHS5050_ND_DNAZol	64020	0.9946892
O3	SHS5050	DNAZol	Yes	R_SHS5050_D_DNAZol	263	0.8365019
O6	SHS5050	DNAZol	No	R_SHS5050_ND_DNAZol	42802	0.9939022
O10	SHS5050	CTAB	No	R_SHS5050_ND_CTAB	3686	0.9750407
O7	SHS5050	CTAB	Yes	R_SHS5050_D_CTAB	148	0.7229730
O1	SHS5050	DNAZol	Yes	R_SHS5050_D_DNAZol	230	0.8391304

SD 6. Permutational Multivariate Variance Analysis (Permanova) of the root bacterial community structure of maize geminated seeds variety SHS 5050. The disinfection factor was significant (p = 0.006), contributing 36% of the variation.

	Df[a]	Sums of Sqs[b]	Mean Sqs[c]	F. Model[d]	R2	Pr(>F)[e]
Disinfection	1	1.563833	1.5638332	5.719781	0.3638588	0.006
Residuals	10	2.734079	0.2734079	NA	0.6361412	NA
Total	11	4.297912	NA	NA	1.0000000	NA

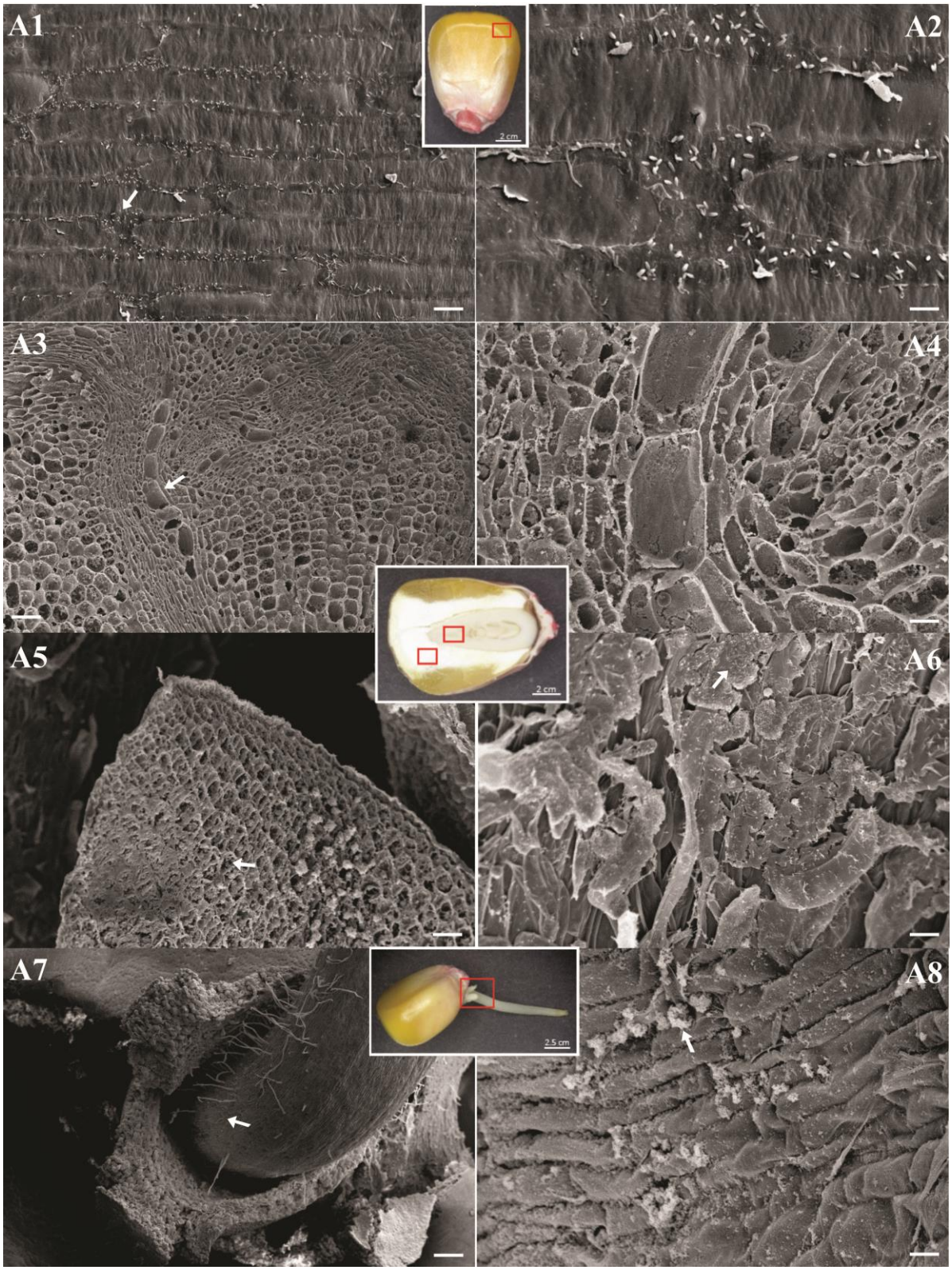
[a]Df: degrees of freedom
[b]Sum of Sqs: sequential sums of squares
[c]Mean Sqs: mean squares
[d]F. Model: F statistics
[e]Pr>(F): partial R-squared and P values

SD 7. Differential abundance of roots associated OTU of maize plantlets variety SHS 5050 from disinfected (DS) *versus* non-disinfected seeds (NDS).

rab.all[a]	rab.win.R_ Root_DS[b]	rab.win.R_ Root_NDS[c]	effect	overlap	we.ep[d]	Family	Genus
14.3029290	8.2025510	16.6679985	3.6336860	0.0003653998	0.0008595304	f_Burkholderiaceae	*g_Burkholderia*
1.0463097	5.3631248	-0.4587697	-2.1410903	0.0052604506	0.0065642852	f_Oxalobacteraceae	*NA*[*]
4.2163306	7.3515686	3.0277565	-1.9246204	0.0003653998	0.0092850702	f_Alcaligenaceae	*NA*[*]
6.6462458	2.1751503	8.8878914	2.4701196	0.0003653998	0.0215379059	f_Burkholderiaceae	*g_Burkholderia*
2.2337604	4.2573698	0.5699101	-1.4411406	0.0518185718	0.0271257626	f_Corynebacteriaceae	*g_Corynebacterium*
8.2251787	2.1263053	10.6305670	2.6413734	0.0003653998	0.0274517615	f_Burkholderiaceae	*g_Burkholderia*
7.3891091	1.7284770	9.3867903	2.7051190	0.0003653998	0.0297697949	f_Burkholderiaceae	*g_Burkholderia*
3.5135809	5.1803706	2.8285070	-1.8998283	0.0259172269	0.0299101109	f_Staphylococcaceae	*g_Staphylococcus*
8.1816592	2.1437384	10.3636854	2.6321218	0.0003653998	0.0353067527	f_Burkholderiaceae	*g_Burkholderia*
6.1747742	0.4890293	6.5897098	1.8652017	0.0104432569	0.0358160707	f_Burkholderiaceae	*g_Burkholderia*
0.9485628	3.6297661	-0.6027736	-1.2680380	0.0466378252	0.0382945172	f_Microbacteriaceae	*g_Cryocola*
5.7504276	0.5893332	6.3364622	1.7958206	0.0207385644	0.0456794252	f_Burkholderiaceae	*g_Burkholderia*
6.1834278	0.2673052	6.6261919	1.8131076	0.0103891482	0.0515493973	f_Burkholderiaceae	*g_Burkholderia*
2.7629464	4.3992226	1.2307604	-0.8633717	0.0937527019	0.0536003332	f_Sinobacteraceae	*NA*[*]

[a] rab.all - median clr value for all samples in the feature
[b] rab.win.No - median clr value for the group of non-disinfected samples
[c] rab.win.Yes - median clr value for the group of disinfected samples
[d] we.ep - Expected P-value of Welch's t-test
[*] NA = unassigned

SD 8. Colonization of disinfected non-germinated seeds of maize with syncom (A). Bacterial cells were visualized by SEM. Seed regions: pericarp (A1 and A2), endosperm (A3 and A4), embryo (A5 and A6), and early emerged radicle (A7 and A8). White arrows indicate biofilms and small bacterial aggregates. Bars represent the following scales: panel A2, A6, and A8: 10 µm; A1 and A4: 20 µm; A3 and A5: 100 µm; A7: 200 µm.



CHAPTER 3:

Altered bacteria community dominance reduces tolerance to resident fungus and seed to seedling growth performance in maize (*Zea mays* L. var. DKB 177)

Chapter 3: Manuscript published

Altered bacteria community dominance reduces tolerance to resident fungus and seed to seedling growth performance in maize (*Zea mays* L. var. DKB 177)

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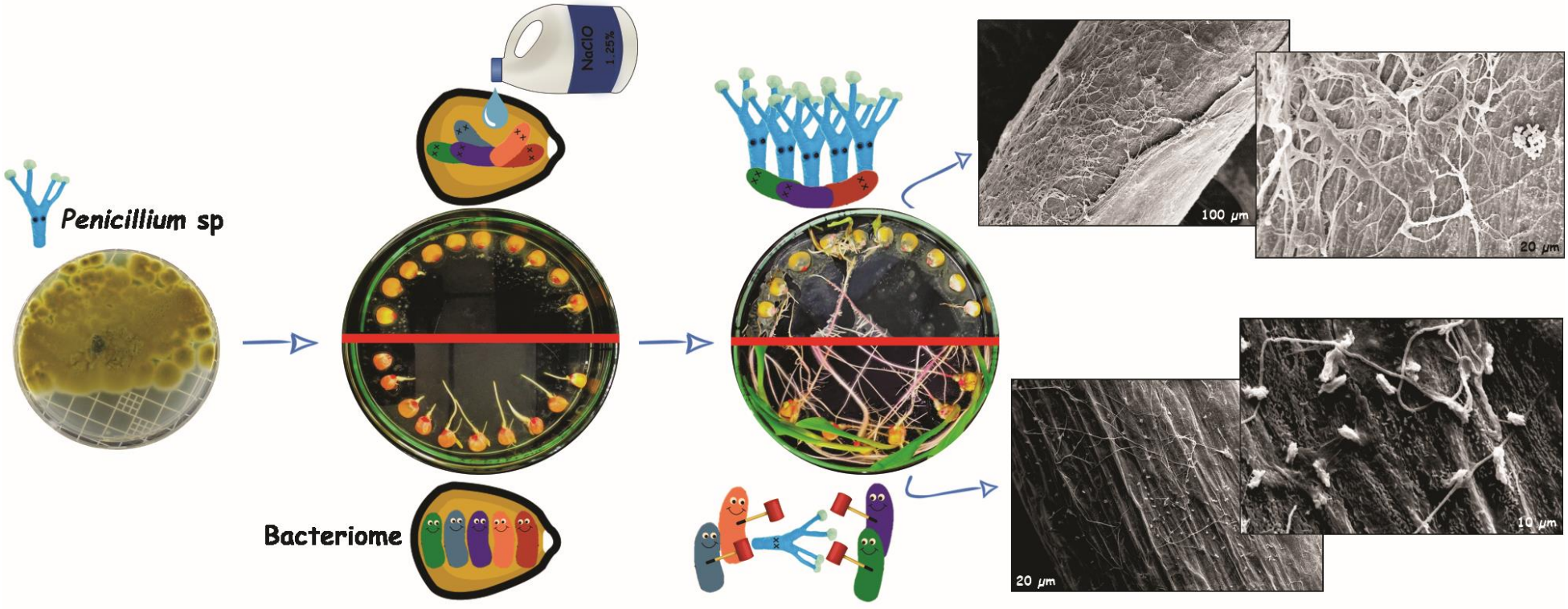
RESUMO

As sementes são reservatórios de micro-organismos benéficos e prejudiciais que modulam o crescimento e a saúde das plantas. Neste estudo, acessamos a montagem do bacterioma da semente em plântulas modificadas por desinfestação das sementes e seu efeito sobre a germinação do milho e a colonização microbiana das raízes. A desinfestação das sementes foi realizada com hipoclorito de sódio (1,25%, 30 min), resultando em redução da fração cultivável da população bacteriana transmitida pela semente, mas não detectada significativamente por PCR em tempo real, microscopia e análise bioquímica das raízes de sementes germinadas. O sequenciamento do rRNA 16S revelou que o bacterioma das sementes não germinadas e das raízes germinadas exibiram, após 5 dias, diversidade semelhante e não diferiram em estrutura após a desinfestação das sementes. Por outro lado, a redução dos gêneros *Azospirillum* e *Acinetobacter* em sementes desinfestadas antes da germinação parece alterar a proeminência de vários novos táxons nas raízes. Curiosamente, esta comunidade bacteriana reconstruída afetou negativamente a velocidade de germinação e o crescimento das plântulas de milho. Além disso, a remodelação do bacterioma aumentou a suscetibilidade da var. DKB 177 ao patógeno vegetal transmitido pela semente *Penicillium* sp. Essas mudanças na composição natural da semente removeram sua barreira natural, aumentando a suscetibilidade a patógenos, impedindo que as sementes desinfestadas germinassem e se desenvolvessem. Concluímos que as bactérias transmitidas pelas sementes modulam a abundância relativa dos táxons que colonizam as raízes emergidas, promovem a germinação, o crescimento das plântulas e protegem o milho contra patógenos fúngicos.

Palavras-chave: Bactéria endofítica, Bactérias transmitidas por sementes, Biocontrole, Desinfestação das sementes, Germinação, *Penicillium* sp., Sequenciamento do rRNA 16S.

Graphical Abstract
(Resumo Gráfico)



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Altered bacteria community dominance reduces tolerance to resident fungus and seed to seedling growth performance in maize (*Zea mays* L. var. DKB 177)

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ABSTRACT

Seeds are reservoirs of beneficial and harmful microorganism that modulates plant growth and health. Here, we access seed to seedling bacteriome assembly modified by seed-disinfection and the underlined effect over maize germination performance and root-seedlings microbial colonization. Seed-disinfection was performed with sodium hypochlorite (1.25 %, 30 min), resulting in a reduction of the cultivable-dependent fraction of seed-borne bacteria population, but not significantly detected by real-time PCR, microscopy, and biochemical analysis of the roots on germinated seeds. 16S rRNA sequencing revealed that bacteriome of non-germinated seeds and roots of 5-d germinated seeds exhibited similar diversity and did not differ in the structure concerning seed-disinfection. On the other hand, the relative abundance reduction of the genera f_Enterobacteriaceae_922761 (unassigned genus), *Azospirillum,* and *Acinetobacter* in disinfected-seed prior germination seems to display changes in prominence of several new taxa in the roots of germinated seeds. Interestingly, this bacteriome community rebuilt negatively affected the germination speed and growth of maize plantlets. Additionally, bacteriome reshape increased the maize var. DKB 177 susceptible to the seed-borne plant pathogen *Penicillium* sp. Such changes in the natural seed-borne composition removed the natural barrier, increasing susceptibility to pathogens, impairing disinfected seeds to germinate, and develop. We conclude that bacteria borne in seeds modulate the relative abundance of taxa colonizing emerged roots, promote germination, seedling growth, and protect the maize against fungal pathogens.

1. Introduction

Plants are colonized by diverse microbial assemblages, known as microbiota (set of microorganisms) or microbiome (set of genomes) (Compant et al., 2019). Regarding the bacterial component of the microbiome (bacteriome), the ability to occupy various niches in the plant (surface and interior of the tissues) (Gopal and Gupta, 2018) and perform various beneficial activities, such as promoting growth and biocontrol of phytopathogens, is highlighted. These bacteria generally promote plant growth by facilitating the acquisition of nutrients (nitrogen, phosphorus, and iron) and producing/modulating phytohormones (auxin, gibberellin, and cytokinin) (direct effect); or by reducing damage caused by fungal and bacterial (harmful) pathogens through compounds they produce (siderophores, antibiotics, lytic enzymes, bacteriocins, lipoproteins, and volatile organic compounds) (indirect effect) (Orozco-Mosqueda et al., 2018; Verma et al., 2019b). Direct and

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indirect interactions of the microbiota with plants are essential to reduce the use of synthetic and pesticide fertilizers and make agriculture more sustainable.

The plant bacteriome has its origin in seeds (endo and epiphytic), considered a natural carrier of microbial inoculants transmitted vertically (Frank et al., 2017). Bacteria have already been isolated from sterilized seeds on the surface of many cultures (Verma et al., 2017, 2018; Verma and White, 2018), which suggests their protection inside the seed or strong adhesion to the surface. During germination, the radicle, already densely colonized by resident seed-bacteria, elongates and emerges from its coating. Then, the primary root grows in contact with the soil becomes a new source of bacteria for the plant host via a horizontal transmission (Bakker et al., 2015). Highlighting only the bacterial associations originating from the seed, we found studies that demonstrated the ability of its prokaryotic inhabitants to promote germination and growth of different plant species, which was confirmed by removing them by chemical and thermal disinfection (Holland, 2016; Irizarry and White, 2017; Verma et al., 2017, 2018; Verma and White, 2018; Holland, 2019; Verma et al., 2019b).

In addition to bacteria, the seeds harbour fungi of a phytopathogenic nature that can develop during the germination period, delaying germination, or killing the seed (Xing et al., 2018). Among the main pathogens transmitted by seed are fungi of the genus *Penicillium*, which infect a wide range of economically important plants (Xing et al., 2018). These fungi often reduce grain yield and quality, in addition to producing mycotoxins (Akonda et al., 2016). Fortunately, seed bacteria are competent biocontrol agents and protect plants from their enemies (Verma et al., 2017; Khalaf and Raizada, 2018; Verma et al., 2018; Verma and White, 2018; White et al., 2017).

Most of the analyzes available on the seed microbiota focus on the diversity or functional abilities of isolated bacteria. In the present study, possible roles for the seed-borne bacteria community were accessed by comparing chemical disinfected and non-disinfected maize seeds growing under axenic conditions. Using cultivable-independent approaches, we evaluated bacteria populations of non-germinated seeds and radicle of germinated seeds throughout 16S rRNA sequencing and population size by real-time PCR. Also, the population size of the cultivable bacteria pool and the structural interaction between the seed-resident microbial community and maize seedling were accessed. Finally, seed germination, seedling growth, and stored reserve remobilization were evaluated, and during these assays, it was observed a differential behaviour for the seed-borne fungus *Penicillium* sp. on disinfected and non-disinfected seeds.

2. Material and methods

2.1. Surface disinfection, germination, and seedlings growth promotion of maize

Maize seeds (*Zea mays* L.) of the DKB 177 variety (Dekalb®, Brazil) were immersed in sterile distilled water for 5 h, with part of the seeds being non-disinfected (*NDS treatment*). The disinfected seeds (*DS treatment*) were treated in 70 % alcohol for 5 min and sodium hypochlorite (NaClO; Butterfly Ecologia, Audax Company) 1.25 % for 30 min. Washings in sterile distilled water were performed between the solutions (1x) and after immersion in hypochlorite (5x). Preliminary tests were carried out to reach the 30 min time of immersion in NaClO (1.25 %). For this, seeds were immersed in this solution for times ranging from zero to 90 min (0, 10, 20, 30, 40, 50, 60, 70, 80 and 90 min) and placed in tubes containing NB liquid medium (Nutrient Broth; 5 mL) for 48 h (180 rpm, up to 30 °C). The optical density (OD) of each treatment was measured at 595 nm in a spectrophotometer (Chameleon, Hidex, model 425−156) and associated with the growth of seed bacteria.

The effect of disinfecting seeds for 30 min was evaluated using the Live/Dead® kit (Thermo Fisher Scientific), which analyzes the viability of bacterial cells. For that, bacterial suspension (2 mL) grown from the seed (zero- and 30-min immersion in hypochlorite) was centrifuged (10,000 × *g* for 4 min) and resuspended in saline (NaCl; 8.5 g L^{-1}) twice. Then, two fluorescent nucleic acid dyes, SYTO9 and propidium iodide (PI) (0.5 µL each) were mixed with the bacterial suspension (100 µL) and incubated at room temperature for 15 min. The stained bacteria were visualized using an Axioplan Zeiss epifluorescence microscope.

Disinfected and non-disinfected seeds were germinated under axenic conditions, being placed in Petri dishes (8 repetitions; 12 seeds per dish) containing agar-water medium (0.5 %) and packed in BOD (30 °C; photoperiod 12/12 h (light/dark)) for 5 days. After this period, germination percentage (%G), germination speed index (GSI), average germination time (AGT), and average germination speed (AGS) (Maguire, 1962) were evaluated using an unpaired *t*-test. Radicles ≥ 5 mm were considered to be germinated.

2.2. Seed-borne bacteria population count

The bacteria population associated with emerged radicle of germinated seedlings under axenic conditions (as described in topic 2.1) of disinfected and non-disinfected seeds were estimated. The diazotrophic population estimation was determined by the Most Probable Number (MPN) technique for positive growth in semi-solid media (data expressed as $\log_{10}$ nº cells. g^{-1} root). The total heterotrophic bacteria was accessed by colony-forming unit in Nutrient Broth (NB) solid medium plates (data expressed as $\log_{10}$ nº cells. g^{-1} root or mL^{-1}) with and without population enrichment in liquid NB medium. For this, roots (1 g) were macerated in saline (NaCl; 99 mL; 8.5 g L^{-1}), subjected to serial dilution (10^{-3} to 10^{-6}) and inoculated with a pipette (100 µL) into a 16 mL glass flask containing 5 mL of N-free semi-solid JNFb and LGI medium (diazotrophic population) or plated in NB medium (heterotrophic bacteria). The flasks and Petri plates were incubated in BOD (30 °C; 5–7 days), and the counts were as described above and following Baldani et al. (2014). Additionally, primary root segments were placed in tubes containing NB liquid medium (5 mL) subjected to “overnight” agitation (180 rpm, at 30 °C). After bacterial growth, serial dilution, plating in solid NB, and colony counting were performed.

2.3. Structural characterization of the maize bacterial microbiota by light microscopy (LM), scanning electron microscopy (SEM) and transmission electron microscopy (TEM)

Maize seeds and seedlings were grown under axenic conditions were collected and processed for light microscopy (LM) and scanning electron microscopy (SEM).

For LM, the primary maize root was visualized after being stained with 2,3,5-triphenyl tetrazolium chloride (0.1 % TTC for 2 h) (TTC; Reagen®), followed by reduction of the tissue background after immersing the root in potassium hydroxide solution (2.5 % KOH for 40 min). Stained roots were placed on slides with sterile distilled water and bacteria visualized for reducing the TTC from the colourless soluble form to the insoluble pink form, with this precipitation around the colonies being recorded by the bright-field microscope Axioplan Zeiss.

For SEM, segments (1 cm) of the primary maize root were fixed in glutaraldehyde (2.5 %) and paraformaldehyde (4 %) in sodium phosphate buffer (0.05 mol L^{-1}, pH 7.0). Then, the segments were washed with the same buffer (3 times for 20 min for root; 30 min for seed) and dehydrated in an alcoholic series (15, 30, 50, 70, 90 and 2 × 100 % at 15 min for each root; 30 min for seed). The samples were dried in a critical point device (Bal-Tec CPD 030), mounted on aluminium stubs, and metalized with ionized platinum in a sputtering coat apparatus (Bal-tec SCD 050). Maize seeds and roots were visualized in SEM Zeiss EVO 40 at 15 kV. Magnifying glass (Zeiss Stemi SV 11). Magnifying glass records (Zeiss Stemi SV 11) were used to indicate macroscopic structures of the seed.

Other root segments were incorporated, after the dehydration phase, in crescent LR White resin (medium grade) until complete tissue

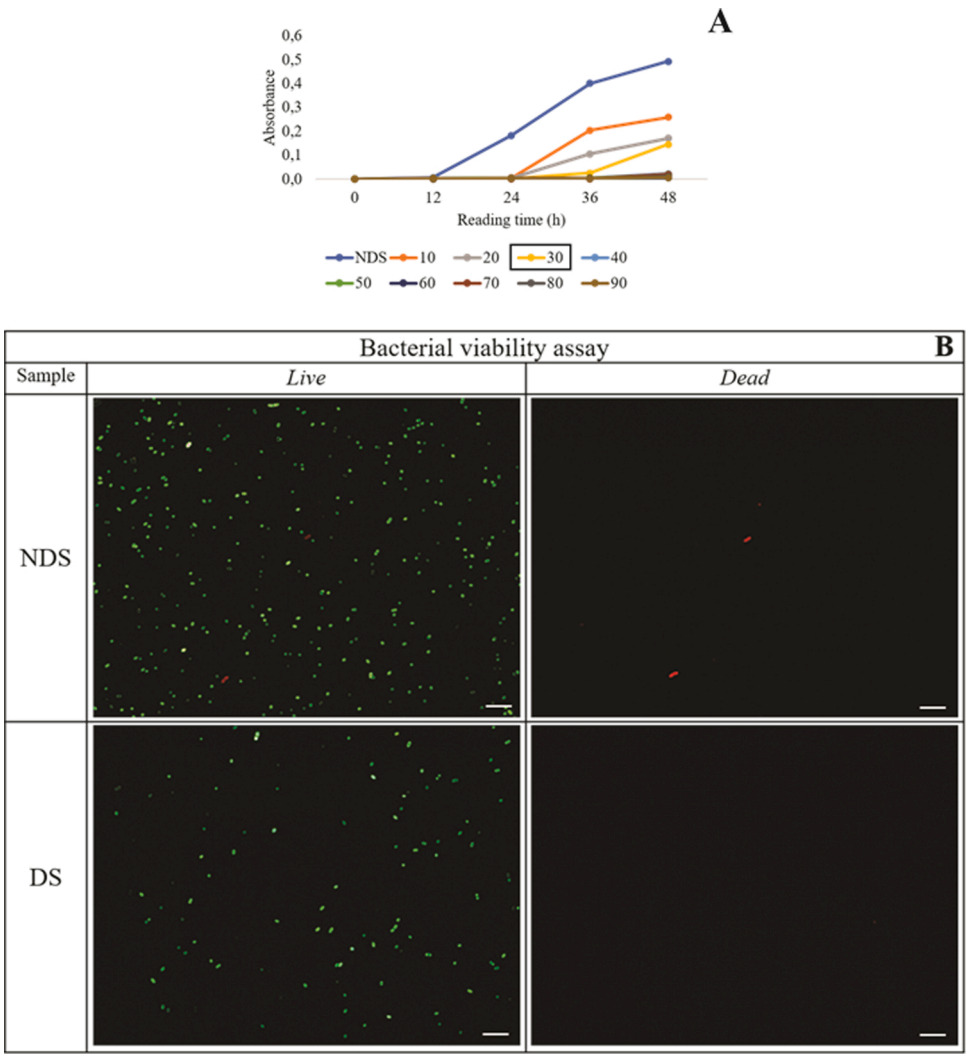

Fig. 1. Bacterial growth reported as optical density increase in NB liquid medium with maize seeds submitted to different disinfection times in sodium hypochlorite (1.25 % for 10, 20, 30, 40, 50, 60, 70, 80 and 90 min) (A) and fluorescence live/dead bacterial viability assay images of seeds non-disinfected (NDS) and disinfected (DS) (B). Scale bar: 10 μm.

replacement of ethanol for resin. After that, individual samples were mounted in transparent gelatin capsules filled with pure fresh resin and then polymerized in an oven at 60 °C for 24 h. Semi-thin sections (0.8–1.0 μm) of cured samples were obtained with the aid of a glass knife and ultramicrotome (Reichert-Jung Ultracut II E). The sections were collected on glass slides heated in a metal plate and stained with toluidine blue (1 %). After staining, the material was mounted in water with a coverslip, and it was observed under a light microscope. For TEM, samples prepared as described above were sectioned in ultra-thin sections (50−90 nm) with the aid of a diamond knife and ultramicrotome (Reichert-Jung Ultracut II E). The sections collected in copper grids (300 mesh), contrasted with uranyl acetate (5 % for 20 min) and lead citrate (5 min) and observed in a transmission electron microscope JEOL 1400 Plus at 80 KV.

2.4. Maize seeds and roots bacteriome

Seeds and roots were sampled from plates of the axenic assay described above and stored at -70 °C until DNA extraction. Frozen samples were macerated in liquid nitrogen to extract genomic DNA (from 0.2 g) using Cetyltrimethylammonium bromide (CTAB) for roots and seeds (Chen and Ronald, 1999; Doyle and Doyle, 1987). The amount of DNA in the samples was determined using NanoDrop 2000® spectrophotometer (Thermo Scientific) and Qubit® fluorometer (Invitrogen), while the quality was confirmed in agarose gel (0.8 %) electrophoresis (80 V, for 70 min). The total DNA was sent to the company "WEMSeq Biotechnology" for sequencing of the 16S rRNA gene in Illumina MiSeq, with three replicates per treatment. The samples were amplified with primers 515F and 806R against the V4 region of the 16S rRNA (Caporaso et al., 2012). PCR products were quantified (Qschd dsDNA HS kit, Invitrogen) and sequenced on the Illumina MiSeq platform (300V2 Kit, Illumina) according to the manufacturer's instructions.

The MiSeq raw sequences were analyzed in QIIME (Caporaso et al., 2010), version 1.9.0, where low-quality readings were filtered, and the rest grouped into Operational Taxonomic Units (OTUs) using a 97 % identity threshold. After grouping, sequences were aligned and classified with the SILVA database (Quast et al., 2012). The quality of the sampling was estimated from Good's coverage (Good, 1953). Subsequent analyzes were performed in the R environment (Team, R. C., 2013) using the phyloseq package (McMurdie and Holmes, 2013) to estimate the alpha and beta diversity. The ordering of the sequencing for beta diversity was performed based on the Bray-Curtis dissimilarity matrix and presented in principal coordinate analysis (PCoA) graphs. Permutational multivariate analysis of variance (Permanova) (Anderson, 2014) was used to assess statistical differences between treatments through the vegan package (Oksanen et al., 2013). The alpha diversity of the treatments

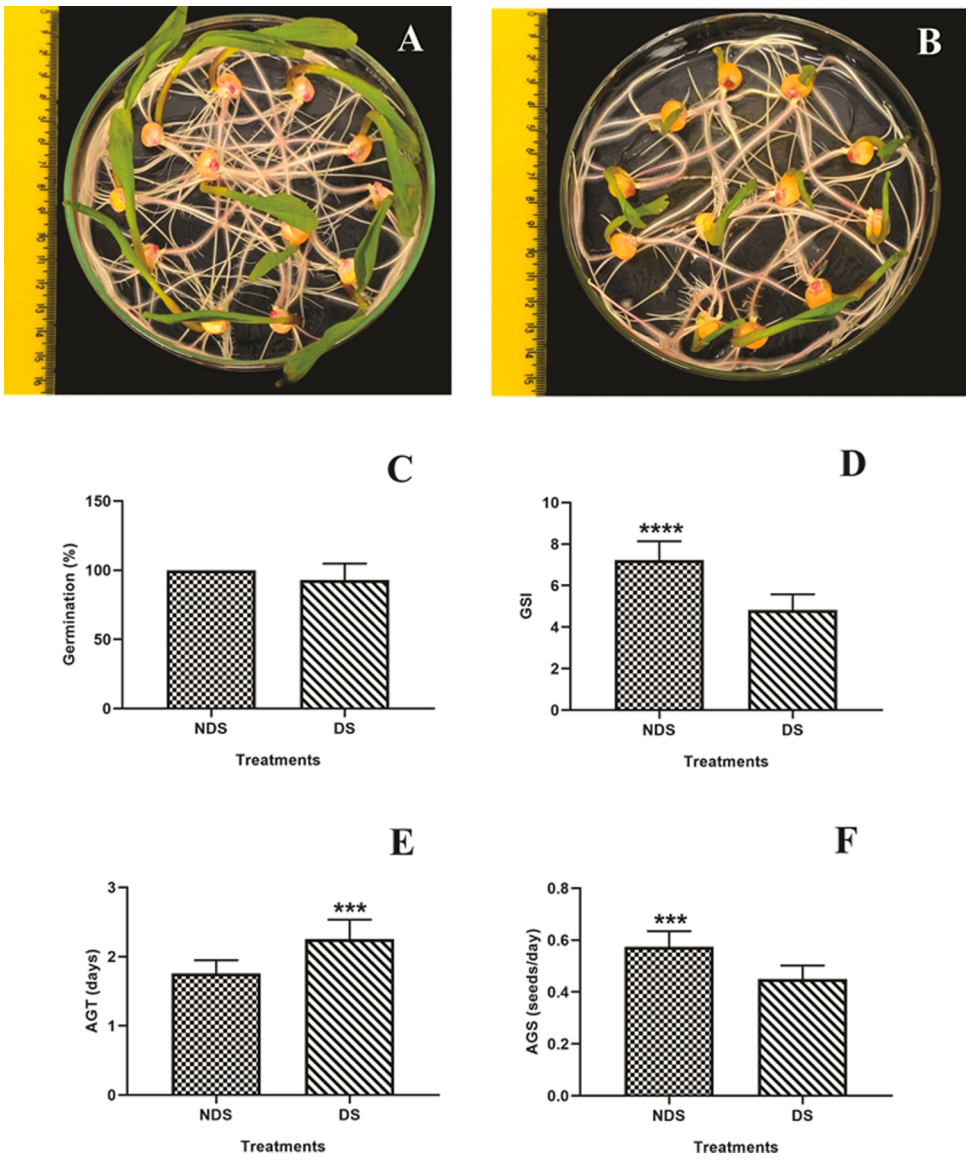

Fig. 2. Germination of non-disinfected (NDS; A) and disinfected (DS; B) maize seeds. Germination percentage (C), germination speed index (D), average germination time (E), and average germination speed (F). *Significant difference between treatments according to the Tukey test ($p \leq 0.05$).

was estimated from the Shannon index, and the results were contrasted using the Wilcoxon nonparametric statistical method. Venn diagrams were created to illustrate the overlap of OTUs between samples. Differential and relative abundance analyzes were performed at the gender level, the latter being represented in heatmaps.

2.5. Quantitative PCR for bacterial microbiome

The abundance of the Eubacteria domain in seed and maize root was measured by real-time PCR from the 16S rRNA. For this, the total DNA of the samples was extracted according to the methodology mentioned in the previous topic (CTAB method) and amplified from primers 926F (AAACTCAAAKGAATTGACGG) and 1062R (CTCACRRCACGAGCTGAC) (De Gregoris et al., 2011). PCR was performed in triplicate and with 15 µL of a reaction containing DNA (100 ng for seed and 40 ng for root), 7.5 µL of SYBR Green (Promega), 0.5 µL of each primer (10 µM) and water. The reaction conditions were 5 min incubation at 95 °C, 40 cycles of 15 s at 95 °C and 1 min at 60 °C in Step-One-Plus Real-Time PCR System (Applied Biosystems). The standard curve was generated by diluting the DNA of the bacterium *Escherichia coli* ATCC 25922 in series of 10^2 - 10^{-8} ($20-2 \times 10^9$ ng of DNA). *E. coli* was grown in NB liquid medium (180 rpm, at 30 °C) and had its DNA extracted with Wizard Genomic DNA Purification Kit (Promega). The number of bacteria in the seed and root was calculated based on the values of Ct (cycle threshold) and the standard curve (Staroscik, 2004).

2.6. Mobilization of seed-maize reserves during germination

To measure the mobilization of reserves during the germination of maize, seeds were treated and germinated according to item 2.1, with some modifications, including the soaking of NDS in sterile distilled water for 35 min. At the same time, DS was immersed for an equal period in alcohol/hypochlorite. Biochemical analyzes on maize were carried out in three distinct stages: 1°) Imbibition (seeds sampled after disinfection); 2°) End of germination (embryonic axis collected 24 (for NDS) and 48 h (for DS) after radicle emission (5 mm); 3°) Seedling stage (root collected after 5 days of germination). In stage 2, the embryonic axis was collected at different times because disinfection reduces the speed of seed germination (Fig. 2). The samples collected in the 3 stages were macerated in liquid nitrogen and analyzed, in triplicate, for protein (Smith et al., 1985), reducing sugar (Miller, 1959), glucose, triglycerides and alpha-amylase activity (Bioclin® K082, K117, K003), according to

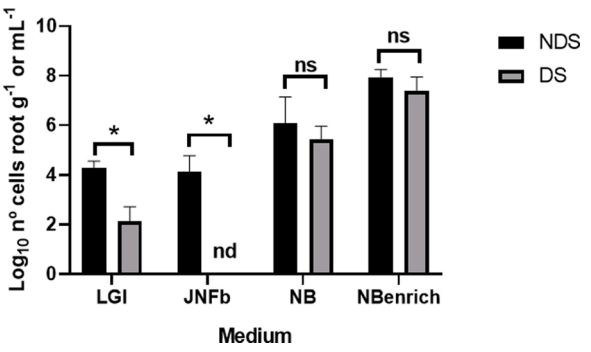

Fig. 3. Influence of seed disinfection (DS versus NDS) on bacterial population counts associated to five days emerged radicle of maize seedling (*Z. mays* DKB 177) under axenic conditions recovered in different culture media. Maize seeds non-disinfected (NDS) and disinfected (DS). nd: not detected. Positive growth in LGI (sucrose as C-source) and JNFb (malic acid as C-source) semi-solid N-free media means the formation of a white sub-superficial white pellicle Data for diazotrophic expressed as $\log_{10}$ nº cells. g^{-1} root with three biological replicates. Data for total bacteria expressed as $\log_{10}$ nº CFU. g^{-1} root or mL^{-1} in NB solid medium.

the cited protocols. The results were analyzed by ANOVA, followed by Tukey test ($p \leq 0.05$).

2.7. Effect of bacterial microbiota on biocontrol

To evaluate the potential of maize seed-borne bacteria in the control of phytopathogenic fungi, disinfected, and non-disinfected seeds were assayed as described in the 2.1. follow inoculation with *Penicillium* sp. This fungus was initially isolated from another maize variety (*Z. mays* var. SHS5050) after inhibiting 100 % of its germination and affected seed reserve remobilization in the previous study with the same experimental setup under the axenic condition described herein.

Once isolated, *Penicillium* sp. was grown in solid potato-dextrose-agar (PDA) in BOD (30 °C; 7 days), followed by new growth in NB liquid medium (180 rpm, at 30 °C) and inoculation in NDS and DS (150 µL per seed; OD595 = ~1; 10 seeds per plate; 4 repetitions). After five days, germination rate and seedling growth (length and mass) were evaluated, while scanning microscopy was used to characterize fungal colonization at the root of the different treatments (methodologies described in topics 2.1 and 2.3, respectively). The results were submitted to analysis of variance (ANOVA) and the means compared by the Tukey test ($p \leq 0.05$).

3. Results

To assess the role of seed-borne bacteria in maize, a time-course chemical seed-disinfection assay with sodium hypochlorite was carried out to remove most of the microbial community without impairing the germination process. Thus, we selected the time of 30 min of seed immersion in 1.25 % sodium hypochlorite solution. Disinfecting the seeds for 30 min removed most of their bacteria (Fig. 1A), without affecting the germination percentage of the maize (Fig. 2C). Live/Dead® viability tests confirmed that the disinfection reduced the number of viable bacteria cells compared to the non-disinfected treatment (Fig. 1B). Both treatments showed green fluorescence (live cells), with few visible (red) dead cells (Fig. 1B).

The seed-disinfection did not affect the germination percentage of the maize (Fig. 2C) but reduced the germination speed (Fig. 2D and F) and increased the time necessary for the seed to germinate (Fig. 2E).

Seed-disinfecting treatment significantly reduced diazotrophic bacteria population associated to emerged radicle of maize seedling (5 days after emergence) in LGI semi-solid N-free medium (sucrose as a C-source) and dramatically reduced the diazotrophic bacteria population grown in JNFb semi-solid N-free medium (malic acid as C-source) to no detected level (Fig. 3). For total heterotrophic bacteria in NB solid medium, it was shown no significant decrease in the root seedling population in disinfect seeds. The same trend was obtained by population size of root segments overnight enriched in NB liquid medium (Fig. 3)

Elongation/differentiation zone cross-section of the emerged radicle from non-disinfected (Supplementary Fig. A1, A1–A3) and disinfected maize seeds (Supplementary Fig. A1, B1–B3) were viewed under light (LM) and transmission electron microscopy (TEM). The tissue system organization of the root tissue can be visualized in images of LM (Supplementary Fig. A1). Under TEM, the regular orientation of the plant cell wall, cytoplasmatic organelles, and the presence of prominent vacuoles in both treatments were noticed (Supplementary Fig. A1). Thus, it seems unlikely that the hypochlorite affected the root anatomy organization of maize root seedling.

Scanning electron microscopy (SEM) was used to characterize the niche occupancy by the bacterial community in water-imbibed seeds before germination (Fig. 4). It was not possible to distinguish population density differences of bacteria in the pericarp coat (Fig. 4A1 and 4B1) and endosperm region (Fig. 4A2 and 4B2) from disinfected and non-disinfected seeds. After 48 h-germination, with radicle protrusion that emerged throughout the micropyle, it was noticed a remarkable densely bacteria populations at the bottom of the radicle surface in both treatments (Fig. 4A3–A4 and 4B3–B4).

After 5 D-germination, bacterial cells were viewed under LM and SEM. In root-segments stained with TTC, few bacteria aggregates were seen in the lateral root emission region (Fig. 5A1 and 5B1). In contrast, no bacteria were visualized directly in the root tip surface of the disinfected and non-disinfected treatments (Fig. 5A2 and 5B2). SEM observations showed many single to small aggregate bacterial cells distributed over root hair zone of NDS-treated (Fig. 5A3), while DS bacteria were viewed as larger aggregate with less frequent colonization pattern over root hair zone (Fig. 5B3). At the elongation zone of DS, bacteria cells were main visualized as single cells, while aggregated or biofilms of bacteria community were observed in the NDS (Fig. 5A4 and 5B4). In the root tip segment, the pattern colonization was similar for both (NDS and DS) with bacteria community more frequently organized as small aggregates (Fig. 5A5 and 5B5).

By sequencing and analyzing the16S rRNA, we characterize the bacteriome of the transition phase of the seed to root seedling emergence under axenic conditions. Data sequencing of 5-h embedded seed resulted in 587 reads in total, with an average sample coverage of 53 % (Supplementary Table 1). PCoA analysis for seed bacteriome showed distinct clustering between some samples independent of disinfected and non-disinfected seeds (Supplementary Fig. A2, A). The seed bacteriome showed similar diversity ($p = 0.66$) (Supplementary Fig. A2, B) and did not differ in structure ($p = 0.6$; $R^2 = 0.195$) (Supplementary Table 2) after the disinfection process.

Venn diagram revealed a low number of OTUs in non-germinated embedded seeds, with two unique OTUs for non-disinfected seeds and 3 for disinfected seeds (Supplementary Fig. A3, A). Only 2 OTUs were shared between treatments. In the bacteriome associated with the seed, we identified ten genera from 3 different phyla, attributed to Proteobacteria (7 genera), Firmicutes (2 genera), and Actinobacteria (1 genus) (Supplementary Fig. A3, B). Among the ten genera, *Azospirillum*, *Acinetobacter,* and f_Enterobacteriaceae_922761 (unassigned genus) were more abundant in non-disinfected seeds, while *Acinetobacter* and *Staphylococcus* were abundant in seeds treated with hypochlorite. Other genera were detected in low numbers. Disinfection appears to reduce the relative abundance of f_Enterobacteriaceae_922761, *Azospirillum,* and *Acinetobacter* in the seed. However, the analysis of differential abundance revealed that no taxon was removed by the hypochlorite (Supplementary Fig. A3, B)

Five days after seed germination, the sequencing of the maize root bacteriome resulted in 2,229.23 reads in total and average sample coverage of 86 % (Supplementary Table 3). PCoA analysis showed that

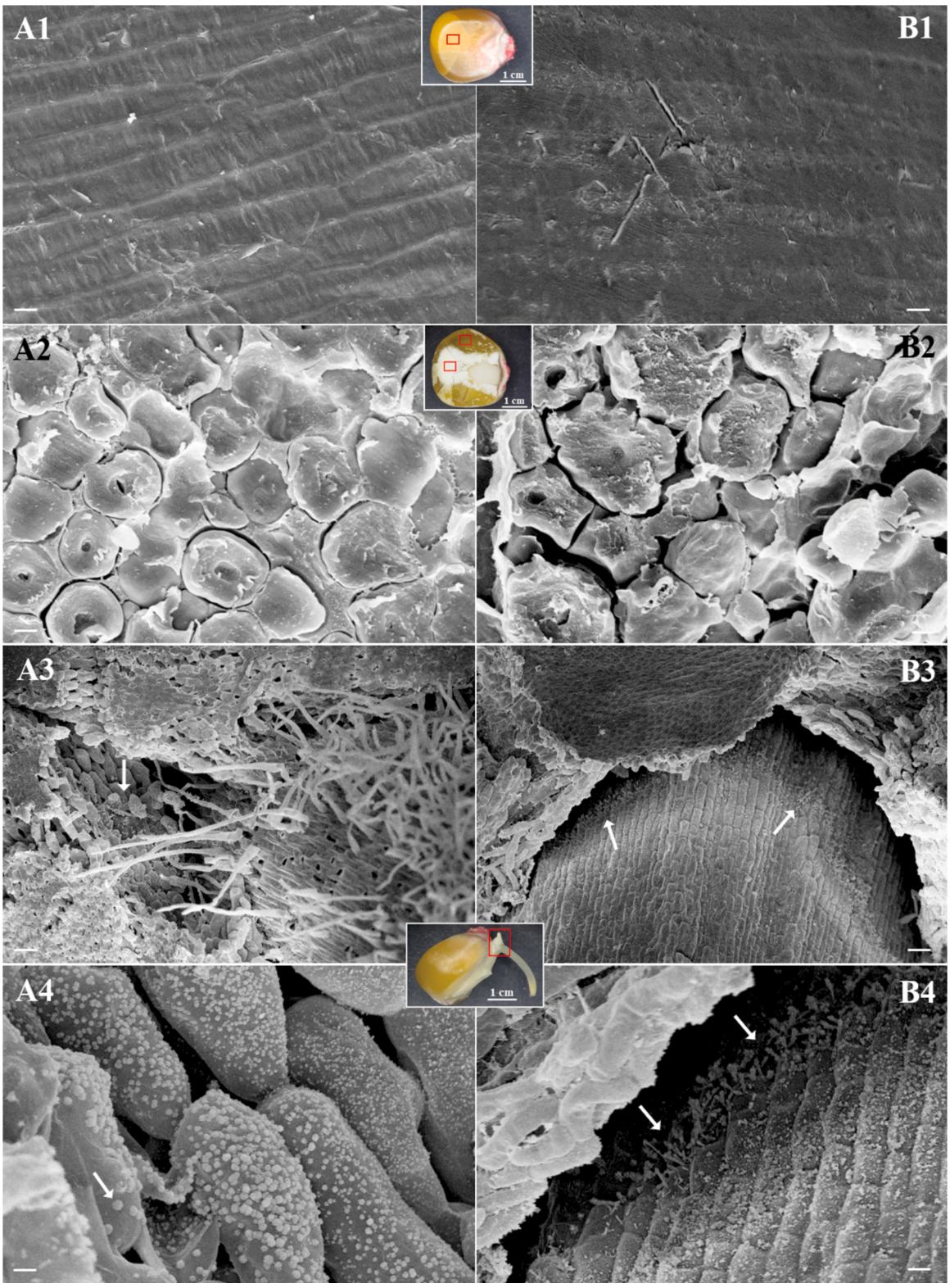

Fig. 4. Colonization of maize seeds by its microbiota. Bacterial cells were visualized by scanning electron microscopy (SEM). Maize seeds non-disinfected (A) and disinfected (B). Seed regions: pericarp (A1 and B1), endosperm (A2 and B2), and radicle (A3, A4, B3, and B4). Biofilms and small bacterial aggregates are indicated by white arrows. Bars represent the following scales: panel B2: 3 μm; A2: 10 μm; A1, A4, B1, and B4: 20 μm; A3: 100 μm; B3: 200 μm.

the beta dispersion of the samples did not differ between the emerged roots of NDS and DS (Fig. 6A), while Permanova indicated that disinfection was not significant for grouping biological replicates ($p = 0.384$; $R2 = 0.091$) (Supplementary Table 4). The bacterial diversity of the root was also not altered by disinfection ($p = 1.0$) (Fig. 6B). We observed a high overlap of OTUs between bacteria communities associated with emerged roots of NDS and DS, with 10 OTUS in common (Fig. 7A). Only 3 and 6 exclusive OTUS were observed for NDS and DS, respectively (Fig. 7A). In the root bacteriome, we identified ten genera, nine classified in the phylum Proteobacteria and 1 in the phylum Bacteroidetes

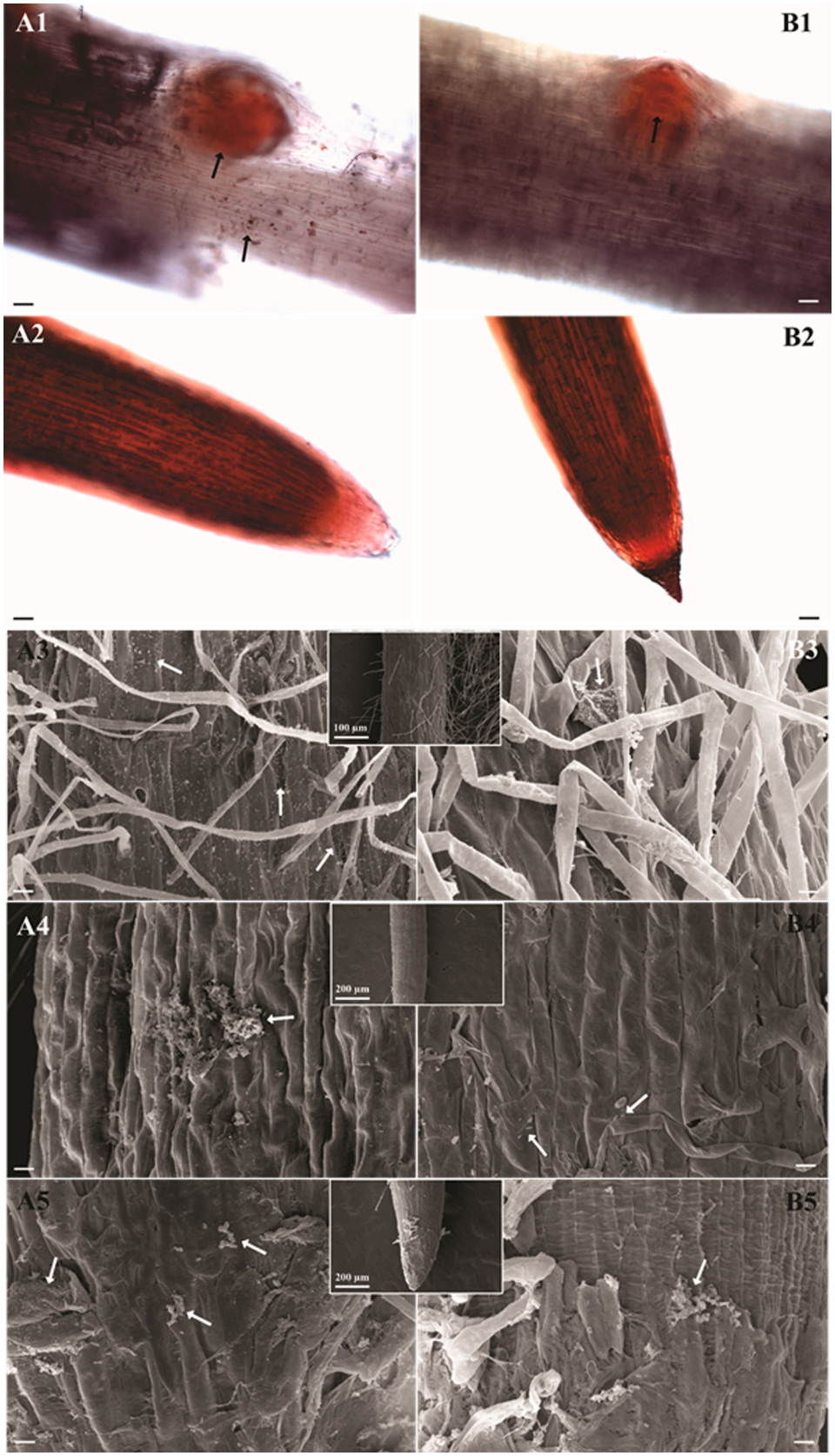

Fig. 5. Bacterial colonization of maize root visualized by light (LM; root stained with TTC) and scanning electron microscopy (SEM). Maize seeds non-disinfected (A) and disinfected (B). Root regions: mitotic sites (A1 and B1), root hair (A3 and B3), the zone of elongation (A4 and B4), root cap (A2, A5, B2, and B5). Bacteria are indicated by arrows. Bars represent the following scales: panel A1, A2, B1, and B2: 10 µm; A3-A5 and B3-B5: 20 µm.

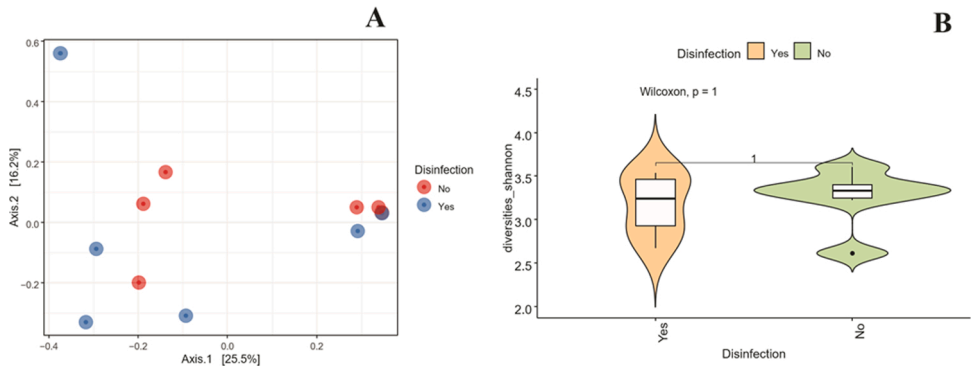

Fig. 6. Principal coordinate analysis (PCoA) plot and alpha diversity of the bacteriome associated with the root of non-disinfected (No) and disinfected (Yes) seeds in axenic systems.

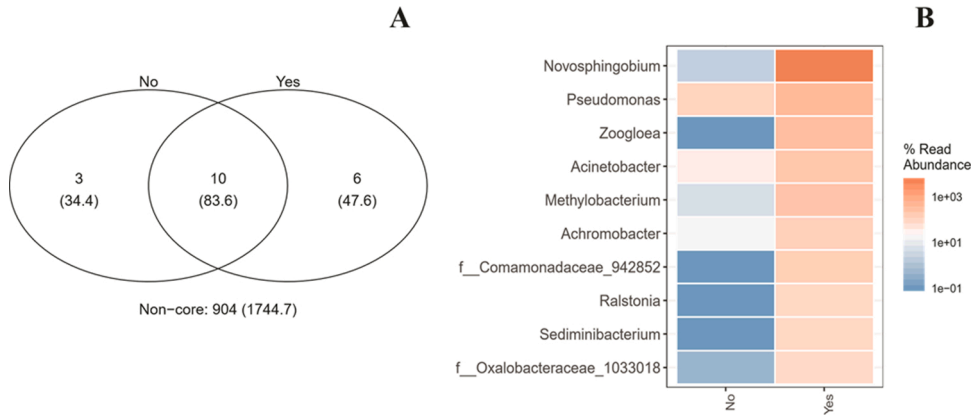

Fig. 7. Venn diagram with shared OTUs and heat map with the taxonomy of the most abundant genera in roots of non-disinfected (No) and disinfected (Yes) seeds maintained in axenic conditions.

(Fig. 7B). Genus differences include a greater abundance of *Pseudomonas*, *Acinetobacter*, *Achromobacter*, *Methylobacterium,* and *Novosphingobium* in the roots of disinfected treatment (Fig. 7B). Interestingly, roots from the disinfected treatment were densely colonized by bacteria abundant in NDS roots, plus the genera *Zoogloea*, f_Comamonadaceae_942852 (unassigned genus), *Ralstonia*, *Sediminibacterium* and f_Oxalobacteraceae_1033018 (unassigned genus) (Fig. 7B). We found 3 OTUs that differed in abundance in disinfected versus non-disinfected seeds and were attributed to the genera *Acinetobacter* and *Ochrobactrium* (Supplementary Table 5). Within *Acinetobacter*, only the species *A. rhizosphaerae* was identified.

The quantification of bacteria via real-time PCR identified a similar number of cells/ng of DNA in roots (Supplementary Fig. A4, A; NDS: 7356 log cell; DS: 7287; $p \geq 0.05$), and roots (Supplementary Fig. A4, B; NDS: 5360 log cell; DS: 5208; $p \geq 0.05$) disinfected and non-disinfected.

Significant differences in the mobilization of maize reserves were only observed in the degradation of triglycerides of the embryonic axis (Fig. 8C) and activity of the alpha-amylase enzyme to the root (Fig. 8E). In both cases, the results were superior in the non-disinfected treatment. The protein, glucose, and reducing sugar content did not differ between treatments (NDS and DS) of the seed compartment, embryonic axis, and root (Fig. 8A, B, and D). In general, the mobilization of reserves changed as germination progressed, with significant differences between the analyzed stages (Supplementary Table 6).

We also evaluated if the observed changes in the seed-borne bacteria community interfere with tolerance against seed-borne phytopathogenic fungi. Maize germination was tested after seed disinfection and inoculation of *Penicillium* sp. In Fig. 9, we observed that fungus significantly reduced the percentage (C) and the germination speed (D) of the disinfected seeds, which had part of their microbiota re-shaped by the action of the hypochlorite. Due to the action of the fungus, many disinfected seeds decay before or just after germination (Fig. 9B2). When non-disinfected, the seeds that received the fungus germinated normally (Fig. 9C and D), with percentage and speed equal to the control (without inoculation of the fungus). The average germination speed and time did not differ between treatments (Fig. 9E and F).

The growth of germinated seedlings from disinfected seeds and inoculated with *Penicillium* sp. was drastically reduced, affecting the length of the aerial part (LAP) and root (LR), as well as the fresh root mass (FMR) (Fig. 10C, D, and F). These results were significantly inferior to the non-disinfected seeds challenged with the phytopathogenic fungus and the disinfected-without-inoculation control (Fig. 10). It is noteworthy that disinfected-inoculated seedlings rotted, showing brown spots over the root axis (Fig 10B2). Seedlings of seeds that were non-disinfected and inoculated with fungus grew normally for all characteristics analyzed (except FMAP), with LAP, LR, and FMR values close to the control without inoculation (Fig. 10C, D, and F). Only the fresh weight of the aerial part (FMAP) did not differ between treatments (10E).

Scanning electron microscopy (SEM) identified the presence of fungal hyphae in maize roots infected with *Penicillium* sp. (Fig. 11). Mycelia densely colonized the elongation/differentiation zone (Fig. 11B3 and B4) and the root tip region(Fig. 11B5) of the germinated roots of the disinfected seeds. The lesser density of bacteria and yeast aggregates were observed in the infected region (Fig. 11B4). In the roots of non-disinfected seeds, SEM showed few hyphae in the elongation/differentiation zone (Fig. 11A3 and A4), and no hyphae were viewed in the root tip region (Fig 11A5). In this treatment, it was observed a high number of bacteria attached to the root surface in monolayer, interacting with fungus hyphae (Fig. 11A4). *Penicillium* sp., yeasts, and

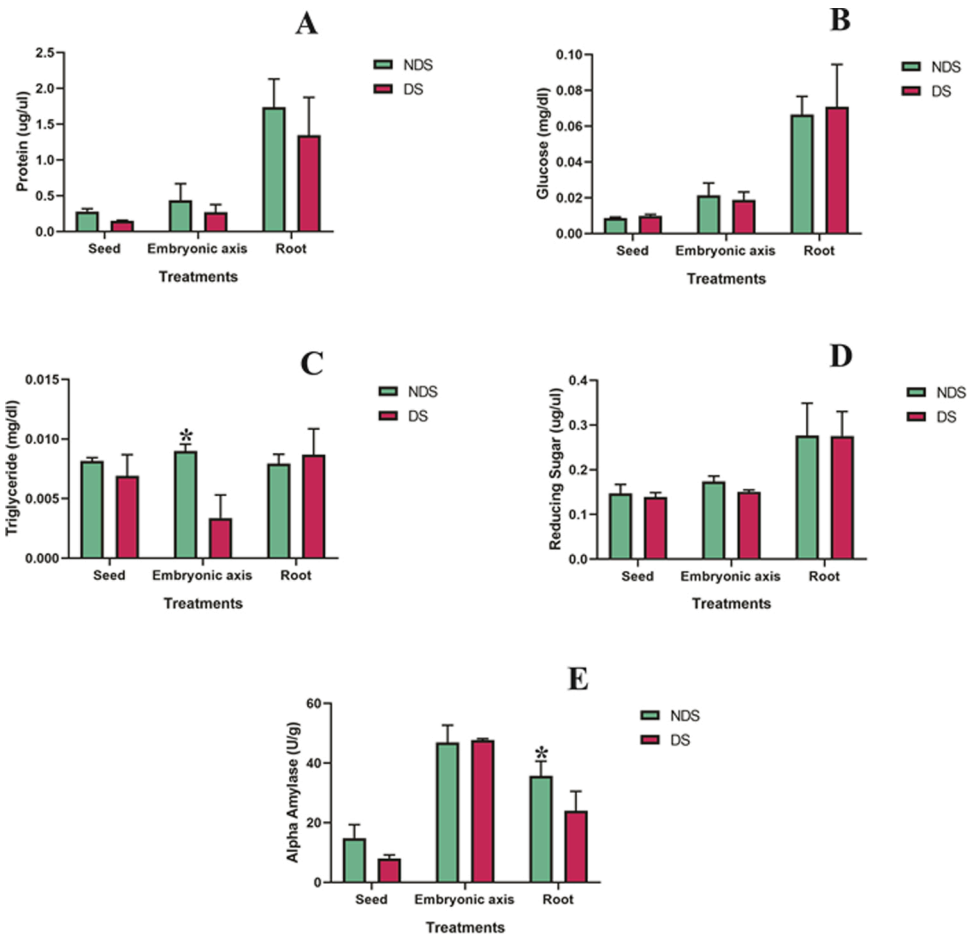

Fig. 8. Dosage of protein (A), glucose (B), triglyceride (C), reducing sugar (D), and alpha-amylase activity (E) in seed, embryonic axis, and root non-disinfected (NDS) and disinfected (DS). *Significant difference between treatments NDS and DS (within each stage) according to the Tukey test ($p \leq 0.05$).

bacteria colonized root areas with lateral root emergence, in the disinfected treatment (Fig. 11B1 and B2), while bacterial biofilms were seen in the non-disinfected treatment in the same niche (Fig. 11A1 and A2).

No filamentous fungal tissue was observed in uninoculated seedling roots (Supplementary Figure A5). In control, bacteria and yeasts were seen colonizing the root tissue in isolation or small aggregates (Suppl. Fig. A5). A higher density of bacteria was detected in the root hood of the non-disinfected treatment (Suppl. Fig. A5).

4. Discussion

For a long time, "sterile" seeds were considered healthy, which contributed to the development of disinfection methods (chemical, mechanical, physical, and biological) in order to remove their "pathogens" (Berg and Raaijmakers, 2018). However, in recent years, studies based on "omics" have shown that the seeds harbour diverse and mostly beneficial microbial communities (Berg and Raaijmakers, 2018). In the present work, we confirmed that axenically germinated maize seeds host several bacteria taxon, which was located, quantified and identified by microscopic analysis, real-time PCR, counting in the culture medium, and sequencing. Also, it was demonstrated that from seed to seedling transition, there was a substantial increase in size and complex of the bacteria community structure, whose functionalities remain to be elucidated.

In this axenic study, soaking maize seeds in sodium hypochlorite solution (1.25 %, 30 min) proved its antimicrobial effect through the Live-Dead viability assay and population estimation by count in the culture medium. However, microscopy analysis of the water embedded seed and emerged roots from germinated maize seeds revealed that the chemical disinfection reduces but does not remove all bacteria from the seed. Pieces of evidence for this are images of SEM and LM with no visible differences in the bacterial density of the treatments (NDS *versus* DS).

After reducing the number of maize bacteria with disinfection, a delay in seed germination speed and seedling growth was observed that reinforces the idea that some bacteria borne in seeds are essential for these physiological processes. Other studies have shown that chemical disinfection (Irizarry and White, 2017; Verma et al., 2017, 2018; Verma and White, 2018) and thermal treatments (Holland, 2016, 2019) have slowed germination and growth of rice, soybean, beans, and millet. It is worth mentioning that disinfection did not affect the germination percentage of maize and did not structurally alter the plant cell and tissue viewed by transmission electron microscopy. Therefore, it seems unlikely that delays in the speed of germination and growth are attributed to sodium hypochlorite.

The idea that disinfection can reduce, but not remove all bacteria from maize, was confirmed by sequencing the 16S rRNA, since there were no significant differences between treatments (NDS versus DS) for Permanova and Shannon diversity, besides the significant number of shared OTUs. Differences in sequencing were restricted to the distinct taxonomic composition between the seed and root compartments, which shared only two genera (*Acinetobacter* and *Novosphingobium*). Within the studied compartments, the variation between treatments is only quantitative; that is, it is based on the abundance of taxa, not on their presence or absence, which justifies the fact that only 3 OTUs have been

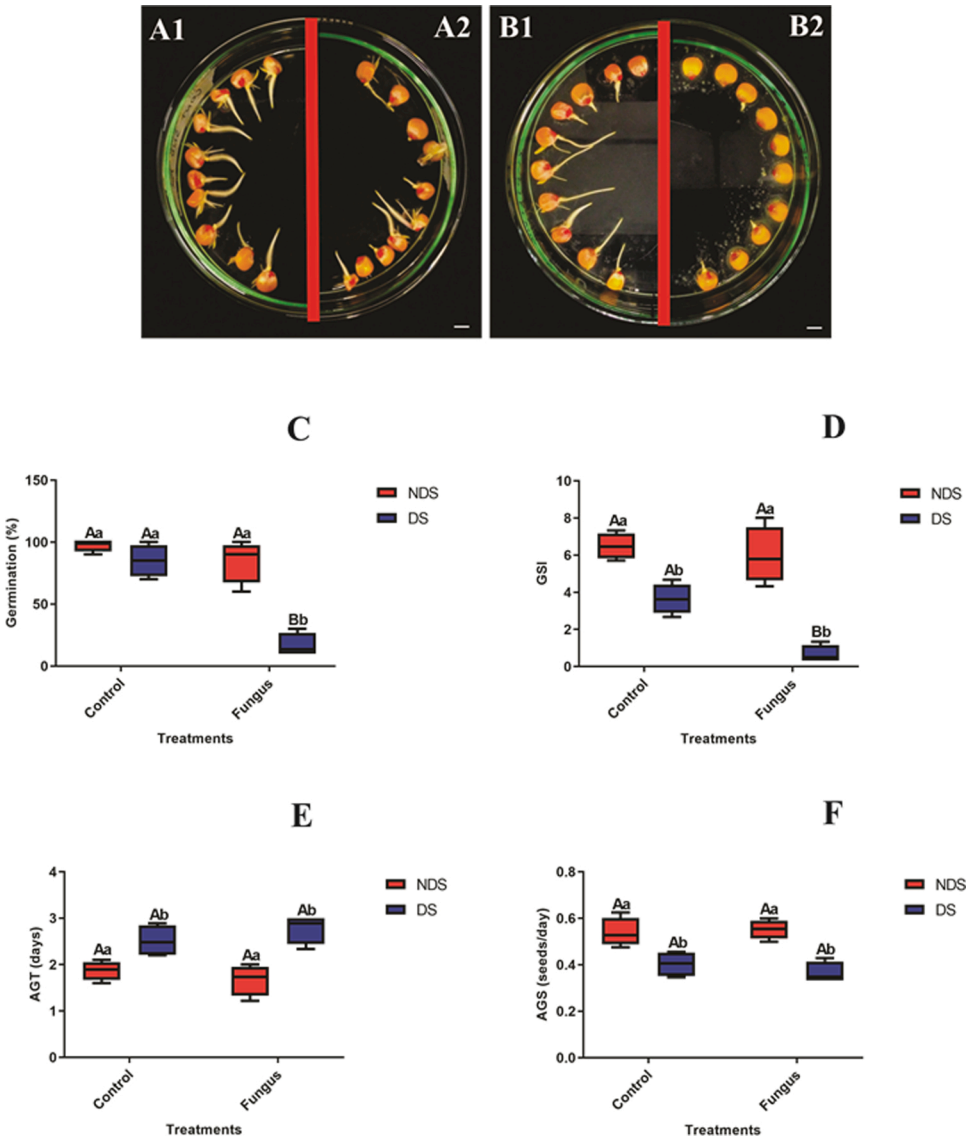

Fig. 9. Germination of non-disinfected (NDS; A1 and B1) and disinfected (DS; A2 and B2) maize seeds inoculated (B1-B2) or not (A1-A2) with fungus *Penicillium* sp. Germination percentage (C), germination speed index (D), average germination time (E), and average germination speed (F). Different capital letters indicate significant differences for the inoculation factor (Control x Fungus), and lowercase letters indicate significant differences for the disinfection factor (NDS x DS) according to the Tukey test ($p \leq 0.05$).

removed by disinfection (*Acinetobacter* (2) and *Ochrobacterium* (1)). In the seed, the reduction of the genera f_Enterobacteriaceae_922761 (unassigned genus), *Azospirillum,* and *Acinetobacter* after disinfection can be related with later emergence of several new taxa (9 genera in all) activated at the emerged root during germination, with emphasis on the genus *Novosphingobium*. These findings suggest that the reduction of dominant genera in the seed reduced the competition for niches or resources in the root, allowing colonization by other bacterial groups (Hardoim, 2019). However, these new groups do not contemplate or contemplate a reduced number of bacteria that promote germination and growth, which affected the development of maize.

Once the bacteriome of maize is characterized, it remains to elucidate how these microorganisms promote germination and plant growth. Most studies attribute bacteria to biostimulatory, biofertilizer, and biocontrol skills (Santos et al., 2019). Some research already reports that bacteria can positively modulate the mobilization of seed reserves during germination. In this study, the changes in compositional bacteria structure do not seem to interfere with this seed-stored mobilization, maybe due to the remaining presence of key taxon after disinfection. On the other hand, if the number of bacteria/ng of DNA (detected via real-time PCR) has not been altered by disinfection, the mobilization of reserves will not be altered either.

After the loss of several germination assays due to the action of the pathogenic fungus *Penicillium* sp. (data not shown from our group for *Z. mays* variety SHS5050), we decided to explore the role of the bacterial microbiota in protecting the seed (maize of the variety DKB 177). Results of this test showed that the partial removal of the microbiota by the hypochlorite rendered the seed more susceptible to the seed-borne fungus *Penicillium* sp., drastically reducing the germination and growth of maize. On the other hand, seeds that were non-disinfected and inoculated with the fungus developed typically, as well as the control seeds without fungus challenger. This finding was confirmed by SEM of germinated roots, with dense fungal colonization in the disinfected-inoculated treatment, while little or no hypha was observed in the treatment non-disinfected-inoculated. Other studies have also shown that seed-bacteria control fungal diseases (Verma et al., 2017; Khalaf and Raizada, 2018; Verma et al., 2018; Verma and White, 2018; White et al., 2017).

We correlated biocontrol results with maize sequencing and observed that the bacteriome acted as a "barrier" against phytopathogens by inhibiting the proliferation of fungi that deteriorate the seeds, also determining the bacterial profile of the root and the growth parameters of the maize seedlings. For this, they had to compete for nutrients and niches, induce plant resistance or produce antifungal and antibacterial metabolites (antibiotics, bacteriocins, lytic enzymes, and volatile compounds) (Verma et al., 2019a). The candidates' bacteria

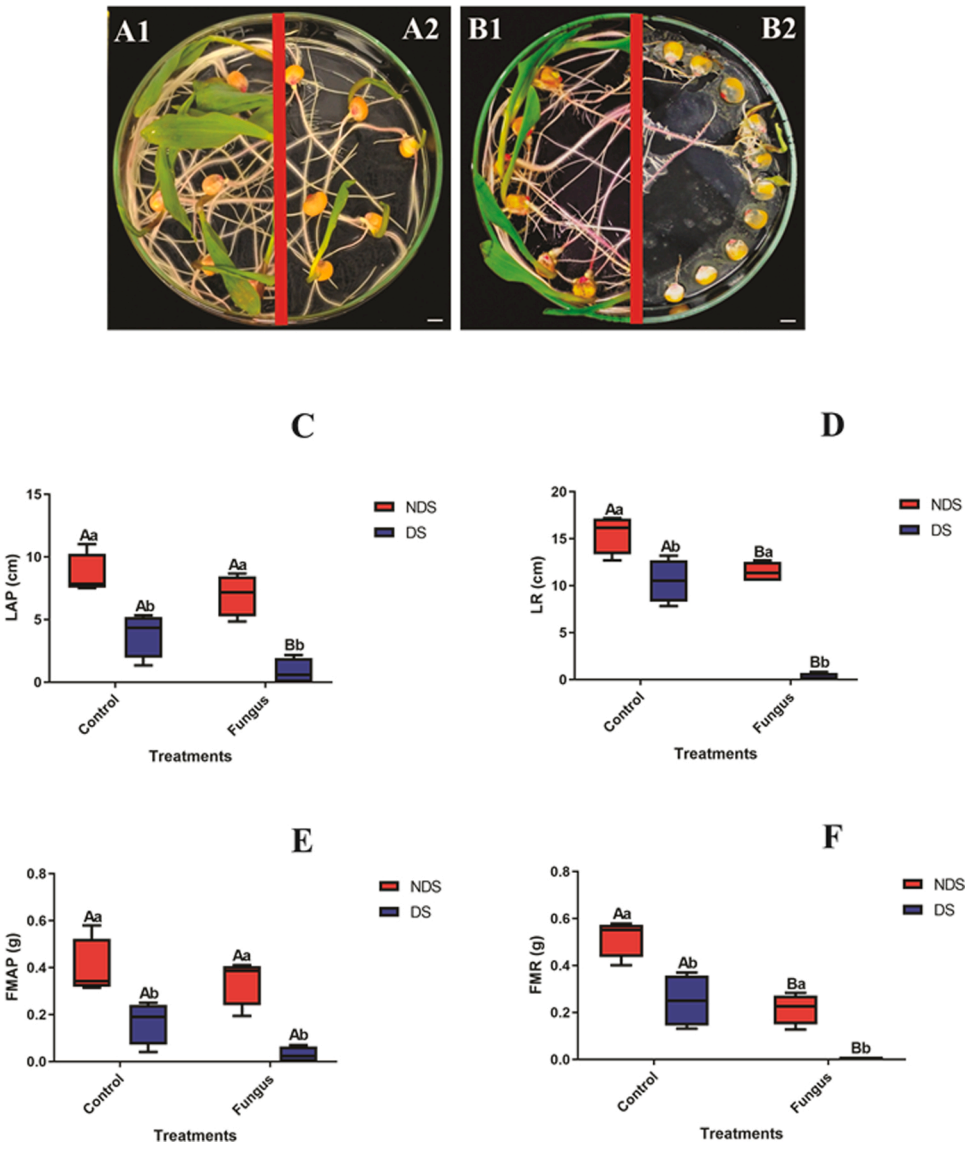

Fig. 10. Growth of maize germinated of non-disinfected (NDS; A1 and B1) and disinfected (DS; A2 and B2) maize seeds inoculated (B1-B2) or not (A1-A2) with fungus *Penicillium* sp. Shoot length (C; LAP) and root (D; LR), shoot fresh weight (E; FMAP), and root (F; FMR). Different capital letters indicate significant differences for the inoculation factor (Control x Fungus), and lowercase letters indicate significant differences for the disinfection factor (NDS x DS) according to the Tukey test ($p \leq 0.05$).

consortium responsible for microbial "barrier" are f_Enterobacteriaceae_922761, *Azospirillum,* and *Acinetobacter*. With disinfection, the abundance of these genera was reduced, changing the root bacteriome, making the seed susceptible to *Penicillium* sp. that harming the germination and seedling growth of maize.

Possible functional abilities of the featured gender have been established in the literature. Recent studies indicate that endophytic strains of *Enterobacter* (classified in the family f_Enterobacteriaceae_922761) can stimulate germination and plant growth (Panigrahi et al., 2019; Vitorino et al., 2019). The underlying mechanisms involve phytohormones (indole-acetic acid-IAA) (Verma et al., 2017; Srisuk et al., 2018; Panigrahi et al., 2019), siderophores (Maleki et al., 2018; Panigrahi et al., 2019) and phosphate solubilization (Verma et al., 2017; Panigrahi et al., 2019; Luduena et al., 2018); and that seeds not treated with these bacteria are susceptible to degradation by fungal phytopathogens, such as *Penicillium*, *Fusarium* and others (Sandhya et al., 2017; Verma et al., 2017; Vitorino et al., 2019). Enterobacteria were able to inhibit the growth of *Aspergillus flavus* and seven other fungal pathogens through volatile compounds produced (Gong et al., 2019).

The *Azospirillum* genus comprises bacteria widely studied and used in agriculture (Santos et al., 2019). Its inoculation in plants promotes growth through different mechanisms, such as biological nitrogen fixation, production of phytohormones (such as IAA, gibberellins), and siderophores (Fukami et al., 2017; López-Reyes et al., 2017). This genus has been attributed to the ability to reduce the incidence of fungal diseases (*Alternaria*, *Bipolaris,* and *Fusarium*) of maize (López-Reyes et al., 2017) through the induction of defence genes (Fukami et al., 2017, 2018).

Other studies have identified in *Acinetobacter* bacteria the ability to produce IAA, siderophores, solubilize phosphate and zinc, fix nitrogen and promote plant growth (Gandhi and Muralidharan, 2016; Kang et al., 2016; Patel et al., 2017); in addition to acting on the control of fungi associated with seeds (*Fusarium* and *Alternaria*) (Medina-de la Rosa et al., 2016) through chitinases they produce (Krithika and Chellaram, 2016).

5. Conclusion

We concluded that the structure of the seed-borne bacteria community is drastically shaped (mainly taxon relative abundance) by the germination process of maize that ultimately influences germination and seedling growth. Additionally, the removal of certain bacteria taxa by chemical seed-disinfection suppress natural seed-borne barrier protection of maize seedlings from fungal pathogens. Understanding the

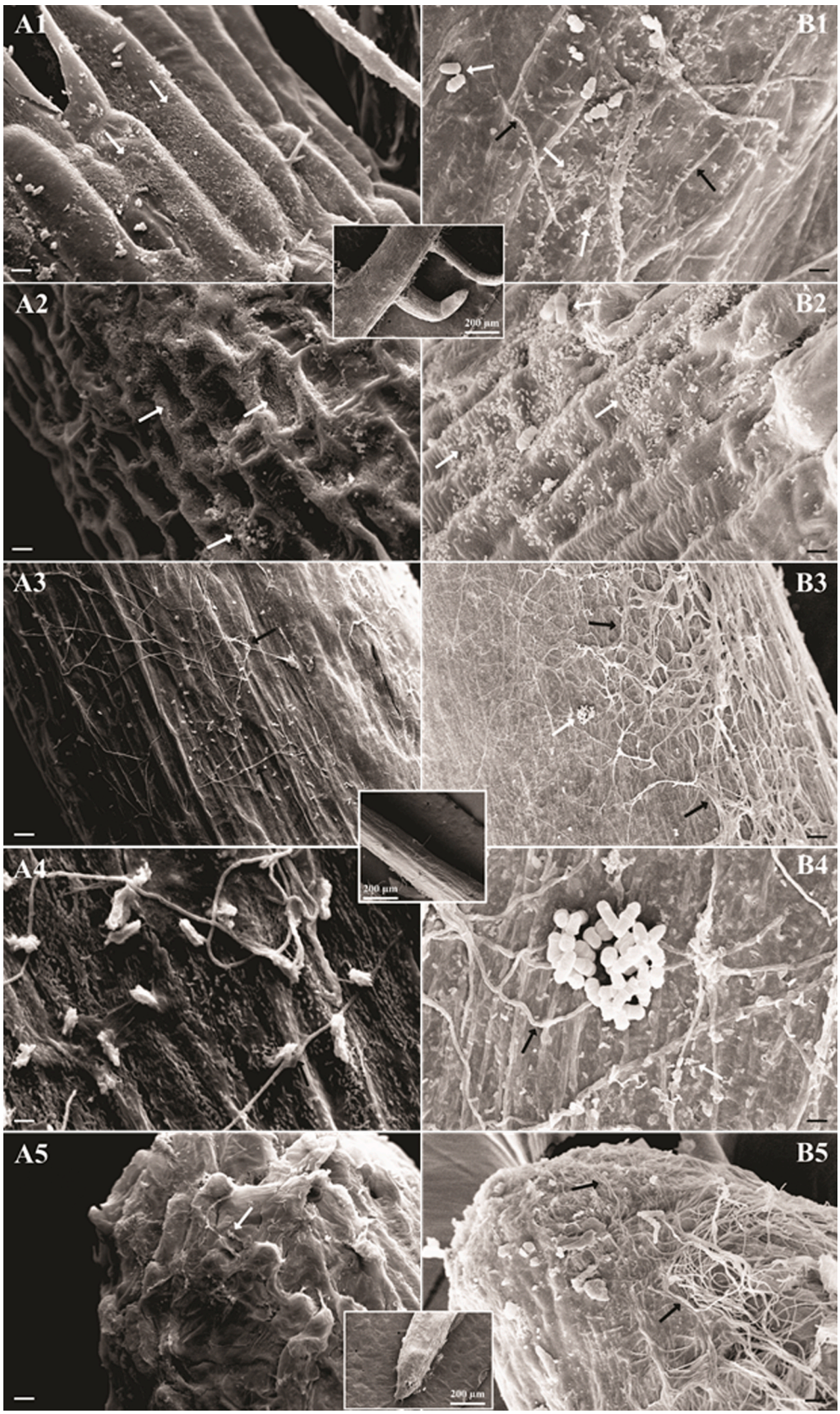

Fig. 11. Colonization of maize root by *Penicillium* sp. Fungi were visualized by scanning electron microscopy (SEM). Maize seeds non-disinfected (A) and disinfected (B). Root regions: lateral root (A1, A2, B1, and B2), the zone of elongation (A3, A4, B3, and B4), root cap (A5 and B5). Bacteria/yeasts and filamentous fungi are indicated by white and black arrows, respectively. Bars represent the following scales: panel A2, A4, B1, B2, and B4: 10 µm; A1, A3, A5, B3, and B5: 20 µm.

successional community balance during the seed germination and critical community members of the microbial network and physiological process will open up new ways for the formulation of inoculants to boost crop productivity and crop protection. Although the strategy of creating seed microbiome-based inoculants has not yet been put into practice, it represents the future of agriculture.

Declaration of Competing Interest

No conflict of interest declared.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.micres.2020.126643.

References

Akonda, M.M.R., Yasmin, M., Hossain, I., 2016. Incidence of seedborne mycoflora and their effects on germination of maize seeds. Int. J. Agron. Agric. Res. 8, 87–92.
Anderson, M.J. 2014. Permutational multivariate analysis of variance (PERMANOVA). Wiley statsref: statistics reference online, 1-15.
Bakker, M.G., Chaparro, J.M., Manter, D.K., Vivanco, J.M., 2015. Impacts of bulk soil microbial community structure on rhizosphere microbiomes of *Zea mays*. Plant Soil 392, 115–126.
Baldani, J.I., Reis, V.M., Videira, S.S., Boddey, L.H., Baldani, V.L.D., 2014. The art of isolating nitrogen-fixing bacteria from non-leguminous plants using N-free semi-solid media: a practical guide for microbiologists. Plant Soil 384, 413–431.
Berg, G., Raaijmakers, J.M., 2018. Saving seed microbiomes. ISME J. 12, 1167–1170.
Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Peña, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., Mcdonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R., 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods 7, 335–336.
Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Huntley, J., Fierer, N., Owens, S.M., Betley, J., Fraser, L., Bauer, M., Gormley, N., Gilbert, J.A., Smith, G., Knight, R., 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J. 6, 1621–1624.
Chen, D.H., Ronald, P.C., 1999. A rapid DNA minipreparation method suitable for AFLP and other PCR applications. Plant Mol. Biol. Rep. 17, 53–57.
Compant, S., Samad, A., Faist, H., Sessitsch, A., 2019. A review on the plant microbiome: ecology, functions, and emerging trends in microbial application. J. Adv. Res. 19, 29–37.
De Gregoris, T.B., Aldred, N., Clare, A.S., Burgess, J.G., 2011. Improvement of phylum- and class-specific primers for real-time PCR quantification of bacterial taxa. J. Microbiol. Methods 86, 351–356.
Doyle, J.J., Doyle, J.L., 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 19, 11–15.
Frank, A.C., Saldierna Guzman, J.P., Shay, J.E., 2017. Transmission of bacterial endophytes. Microorganisms 5, 70.
Fukami, J., Ollero, F.J., Megias, M., Hungria, M., 2017. Phytohormones and induction of plant-stress tolerance and defense genes by seed and foliar inoculation with *Azospirillum brasilense* cells and metabolites promote maize growth. AMB Express 7, 153.
Fukami, J., Cerezini, P., Hungria, M., 2018. *Azospirillum*: benefits that go far beyond biological nitrogen fixation. AMB Express 8, 73.
Gandhi, A., Muralidharan, G., 2016. Assessment of zinc solubilizing potentiality of *Acinetobacter* sp. Isolated from rice rhizosphere. Eur. J. Soil Biol. 76, 1–8.
Gong, A.D., Dong, F.Y., Hu, M.J., Kong, X.W., Wei, F.F., Gong, S.J., Zhang, Y.M., Zhang, J.B., Wu, A.B., Liao, Y.C., 2019. Antifungal activity of volatile emitted from *Enterobacter asburiae* Vt-7 against *Aspergillus flavus* and aflatoxins in peanuts during storage. Food Control 106.
Good, I.J., 1953. The population frequencies of species and the estimation of population parameters. Biometrika 40, 237–264.
Gopal, M., Gupta, A., 2018. Building plant microbiome vault: a future biotechnological resource. Symbiosis 77, 1–8.
Hardoim, P., 2019. The ecology of seed Microbiota. In: VERMA, S.K., WHITE, J.J.F. (Eds.), Seed Endophytes: Biology and Biotechnology. Cham: Springer International Publishing..
Holland, M.A., 2016. Probiotics for Plants? What the PPFMs told us and some ideas about how to use them. J. Washington Acad. Sci. 102, 31–42.
Holland, M.A., 2019. Thinking about PPFM bacteria as a model of seed endophytes: who are they? Where did they come from? What are they doing for the plant? What can they Do for Us? In: VERMA, S.K., WHITE, J.J.F. (Eds.), Seed Endophytes: Biology and Biotechnology. Springer International Publishing, Cham.
Irizarry, I., White, J.F., 2017. Application of bacteria from non-cultivated plants to promote growth, alter root architecture and alleviate salt stress of cotton. J. Appl. Microbiol. 122, 1110–1120.
Kang, S.M., Radhakrishnan, R., You, Y.H., Lee, K.E., Kim, J.H., Joo, G.J., Kim, J.G., Lee, I. J., 2016. Mustard and Chinese cabbage plant growth promotion by optimal-medium-cultured *Acinetobacter calcoaceticus* SE370. J. Pure Appl. Microbiol. 10, 1693–1699.
Khalaf, E.M., Raizada, M.N., 2018. Bacterial seed endophytes of domesticated cucurbits antagonize fungal and oomycete pathogens including powdery mildew. Front. Microbiol. 9, 42.
Krithika, S., Chellaram, C., 2016. Isolation, screening, and characterization of chitinase producing bacteria from marine wastes. Int. J. Pharm. Pharm. Sci. 8.
López-Reyes, L., Carcaño-Montiel, M.G., Lilia, T.L., Medina-De La Rosa, G., Armando, T. H.R., 2017. Antifungal and growth-promoting activity of *Azospirillum brasilense* in *Zea mays* L. Ssp. Mexicana. Arch. Phytopathol. Plant Prot. 50, 727–743.
Luduena, L.M., Anzuay, M.S., Angelini, J.G., Mcintosh, M., Becker, A., Rupp, O., Goesmann, A., Blom, J., Fabra, A., Taurian, T., 2018. Genome sequence of the endophytic strain *Enterobacter* sp. J49, a potential biofertilizer for peanut and maize. Genomics 111, 913–920.
Maguire, Jd., 1962. Speed of germination aid in selection and evaluation for seedling emergence and vigor. Crop Sci. 2, 176–177.
Maleki, M., Norouzpour, S., Rezvannejad, E., Shakeri, S., 2018. Novel strains of *Bacillus cereus* Wah1 and *Enterobacter cloacae* Wkh with high potential for production of siderophores. Biol. J. Microorg. 6, 1–11.
Mcmurdie, P.J., Holmes, S.O., 2013. Phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One 8.
Medina-De La Rosa, G., López-Reyes, L., Carcaño-Montiel, M.G., López-Olguín, J.F., Hernández-Espinosa, M.A., Rivera-Tapia, J.A., 2016. Rhizosphere bacteria of maize with chitinolytic activity and its potential in the control of phytopathogenic fungi. Arch. Phytopathol. Plant Prot. 49, 310–321.
Miller, G.L., 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem. 31, 426–428.
Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Wagner, H., 2013. Vegan: Community Ecology Package. R Package Version 2.0-8. package=vegan (access on 30 March 2020). http://CRAN.R-project.org/.
Orozco-Mosqueda, M., Rocha-Granados, M., Glick, B., Santoyo, G., 2018. Microbiome engineering to improve biocontrol and plant growth-promoting mechanisms. Microbiol. Res. 208, 25–31.
Panigrahi, S., Mohanty, S., Rath, C.C., 2019. Characterization of endophytic bacteria *Enterobacter cloacae* MG00145 isolated from *Ocimum sanctum* with Indole Acetic Acid (IAA) production and plant growth promoting capabilities against selected crops. South Afr. J. Bot.
Patel, P., Shah, R., Modi, K., 2017. Isolation and characterization of plant growth promoting potential of *Acinetobacter*sp. RSC7 isolated from *Saccharum officinarum* cultivar Co 671. J. Exp. Biol. Agric. Sci. 5, 483–491.
Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O., 2012. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. J. Nucleic acids Res. 41, D590–D596.
Sandhya, V., Shrivastava, M., Ali, S.Z., Sai Shiva Krishna Prasad, V., 2017. Endophytes from maize with plant growth promotion and biocontrol activity under drought stress. Russ. Agric. Sci. 43, 22–34.
Santos, M.S., Nogueira, M.A., Hungria, M., 2019. Microbial inoculants: reviewing the past, discussing the present and previewing an outstanding future for the use of beneficial bacteria in agriculture. AMB Express 9, 205.
Smith, P.K., Krohn, R.I., Hermanson, G.T., Mallia, A.K., Gartner, F.H., Provenzano, M.D., Fujimoto, E.K., Goeke, N.M., Olson, B.J., Klenk, D.C., 1985. Measurement of protein using bicinchoninic acid. Anal. Biochem. 150, 76–85.
Srisuk, N., Sakpuntoon, V., Nutaratat, P., 2018. Production of Indole-3-Acetic Acid by *Enterobacter* sp. DMKU-RP206 Using Sweet Whey as a Low-Cost Feed Stock. J. Microbiol. Biotechnol. 28, 1511–1516.
Staroscik, A., 2004. Calculator for determining the number of copies of a template. URI Genomics Sequenc. Center 19, 2012.
Team, R. C, 2013. . R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
Verma, S.K., White, J.F., 2018. Indigenous endophytic seed bacteria promote seedling development and defend against fungal disease in browntop millet (*Urochloa ramosa* L.). J. Appl. Microbiol. 124, 764–778.
Verma, S.K., Kingsley, K., Irizarry, I., Bergen, M., Kharwar, R.N., White Jr., J.F., 2017. Seed-vectored endophytic bacteria modulate development of rice seedlings. J. Appl. Microbiol. 122, 1680–1691.

Verma, S.K., Kingsley, K., Bergen, M., English, C., Elmore, M., Kharwar, R.N., White, J.F., 2018. Bacterial endophytes from rice cut grass (*Leersia oryzoides* L.) increase growth, promote root gravitropic response, stimulate root hair formation, and protect rice seedlings from disease. Plant Soil 422, 223–238.

Verma, P.P., Shelake, R.M., Das, S., Sharma, P., Kim, J.Y., 2019a. Plant growth-promoting rhizobacteria (PGPR) and fungi (PGPF): potential biological control agents of diseases and pests. In: SINGH, D.P., GUPTA, V.K., PRABHA, R. (Eds.), Microbial Interventions in Agriculture and Environment: Volume 1 : Research Trends, Priorities and Prospects. Singapore: Springer, Singapore.

Verma, S.K., Kharwar, R.N., White, J.F., 2019b. The role of seed-vectored endophytes in seedling development and establishment. Symbiosis 78, 107–113.

Vitorino, L.C., Palharini, K.M.Z., Rocha, A.F.D.S., Prates, L.S., Goulart, L.G., Silva, A.L., Bessa, L.A., 2019. Application of bacteria symbiotic with *Butia archeri* (Arecaceae) to the biocontrol of the phytopathogenic fungi that deteriorate seeds of *Glycine max*. Seed Sci. Technol. 47, 325–341.

White, J.F., Kingsley, K.I., Kowalski, K.P., Irizarry, I., Micci, A., Soares, M.A., Bergen, M. S., 2017. Disease protection and allelopathic interactions of seed-transmitted endophytic pseudomonads of invasive reed grass (*Phragmites australis*). Plant Soil 422, 195–208.

Xing, H.Q., Ma, J.C., Xu, B.L., Zhang, S.W., Wang, J., Cao, L., Yang, X.M., 2018. Mycobiota of maize seeds revealed by rDNA-ITS sequence analysis of samples with varying storage times. Microbiologyopen 7, e00609.

Supplementary Material

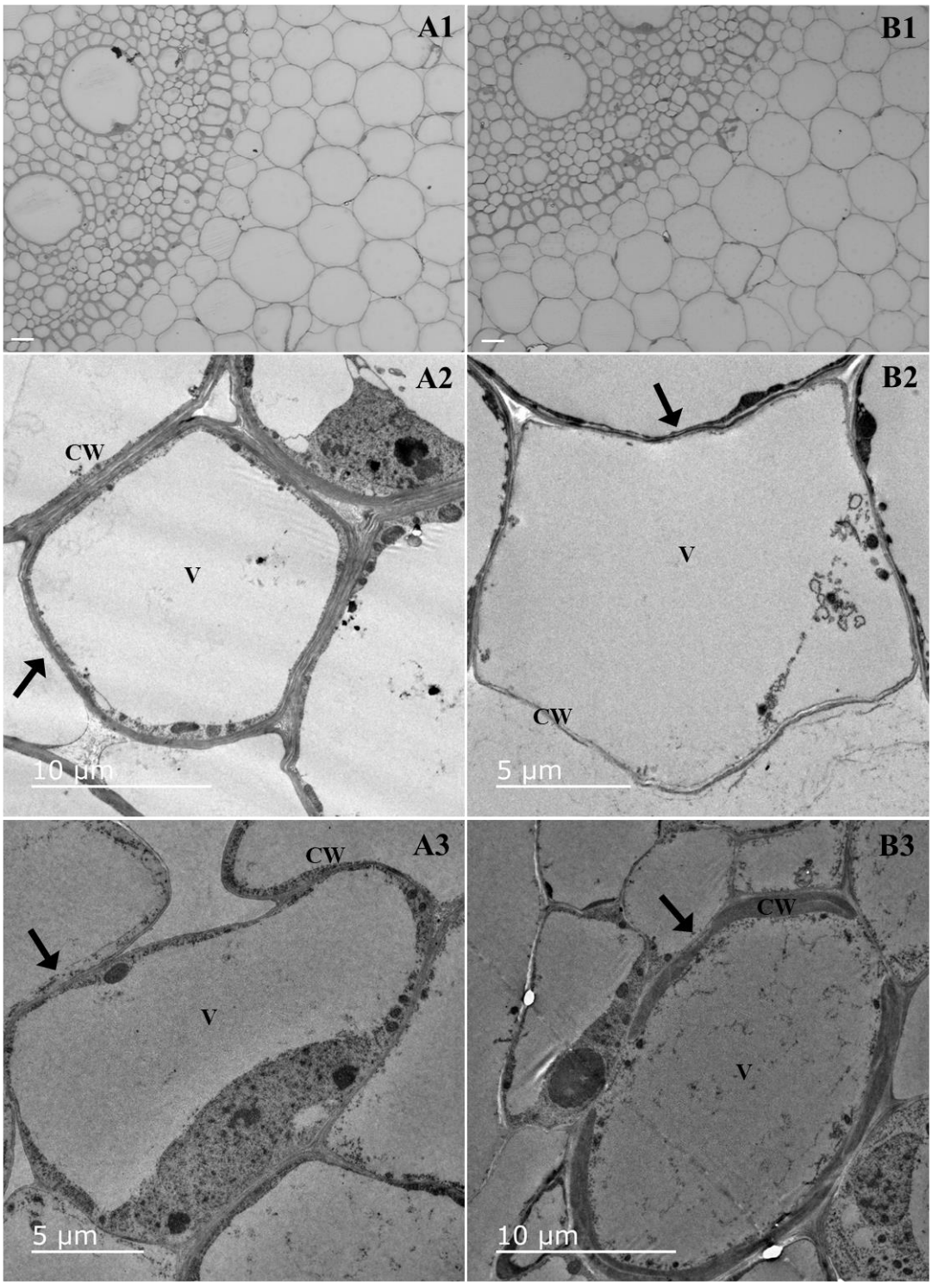

Fig. A.1 Root tip cross-sections of non-disinfected (A) and disinfected (B) maize seeds viewed by light microscopy (A1 and B1) and transmission electron microscopy (A2, A3, B2, and B3). Black arrows indicate cell wall shape. Plant cell organelles: cell wall (CW) and vacuole (V). Bars represent the following scales: panel A1 and B1: 20 µm; A2 and B3: 10 µm; B2 and A3: 5 µm.

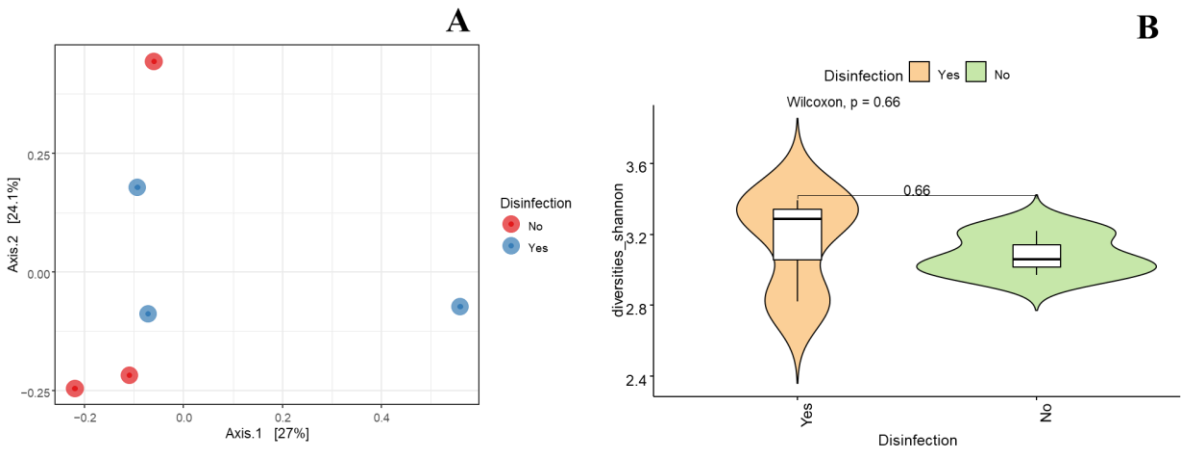

Fig. A.2 Principal coordinate analysis (PCoA) plot and alpha diversity of the bacteriome associated with non-disinfected (No) and disinfected (Yes) seeds in axenic systems.

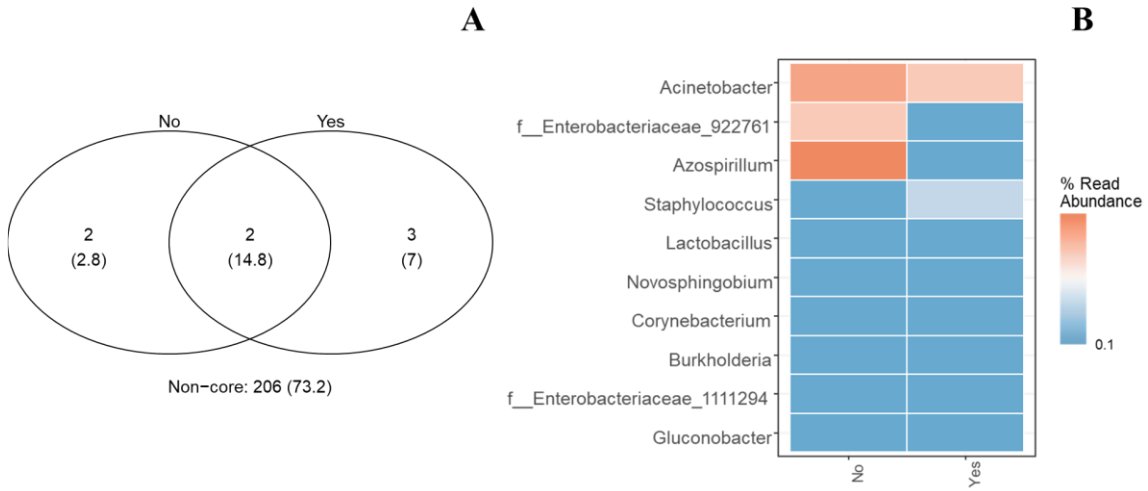

Fig. A.3 Venn diagram with shared OTUs and heat map with the taxonomy of the most abundant genera of non-disinfected (No) and disinfected (Yes) seeds maintained in axenic conditions.

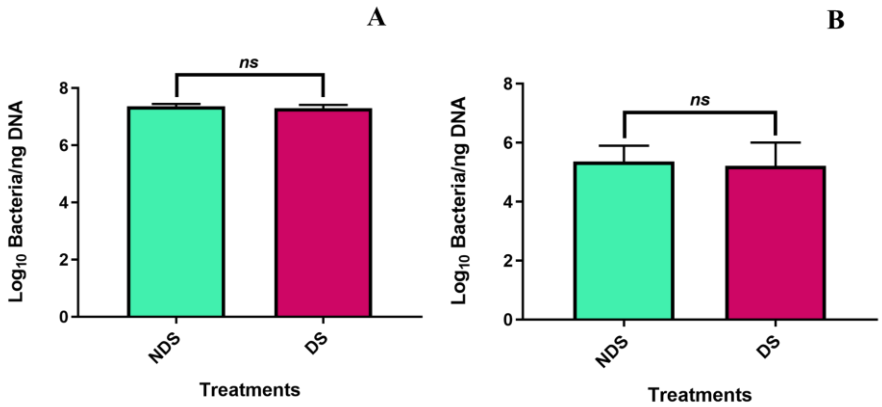

Fig. A.4 Quantification of bacteriome by real-time PCR in non-disinfected (NDS) and disinfected (DS) roots (A) and seeds (B). *Significant difference between treatments according to the Tukey test ($p \leq 0.05$).

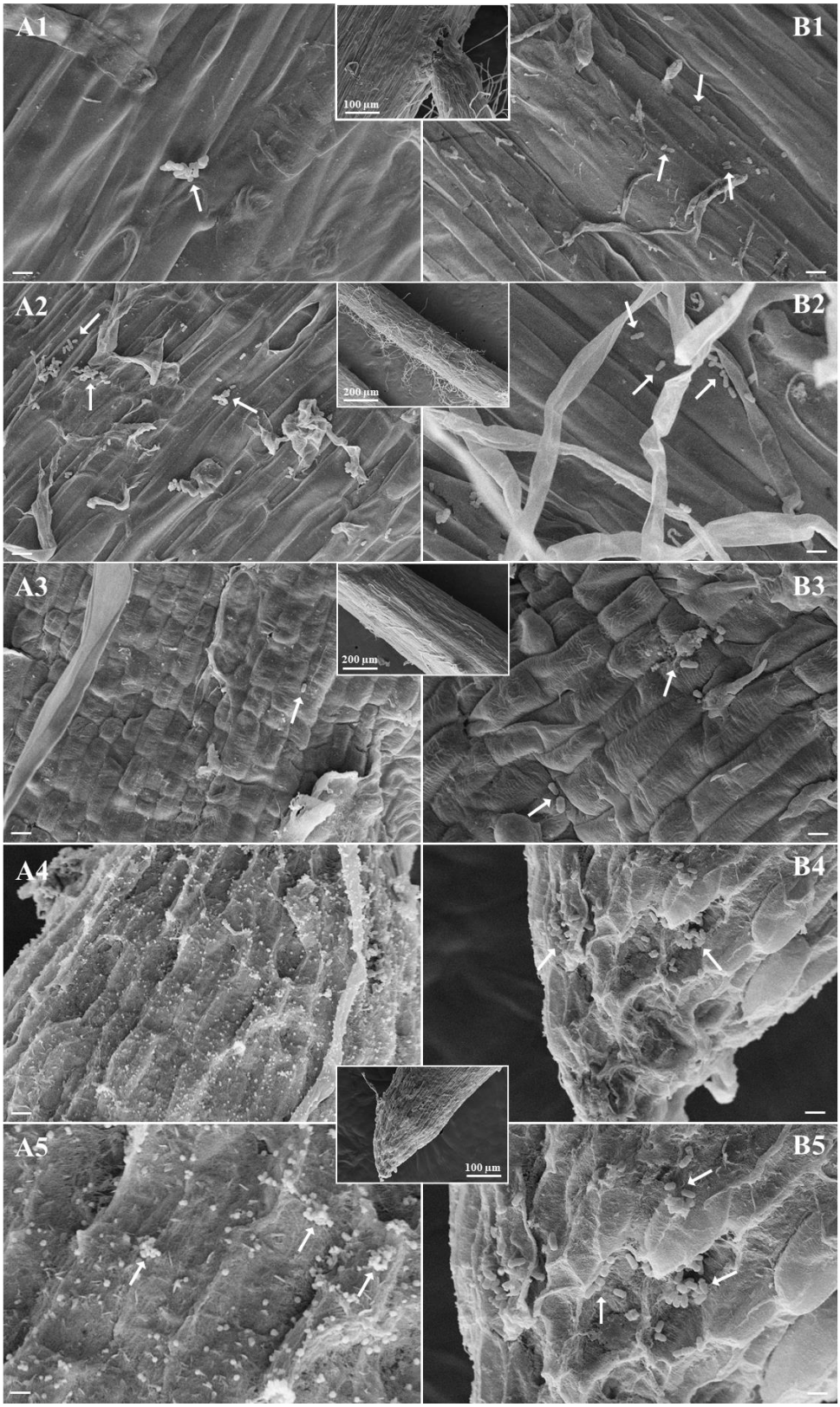

Fig. A.5 Bacterial colonization of maize root visualized by scanning electron microscopy (SEM). Maize seeds non-disinfected (A) and disinfected (B). Root regions: lateral root (A1 and B1), root hair (A2 and B2), zone of elongation (A3 and B3), root cap (A4, A5, B4, and B5). Bacteria and yeast are indicated by arrows. Bars represent the following scales: panel A1 and A5: 20 µm; A2-A4 and B1-B5: 20 µm.

Supplementary Table 1. Distribution of reads per sample of the 5-h water embedded non germinated seed.

X.SampleID	Genotype	Extraction	Disinfection	Treatment	TotalReads	Coverage
O34	DKB177	CTAB	No	S_DKB177_ND_CTAB	106	0.6509434
O33	DKB177	CTAB	Yes	S_DKB177_D_CTAB	92	0.4239130
O32	DKB177	CTAB	Yes	S_DKB177_D_CTAB	183	0.7868852
O36	DKB177	CTAB	No	S_DKB177_ND_CTAB	61	0.4262295
O35	DKB177	CTAB	No	S_DKB177_ND_CTAB	62	0.4516129
O31	DKB177	CTAB	Yes	S_DKB177_D_CTAB	83	0.4216867

Supplementary Table 2. Permanova of the seed bacteriome.

	Df[a]	SumsOfSqs[b]	MeanSqs[c]	F.Model[d]	R^2	Pr(>F)[e]
Disinfection	1	0.282091	0.2820910	0.9659997	0.1945227	0.6
Residuals	4	1.168079	0.2920197	NA	0.8054773	NA
Total	5	1.450170	NA	NA	1.0000000	NA

[a]Df: degrees of freedom
[b]Sum of Sqs: sequential sums of squares
[c]Mean Sqs: mean squares
[d]F. Model: F statistics
[e]Pr>(F): partial R-squared and P values

Supplementary Table 3. Distribution of reads per sample of the 5-d emerged root from germinated seeds.

X.SampleID	Genotype	Extraction	Disinfection	Treatment	TotalReads	Coverage
O18	DKB177	CTAB	No	R_DKB177_ND_CTAB	954	0.9245283
O24	DKB177	CTAB	No	R_DKB177_ND_CTAB	126	0.7222222
O19	DKB177	CTAB	Yes	R_DKB177_D_CTAB	274	0.8686131
O16	DKB177	CTAB	No	R_DKB177_ND_CTAB	373	0.8176944
O15	DKB177	CTAB	Yes	R_DKB177_D_CTAB	409	0.8459658
O14	DKB177	CTAB	Yes	R_DKB177_D_CTAB	492	0.8231707
O21	DKB177	CTAB	Yes	R_DKB177_D_CTAB	582	0.9123711
O20	DKB177	CTAB	Yes	R_DKB177_D_CTAB	17979	0.9987764
O13	DKB177	CTAB	Yes	R_DKB177_D_CTAB	435	0.8459770
O17	DKB177	CTAB	No	R_DKB177_ND_CTAB	493	0.8194726
O23	DKB177	CTAB	No	R_DKB177_ND_CTAB	417	0.8201439
O22	DKB177	CTAB	No	R_DKB177_ND_CTAB	389	0.8868895

Suplementary Table 4. Permanova of the root bacteriome.

	Df[a]	SumsOfSqs[b]	MeanSqs[c]	F.Model[d]	R2	Pr(>F)[e]
Disinfection	1	0.3394742	0.3394742	0.9995477	0.0908717	0.384
Residuals	10	3.3962776	0.3396278	NA	0.9091283	NA
Total	11	3.7357517	NA	NA	1.0000000	NA

[a]Df: degrees of freedom
[b]Sum of Sqs: sequential sums of squares
[c]Mean Sqs: mean squares
[d]F. Model: F statistics
[e]Pr>(F): partial R-squared and P values

Supplementary Table 5. Differential abundance of roots from disinfected versus non-disinfected seeds.

rab.all	rab.win.R_ DKB177_D	rab.win.R_ DKB177_ND	effect	overlap	we.ep	Family	Genus	Species
3.987.430	58.208.770	177.209	-10.068.706	8.072.996	312.351	f__Moraxellaceae	g__*Acinetobacter*	NA*
4.928.825	61.503.817	437.454	-5.952.440	24.739.603	1.441.941	f__Moraxellaceae	g__*Acinetobacter*	s__*rhizosphaerae*
1.314.203	-3.024.789	314.261	6.797.912	18.701.328	1.500.234	f__Brucellaceae	g__*Ochrobactrum*	NA*

*NA = unassigned species

Supplementary Table 6. Differences in protein, glucose, triglyceride, reducing sugar and alpha-amylase activity from different stages of maize germination (seed, embryo axis and root).

	Treatments	
	NDS	**DS**
Protein (ug/ul)		
Seed	0.2805[b]	0.1494[b]
Embryonic axis	0.4385[b]	0.2706[b]
Root	1.7384[a]	1.3464[a]
Glucose (mg/dl)		
Seed	0.0087[b]	0.0099[b]
Embryonic axis	0.0213[b]	0.0189[b]
Root	0.0664[a]	0.0708[a]
Triglyceride (mg/dl)		
Seed	0.0079[a]	0.0034[b]
Embryonic axis	0.0082[a]	0.0069[a]
Root	0.0090[a]	0.0087[a]
Reducing Sugar (ug/ul)		
Seed	0.1471[b]	0.1390[b]
Embryonic axis	0.1738[b]	0.1510[b]
Root	0.2765[a]	0.2756[a]
Alpha-Amylase (U/g)		
Seed	14.8333[c]	8.0000[c]
Embryonic axis	35.6667[b]	24.0000[b]
Root	46.9167[a]	47.6667[a]

Maize seeds non-disinfected (NDS) and disinfected (DS). Different letters in the same column indicate significant differences between stages according to the Tukey test ($p \leq 0.05$).

CHAPTER 4:

Microbial Inoculants in Agriculture and its Effects on Plant Microbiome

Chapter 4: Book chapter

Microbial inoculants in agriculture and its effects on plant microbiome

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Resumo

Produtos microbianos vêm atraindo atenção mundial como tecnologia sustentável para a agricultura. A inoculação microbiana bem-sucedida envolve a formulação adequada e métodos de entrega para superar a competição com a comunidade microbiana natural do solo e das plantas. Por muitos anos, os cientistas relacionaram os efeitos dos bioinoculantes aos seus mecanismos de ação direta nas plantas (biofertilização, bioestimulação, biocontrole e mitigação do estresse abiótico) e excluíram seus efeitos na microbiota indígena. Existem evidências de que a inoculação de bactérias na semente, solo ou planta afeta o crescimento vegetal ao modular a estrutura e a função do microbioma. Neste capítulo, discutimos estudos que lançam luz sobre os efeitos não-alvo dos bioinoculantes na estrutura do bacterioma e correlacionamos esses efeitos com a promoção do crescimento vegetal e o controle de patógenos. Finalmente, destacamos os possíveis mecanismos que conduzem as interações bioinoculante-microbioma e os desafios futuros.

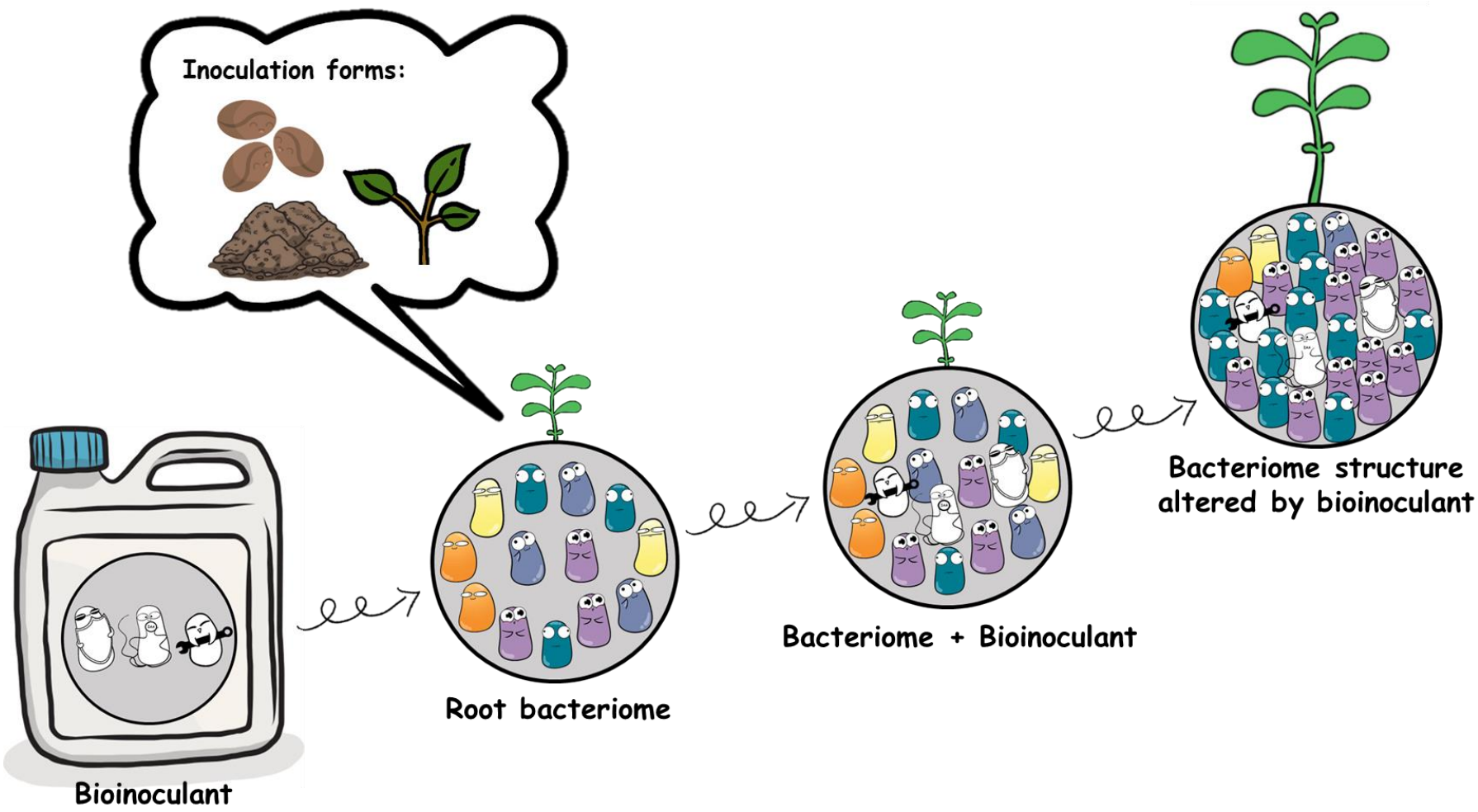
Palavras-chave: Agricultura; Bacterioma; Biocontrole; Bioinoculante; Biofertilização; Bioestimulação; Microbioma; Crescimento vegetal.

Abstract

Microbial products have been attracting attention worldwide as a sustainable technology for agriculture. Successful microbial inoculation involves the proper formulation and delivery methods to overpass the competition with the natural soil-plant microbial community. For many years, scientists linked the effects of bioinoculants on their mechanisms of direct-action on plants (biofertilization, biostimulation, biocontrol and abiotic stress mitigation) and excluded their effects on the indigenous microbiota. There are pieces of evidence that the inoculation of bacteria in the seed, soil or plant affects plant growth by modulating its microbiome structure and function. In this chapter, we discuss the studies that shed light on the non-target effects of bioinoculants on the bacteriome structure and correlate these effects with the promotion of plant growth and the control of pathogens. Finally, we highlight the possible mechanisms that drive bioinoculant-microbiome interactions and future challenges.

Keywords: Agriculture; Bacteriome; Biocontrol; Bioinoculant; Biofertilization; Biostimulation; Microbiome; Plant growth.

Graphical Abstract
(Resumo Gráfico)



1. Introduction

In recent years, with the increase in population, the scarcity of resources and the growing concern about the costs and long-term effects of pesticides and synthetic fertilizers, agriculture has resorted to environmentally sustainable means of increasing crop productivity (Sharma et al., 2016). On the other hand, plants have developed associations with seed and soil microbiomes capable of promoting germination and growth through the secretion of phytohormones, suppression of phytopathogens and increased availability of nutrients (Compant et al., 2019; Glick, 2020; Dos Santos et al., 2020; Santos et al., 2021). In this sense, the use of microbiomes to increase crop productivity seems to be a feasible alternative concerning conventional industrial fertilizers and pesticides. Even recognizing the vast potential of the microbiome, the fundamental and applied researches have been centred on the use of a single strain of certain selected microbes or an empirical mixture of some microorganisms formulated as bioinoculants.

Bioinoculants are the products based on living microorganisms that promote plant growth (Brasil, 2004) by several underlined mechanisms of action that include biocontrol, biostimulation and biofertilization (Sharma et al., 2016). Recently, scientists have begun to realize that these bioinoculants not only have direct effects on plants but also their interaction with the microbiome is a possible mechanism for promoting plant growth (Table 1). Bioinoculant's actions range from deleterious to beneficial effects on plant-resident microbes, contributing to open-field failures and highly variable plant response results, despite their evident efficiency under laboratory and greenhouse conditions (Mohanram & Kumar, 2019; Sessitsch et al., 2019; Misra et al., 2020).

The use of bioinoculants aims to enhance the effect of one or more microorganisms by applying them to the soil or plant in a much higher population size than those found under natural conditions, a technology called microbial enrichment. However, even in higher microbial numbers, the bioinoculant needs to compete and adjust to an already established and locally adapted microbial community (Eisenhauer et al., 2013; Banerjee et al., 2018). Besides the niche occupancy challenger, it is also possible that the inoculant had shown similar microbial function already present in the microbiome community, and then does not provide new or complementary functions (Ambrosini et al., 2015). Nevertheless, if the introduced microorganism can establish itself in the soil or the plant, it can interact with the native microbiota and promote benefits to the plant (Sessitsch et al., 2019).

Although the direct effect of bioinoculant on plants has been vastly studied and critically reviewed (Kour et al., 2020; Sammauria et al., 2020; Tian et al., 2020), the indirect effect on plants by changing the structure of native bacterial communities (plant bacteriome) has been scarcely explored and overlooked until recently. Understanding the underlined mechanism of the interactions of an introduced bacterium with the indigenous microbiota is essential to ensure the bioinoculant's effectiveness. This chapter shows the bioinoculant's impact on plant bacteriome structure and the effects of this modulation on the promotion of growth and control of the pathogens.

2. Plant microbiomes

In the last decade, research on microbiomes has received more attention and has shown their pivotal roles, including plant-protection and plant-growth (Sessitsch et al., 2019). This native microbiota can be found in the plant's different ecological niches within seeds, leaves, and roots (rhizosphere) being the most studied.

The seeds have their microbiome, where they harbour epiphytic microorganisms, located on the surface of the seed, and endophytic microorganisms, which inhabit its interior (Glick, 2020). Seed bacteria can be inherited from the mother plant by vertical transfer and are often associated with the phyla Proteobacteria, Actinobacteria, Firmicutes and Bacteroidetes (Glick, 2020). Currently, 155 bacterial genera have been detected in seeds of different species, and some of them are not found anywhere else in the plant body (Hardoim, 2019). The seed-exclusive bacterial taxon suggests the seed bacteriome due to vertical and horizontal transfers (bacteria from the environment) acting concurrently (Hardoim, 2019). Regarding the contribution of seed microbes, studies show that their bacteria act in germination, protection and plant growth, in addition to controlling the establishment of microbiomes in other organs of the plant (Glick, 2020; Dos Santos et al., 2020; Santos et al., 2021). Hardoim (2019) pointed out that seed endophytes promote benefits by the ability to fix nitrogen, synthesize phytohormones, solubilize phosphate and iron, tolerate adverse conditions and inhibit phytopathogens (through the production of antibiotics, enzymes, and activation of the plant's defense system).

The leaves are also inhabited, on the surface (phyllosphere) and inside (endosphere), by heterogeneous microbial groups, distributed in their numerous microenvironments (Sivakumar et al., 2020). As the leaves are subject to adverse environmental conditions (pathogens, herbivory, temperature, humidity, radiation, and rain), their surface is considered

an oligotrophic environment, which explains their particular microbiota (Liu et al., 2020). In the leaf microbiome, bacteria are predominant and dominated by consistent phylotypes, including Proteobacteria, Actinobacteria, Bacteroidetes, and Firmicutes (Liu et al., 2020). This bacteriome is attributed to the function of controlling pathogens and abiotic stresses, synthesizing phytohormones, degrading pollutants and influencing the functions of the plant ecosystem (Laforest-Lapointe & Whitaker, 2019).

The third compartment of the plant is in direct contact with the soil, which houses a rich microbial life collection. There is a complex "dialogue" between this microbial life and the plants, mediated by the root. The growth of a root in the soil contributes to the formation of the rhizosphere, a region that encompasses three niches: rhizospheric soil, rhizoplane (root surface), and endosphere (root interior) (De la Fuente Cantó et al., 2020). The root and, consequently, its rhizosphere are considered the "microbial hotspots", with the high microbial activity of this region attributed to the process of rhizodeposition (Mohanram and Kumar, 2019). In this process, plant roots secrete various organic compounds in the surrounding soil, including primary and secondary metabolic products, which attract microbes to it (Mohanram & Kumar, 2019; De la Fuente Cantó et al., 2020). The diversity of C-exudates is the main factor responsible for microbial diversity changes of the root and maintains the specific microbial populations (Kalia et al., 2020). Amidst the diversity of the rhizosphere, bacteria are more abundant and often attributed to the phyla Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria (Kour et al., 2019). For plants, the establishment of bacterial communities around the root has positive feedback on the acquisition of nutrients and water, resistance to biotic and abiotic stresses, and morpho-physiological characteristics that increased the plants' ecological fitness (De la Fuente Cantó et al., 2020).

Although the described compartments, i.e. seed, leaf and root are dominated by the same bacterial phyla (Actinobacteria, Bacteroidetes, Firmicutes and Proteobacteria), yet these groups are dynamically shaped by several environmental and host factors, such as soil, climate, plant species, agricultural development and practices (Rodriguez et al., 2019).

Due to their critical roles in agricultural systems, members of the soil and plant microbiome have widely been used as bioinoculants, including plant growth-promoting bacteria (PGPRs) and biocontrol agents.

3. Bioinoculants in agriculture

The functions performed by microorganisms can be used in sustainable food production by reducing or replacing the use of fertilizers and pesticides with bioinoculants, products based on one or some living microorganisms are capable of fixing, solubilizing, mobilizing or decomposing nutrient sources (Mohanram & Kumar, 2019; Sessitsch et al., 2019; Verma et al., 2019). The production of a bioinoculant occurs in several stages, including the selection of useful microorganisms, determination of the functional abilities of the selected microorganisms, an increase of microbial biomass, selection of a carrier, formulation of the bioinoculant, field tests, large-scale production and establishment of quality control, storage and transportation system (Chaudhary et al., 2020). Each step mentioned is decisive for the quality of microbial inoculants.

Microorganisms used in inoculants must promote plant growth by different mechanisms, be compatible with the soil and plant's native microbiota, multiply easily and present no risks to the environment (Macik et al., 2020). Microbes with these characteristics are grown (on a large scale) in an accessible culture medium (low cost and easily found) and nutritious for the strains (Macik et al., 2020). Then, the grown microorganisms are unified with a carrier/vehicle (material that carries and protects the microorganism) of liquid or solid origin, tested in a greenhouse or field (to confirm the benefits and the absence of eco-toxicological effects) and registered (Macik et al., 2020).

Bioinoculants can be applied to the seeds, plants or soil, which vary according to the type of bioinoculant, plant species, environmental, and farmer conditions. Bioinoculants can be classified according to the microorganisms they contain, and their mechanisms of action include three categories: biofertilizer, biostimulant and biocontrol. Currently, these mechanisms are used to explain the direct effects of bioinoculants on plants.

4. Direct effect of bioinoculant on plants

For many years, the benefits of bioinoculants in agricultural production have been attributed solely to their direct effect on plants, achieved through biofertilization, biostimulation and biocontrol (Figure 1).

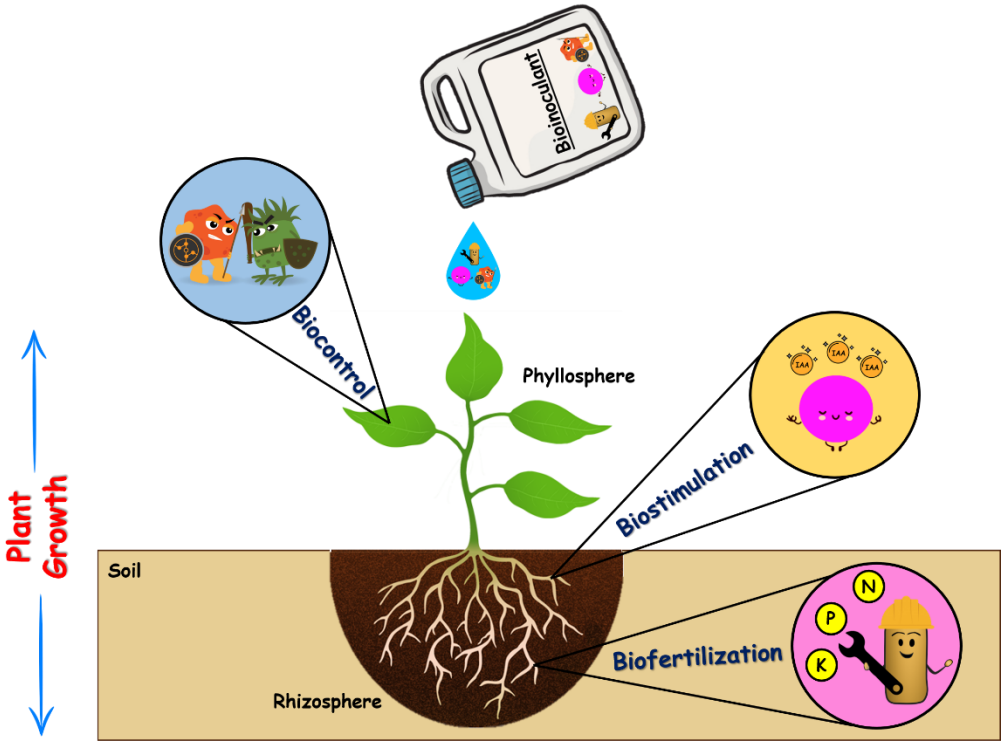


Figure 1. The direct effect of bioinoculant on plant growth through biofertilization biostimulation and biocontrol.

The principle of biofertilization is related to the higher acquisition of nutrients by the plant. These depend on microorganisms to fix, solubilize, mineralize and chelate, respectively, gaseous, inorganic, and organic forms of nitrogen, phosphorus, potassium, zinc, and iron are minimally available to plants (Saad et al., 2020).

Nitrogen is the most abundant element in the atmosphere and a crucial macronutrient for plant growth and development. Although abundant in the atmosphere, it is not in a form available to all living beings. Only bacteria, called diazotrophic, can convert atmospheric nitrogen into a form assimilable by plants and animals, and this process is called biological nitrogen fixation (FBN) (Mahmud et al., 2020). Some of these bacteria can live in symbiotic association with leguminous plants (forming nodules) (examples: *Bradyrhizobium, Mesorhizobium, Rhizobium, Sinorhizobium,* and *Frankia*), while others fix nitrogen in non-legumes and can be free-living or associate with rhizospheric niches and plant endophytes (*Azotobacter, Azospirillum, Azoarcus, Anabaena, Nostoc, Gluconacetobacter, Acetobacter, Burkholderia, Enterobacter, Pseudomonas, Klebsiella* and *Bacillus*) (Glick, 2020; Mahmud et al., 2020; Rawat et al., 2020). Allito et al. (2020) reported that *Rhizobium* inoculation significantly increased nodulation, nitrogen fixation, nutrient absorption and nitrogen balance from the soil. In mung beans and soybeans, Htwe et al. (2019) observed an increase in

growth, nodulation, nitrogen fixation, nitrogen, phosphorus and potassium (NPK) absorption, and seed yield after the inoculation of *Bradyrhizobium* and *Streptomyces griseoflavus*. Biofertilizers based on *Bacillus, Pseudomonas* and *Sinorhizobium meliloti* also increased the nitrogen content in the rhizospheric soil of chilli peppers and improved growth, yield and quality of the fruits (Gou et al., 2020).

After nitrogen, phosphorus is the second most crucial element for plants and is also not readily available in the soil for absorption. However, some bacteria like *Pseudomonas, Bacillus, Agrobacterium, Arthrobacter, Azotobacter, Burkholderia, Enterobacter, Erwinia, Duganella, Pseudoduganella, Variovorax, Kushneria, Paenibacillus, Pantoea, Ralstonia, Rhizobium, Rhodobacter, Rhodococcus, Serratia, Bradyrhizobium, Sinomonas, Thiobacillus* are capable of solubilizing the insoluble phosphate and enriching the soil with this macronutrient (Srivastava et al., 2020). For this, bacteria produce organic acids (such as gluconic acid and 2-ketogluconic acid) and extracellular enzymes (phosphatases and phytases) (Glick, 2020; Rawat et al., 2020). The beneficial impact of phosphorus-solubilizing bacteria has been documented in several plant species. Sarikhani et al. (2020), when using biofertilizer based on bacteria of the genera *Pseudomonas, Enterobacter, Bacillus* and *Pantoe* in maize, observed increases in the fresh and dry weight of the aerial part and root, as well as in the concentration of phosphorus in these regions. Similar results were also found by Zineb et al. (2020) and Borgi et al. (2020) by inoculating, respectively, *Pseudomonas* and *Serratia* in soil with rock phosphate. In both studies, the bacteria increased phosphorus absorption and the growth of *Medicago truncatula* and *Vicia faba*.

Potassium is the third most essential macronutrient for vegetables and is present in the soil as mineral K, making it inaccessible to plants. However, several bacteria can solubilize it and supply the demand for this element for crops (Srivastava et al., 2020). *Pseudomonas, Bacillus, Arthrobacter, Thiobacillus, Burkholderia, Azotobacter, Rhizobium* and *Flavobacterium* are known potassium solubilizing bacteria, and they do this through the secretion of organic acids and acid hydrolysis (Rawat et al., 2020). In a study by Zhao et al. (2019), the inoculant based on *Bacillus megaterium* and *Bacillus mucilaginous* improved the availability of potassium and the growth of chilli peppers. The application of *Bacillus pseudomycoides* with mica significantly increased potassium availability in the soil, which facilitated the absorption of this nutrient by *Camellia sinensis* (Pramanik et al., 2019). According to Khanghahi et al. (2019), inoculation of *Pantoea agglomerans, Rahnella*

aquatilis, and *Pseudomonasorientalis*, alone or combined with half of the recommended K fertilizer, increased the yield of rice grains.

Iron and zinc are the essential micronutrients for plants. Even if required in smaller quantities, the availability of these soil elements is low, which results in deficiencies in crops. Some bacteria (*Pseudomonas, Azotobacter, Bacillus, Enterobacter, Serratia, Azospirillum* and *Rhizobium*) secrete iron-chelating compounds called siderophores supplying iron directly to plants, while the others (*Pseudomonas, Bacillus, Burkholderia, Gluconacetobacter* and *Serratia*) promote the solubilization of zinc through exchange reactions, synthesis of siderophores and organic acids (Macik et al., 2020; Saad et al., 2020; Rawat et al., 2020). In recent studies, Sarwar et al. (2020) showed an increase in the release of iron in the soil after the inoculation of the genus *Bacillus*. Rahimi et al. (2020) found higher iron concentrations in *Cydonia oblonga* treated with the rhizobacterium *Microccucuce yunnanensis*. When inoculating *Bacillus megaterium* in *Capsicum annuum*, Bhatt and Maheshwari (2020) observed increases in plant growth parameters and maximum zinc content in fruits. In another study, *Bacillus* and *Pseudomonas* increased the absorption of zinc and chickpeas' productivity (Zaheer et al., 2019).

The biostimulating action of some microbial inoculants is related to the production of substances that promote plant growth, such as phytohormones, which is the most common mechanism to explain the positive effects of inoculated bacteria. Most plant physiological activities are regulated by phytohormones, including auxins, gibberellins, cytokinins, ethylene, abscisic acid, salicylic acid, jasmonic acid, and brassinosteroids (Glick, 2020). In addition to plants, bacteria also synthesize and/or modulate some phytohormones, such as auxins (indole-3-acetic acid - IAA), gibberellins, cytokinins and ethylene (Glick, 2020). They operate in complex networks to regulate germination, leaf growth, stem, root, flowering, ripening, and senescence (Glick, 2020). An endophytic bacterium and IAA producer, *Mixta theicola*, when inoculated in maize seed, increased germination, root elongation, vigour and fresh and dry seedling biomass (Hagaggi & Mohamed, 2020). The application of the efficient IAA producer *Pantoea agglomerans* in *Corylus avellana* improved the percentage of rooted explant, the number of adventitious roots, survival, and vigour of the plant, in addition to increasing its leaf area (Luziatelli et al., 2020). In addition to IAA, *Bacillus methylotrophicus* is a producer of gibberellin. The inoculation of this bacterium in lettuce increased its germination and biomass (Radhakrishnan & Lee, 2016). A species of seed-borne *Bacillus* (*Bacillus amyloliquefaciens*) also produces gibberellins and promotes rice growth (Shahzad et

al., 2016). Some bacteria promote plant growth under stress conditions through the production or regulation of cytokinin and ethylene phytohormones. According to Jorge et al. (2019), lentils stressed by drought and inoculated with *Methylobacterium oryzae* showed the high levels of cytokinin, better water management and growth; while *Bacillus* producer of ACC (1-aminocyclopropane-1-carboxylate) deaminase was able to mitigate salt stress and promote maize growth by modulating ethylene levels in the plant (Misra & Chauhan, 2020).

For biocontrol, some bacteria (such as *Bacillus, Pseudomonas*, *Streptomyces*, and *Serratia*) defend plants from the infection of various phytopathogens (Rawat et al., 2020). For this, they produce antifungal and antibacterial compounds, enzymes that degrade cell walls, and siderophores. These bacteria can also compete with pathogens for plant nutrients and niches, extinguish quorum, reduce ethylene levels, and induce systemic resistance (Glick, 2020). In a study by Im et al. (2020), *Bacillus methylotrophicus* strongly inhibited the growth of *Ralstonia solanacearum*, which causes tomato wilt, through the production of antibacterial compounds. Similarly, antifungal compounds (diffusible, volatile, and extracellular) from *Bacillus velezensis* inhibited the growth of *Verticillium dahliae* in olive trees (Azabou et al., 2020). The *quorum quenching* activity of *Pseudomonas segetis* reduced the symptoms of soft rot caused by *Dickeya solani*, *Pectobacterium atrosepticum* and *Pectobacterium carotovorum* in potatoes and carrots, in addition to protecting the tomato against *Pseudomonas syringae* (Rodríguez et al., 2020).

The above studies showed that the benefits of bioinoculants have been attributed to its direct effects on crops and have disregarded the indirect effects, which involve the complexity and behaviour of the plants' native microbiota, i.e. microbiomes.

5. Effect of bioinoculants on the structure of the bacteriome with benefits for plants

Bioinoculants are applied in the field in much higher quantities than those found naturally in soils and plants. Although the direct effects of this application on plant growth are well established, few studies have considered the non-target effects of bioinoculants and their beneficial impact on crops. In this interaction, the bioinoculant effect on the native microbiota is variable, where some bacterial groups can be stimulated, others can be inhibited, and in some cases, no changes are observed (Macik et al., 2020). These effects can be transient or long-term (Trabelsi & Mhamdi, 2013; Ambrosini et al., 2016). The result of these interactions of a repressive or inducing nature can be decisive for plant growth (Trabelsi & Mhamdi, 2013; Ambrosini et al., 2016).

Research conducted recently (between 2017 and 2020), in controlled and open field conditions has evaluated the effects of bioinoculants on the resident bacterial community and has found changes in diversity, abundance, and composition of taxonomic groups (Table 1). Several conventional and modern techniques have been applied to access these effects (Sharma et al., 2016), but currently, sequence analyses are by far the most used method. In a survey conducted by Mawarda et al. (2020), changes in the soil microbial community's composition were identified in 96% of the 26 studies with microbial inoculation (all studies used high-throughput sequencing).

Studies that report the effects of bioinoculants on microbiomes are summarized in Table 1. In these studies, one or more combinations of bacteria that act on the growth or defence of several plant species are used. In one of these studies, bionoculants intended to promote growth (*Burkholderia* and *Rhizobium*) and control of pathogens (*Actinomyces*, *Bacillus*and *Aspergillus*) applied to ginseng regulated the soil microbial community by increasing the abundance of beneficial taxa (*Bacillus*, *Burkholderia, Rhizobium, Streptomyces* and *Mycobacterium*) and reduce harmful taxa (*Fusarium*), in addition to increasing the crop yield (Dong et al., 2019). Gu et al. (2020), while using microbial inoculants isolated from forest soil, also observed the changes in the rhizospheric bacterial community structure and positive effects on tomato growth. In the same study, they identified the genera *Streptomyces* and *Enterobacter* as responsible for growth. In another study, inoculants based on *Ensifer*, *Acinetobacter* and *Flavobacterium* promoted the changes in the bacterial community of the indigenous soil with an increase in Gamma-proteobacteria, Acidobacteria, Nitrospirae and Armatimonadetes and a reduction in Actinobacteria and Firmicutes, plus positive effects on cucumber productivity (Wang et al., 2018). The abundance of *Rhodanobacter* spp. and *Mycobacterium* spp., known to cause N losses and plant diseases, was reduced in soybeans after the inoculation of *Paenibacillus mucilaginosus*, while the abundance of beneficial bacteria *Bradyrhizobium* spp. and *Pseudomonas* spp. was increased (Ma et al., 2018). Beneficial effects accompanied changes in the relative abundance of certain taxa on growth, symbiotic nodulation and soybean productivity. Finally, the co-inoculation of *Paenibacillus mucilaginosus* and *Sinorhizobium meliloti* increased the abundance of Firmicutes (including *Bacillus*) and Acidobacteria in the alfalfa rhizosphere and improved its growth (Ju et al., 2020).

The genus *Bacillus* is commonly being used as bioinoculants, a fact attributed to their well-documented agronomic effect (Nambirajan et al., 2020). Since its inoculation directly

affects plant growth, its use will likely also modify the structure of microbiomes. For example, the application of *Bacillus megaterium* and *Bacillus mucilaginous* in pepper increased the abundance of the genus *Flavobacterium* and the availability of phosphorus, potassium (in the soil and plant) and the growth of plants (Zhao et al., 2019). When treated only with *Bacillus megaterium*, rice plants had its beneficial bacteria enriched in the presence of the endophyte (Cheng et al., 2020). The authors correlated microbial changes in the rhizosphere with plant resistance to spikelet rot disease. Inoculation of another species, *Bacillus velezensis*, increased the abundance of beneficial bacteria and reduce pathogens in *Anoectochilus* (Wei et al., 2020) leading to a distinct succession of the rhizospheric microbiota linked to increases in pepper yield (Zhang et al., 2019). Similar results were also found by the other authors when inhibiting the pathogen *Fusarium* sp. with the inoculation of *Bacillus amyloliquefaciens* and *Bacillus* sp., which in turn increased the abundance of disease-suppressing taxa (*Sphingobium, Dyadobacter, Cryptococcus, Lysobacter, Gemmatimonas, Sphingomonas* and *Pseudomonas*) (Fu et al., 2017; Xiong et al., 2017; Shen et al., 2019). With the application of *Bacillus subtilis* in maize, the phyla Bacteroidetes and Chloroflexi became abundant and increased in the use efficiency of nitrogen and culture yield (Sun et al., 2020). The higher abundance of Proteobacteria and Acidobacteria and reduction of Actinobacteria was observed in the rhizosphere of *Prosopis articulata* when receiving *Bacillus pumilus* (Galaviz et al., 2018).

The genus *Pseudomonas* also contributes positively to agriculture and, therefore, is used as bioinoculant; but studies have already pointed out their effect on the plant bacteriome structure. Sharma et al. (2020), when evaluating the total resident (DNA) and active (cDNA) bacterial community of the pigeon pea rhizosphere after receiving *Azotobacter chroococcum*, *Bacillus megaterium* and *Pseudomonas fluorescens*, concluded that the bioinoculant influenced the active community and the absorption of nutrients by the plant. Using the same bioinoculant, Sharma et al. (2017a) observed increases in the abundance of nitrogen-fixing bacteria, *Pseudomonas* and Actinomycetes. The isolated inoculation of *Pseudomonas putida* and *Pseudomonas stutzeri* increased the abundance of several beneficial bacteria in the soil and the rhizosphere, in addition to improving the availability of nutrients and the growth of different cultures (He et al., 2019; Ke et al., 2019; Lu et al., 2020).

The application of *Rhizobium* as a bioinoculant has been carried out for decades to improve plant growth (Sharma et al., 2017b). However, these applications can result in variable effects on the soil and plant resident microbiota (Sharma et al., 2017b). Zhong et al.

(2019) reported that inoculation of *Rhizobium* modulated soybean rhizobacteria communities by increasing the abundance of *Streptomyces, Bradyrhizobium* and *Chryseobacterium* in the rhizosphere. In rice root, the inoculation of *Rhizobium leguminosarum* generated a higher number of operational taxonomic units (OTUs) of *Rhizobium, Azospirillum* and other bacteria of the order Rhizobiales, in addition to contributing to the growth of the crop (Jha et al., 2020).

Like Jha et al. (2020), the other researchers investigated the effect of bioinoculants on the structure of the plant microbiome, especially in the root endophytic environment. Among these studies, Thokchom et al. (2017) showed that the colonization of tangerine roots by *Enterobacter hormaechei*, *Enterobacter asburiae*, *Enterobacter ludwigii* and *Klebsiella pneumoniae* influenced the composition of endophytic and rhizospheric bacterial communities (dominance of Gammaproteobacteria, Firmicutes and Deinococcus-Thermu) and the growth of plants. In *Brassica juncea*, the inoculation of *Serratia marcescens* and *Arthrobacter ginsengisoli* reduced the relative abundance of *Bacillus* and *Fictibacillus* and increased the abundance of *Pseudomonas* and *Microbacterium* in the root, in addition to increasing the root biomass (Wang et al., 2020). Wheat roots inoculated with *Massilia* sp. also had the altered bacterial community, and the increased root biomass (Liu et al., 2017). The inoculation of "living soil" in chrysanthemum increased the abundance of *Pseudomonas* and *Acinetobacter* in the leaves, while *Enterobacter* and *Luteibacter* were abundant in the root (Pangesti et al., 2020).

In general, 26 studies showed bioinoculants (with varying composition) changing the abundance of bacterial taxa residing in the soil and plants (rhizospheric and root endophytic region) (Table 1). Also, those studies related the observed plant growth with an increased relative abundance of specific microbial taxa. Nevertheless, it is not yet clear how inoculation impacts microbiomes.

Table 1. The effect of bioinoculants on the structure of microbiomes. The studies presented are recent (2017-2020), and all used high-throughput sequencing.

Bioinoculant	***Effect on the microbiome***	***Microbiome analyzed***	***Plant species***	***Growth regulation***	***Reference***
Bacillus amyloliquefaciens	Greater abundance of *Sphingobium*, *Dyadobacter* and *Cryptococcus* and lower abundance of *Fusarium*, *Ralstonia* and *Burkholderia*	Rhizosphere	Banana (*Musa* spp.)	Biocontrol of *Fusarium oxysporum*	Fu et al. (2017)
Azotobacter chroococcum, *Bacillus megaterium*, and *Pseudomonas fluorescens*	Abundance of nitrogen-fixing bacteria, *Pseudomonas* and Actinomycetes	Rhizosphere	Pigeon pea (*Cajanus cajan*)	Greater growth	Sharma et al. (2017a)
Bacillus amyloliquefaciens	Greater abundance of *Lysobacter*	Soil	-	Biocontrol of *Fusarium oxysporum*	Xiong et al. (2017)
Massilia sp.	Dominance of *Pseudomonas*	Root	Wheat (*Triticum aestivum*)	Increase in root biomass	Liu et al. (2017)
Enterobacter hormaechei, *Enterobacter asburiae*, *Enterobacter ludwigii*, and *Klebsiella pneumoniae*	The dominance of Gammaproteobacteria, Firmicutes and Deinococcus-Thermus	Root Rhizosphere	Mandarin orange (*Citrus reticulata*)	Greater growth	Thokchom et al. (2017)
Ensifer sp., *Acinetobacter* sp., and *Flavobacterium* sp.	Increase of Gammaproteobacteria, Acidobacteria, Nitrospirae and Armatimonadetes; and reduction of Actinobacteria and Firmicutes	Soil	Cucumber (*Cucumis sativus*)	Increased production	Wang et al. (2018)
Paenibacillus mucilaginosus	Greater abundance of *Bradyrhizobium* spp. And *Pseudomonas* spp., while the abundance of *Rhodanobacter* spp. And *Mycobacterium* spp. decreased	Soil	Soy (*Glycine max*)	Growth, symbiotic nodulation, and productivity	Ma et al. (2018)
Bacillus pumilus	Greater abundance of Proteobacteria and Acidobacteria and reduction of Actinobacteria	Rhizosphere	Mesquite (*Prosopis articulata*)	Increase in root biomass	Galaviz et al. (2018)
Bacillus cereus, *Bacillus subtilis*, and *Bacillus*	Abundance reduction of *Bacillus* (inoculated bacteria), *Lysobacter* and	Root	Broccoli (*Brassica oleracea*)	-	Gadhave et al. (2018)

amyloliquefaciens	*Acidovorax*, while the abundance of *Acinetobacter* increased				
Burkholderia and *Rhizobium*	Increased abundance of beneficial bacteria *Bacillus*, *Burkholderia*, *Rhizobium*, *Streptomyces* and *Mycobacterium*	Soil	Ginseng (*Panax ginseng*)	Biocontrol of *Fusarium oxysporum* and increased production	Dong et al. (2019)
Actinomyces, *Bacillus*, and *Aspergillus*					
Bacillus megaterium and *Bacillus mucilaginous*	Greater relative abundance of the genus *Flavobacterium*	Soil	Chilli (*Capsicum annuum*)	Increase in available phosphorus and potassium and increased growth	Zhao et al. (2019)
Pseudomonas putida	Abundance of the genera *Blastococcus*, AKYG587 and *Pseudomonas* was increased after inoculation, while the abundance of *Solirubrobacter*, *Roseiflexus*, *Actinoplanes* and *Skermanella* decreased	Soil Rhizosphere	Chilli (*Capsicum annuum*)	Increased absorption of nitrogen, phosphorus, and potassium and increased biomass	He et al. (2019)
Bacillus velezensis	Greater abundance of genera *Sphingomonas*, *Sphingopyxis*, *Bradyrhizobium*, *Chitinophaga*, *Dyadobacter*, *Streptomyces*, *Lysobacter*, *Pseudomonas* and *Rhizomicrobium*	Rhizosphere	Chilli (*Capsicum annuum*)	Increased yield	Zhang et al. (2019)
Pseudomonas stutzeri	Increase of nitrogen-fixing communities and ammonia oxidants	Soil Rhizosphere	Maize (*Zea mays*)	Growth and accumulation of nitrogen	Ke et al. (2019)
Bacillus sp.	Stimulation of *Gemmatimonas*, *Sphingomonas*, *Pseudomonas*, *Lysobacter* and *Bacillus*	Soil Rhizosphere	Banana (*Musa acuminata*)	Biocontrol of *Fusarium oxysporum*	Shen et al. (2019)
Rhizobium	Greater abundance of *Streptomyces*, *Bradyrhizobium*, and *Chryseobacterium*	Rhizosphere	Soy (*Glycine max*)	Height increase, biomass, and number of nodules	Zhong et al. (2019)
Microbial inoculants from forest soil	*Streptomyces* and *Enterobacter* genera were positively associated with plant growth	Rhizosphere	Tomato (*Solanum lycopersicum*)	Positive effect on the assimilation of nutrients and biomass	Gu et al. (2020)

Azotobacter chroococcum, *Bacillus megaterium*, and *Pseudomonas fluorescens*	Active bacterial community (cDNA study) was influenced by inoculation	Rhizosphere	Pigeon pea (*Cajanus cajan*)	Better nutrient absorption	Sharma et al. (2020)
Pseudomonas putida	The relative abundance of *Bacillus, Halomonas, Delftia, Brevibacterium, Acinetobacter, Pseudomonas, Aquicella, Flavobacterium, Niastella, Arenimonas, Pontibacter, Reyranella, Mesorhizobium, Phaselicystis* and *Acidibacter* was increased, while *Nitrospira, Aeromicrobium* and *Polycyclovorans* were reduced	Soil	Grape (*Vitis vinífera*)	Increase in the amount of available phosphorus and better growth	Lu et al. (2020)
Bacillus megaterium	Enrichment of beneficial bacteria	Rhizosphere	Rice (*Oryza sativa*)	Reduced incidence of spikelet rot disease	Cheng et al. (2020)
Bacillus subtilis	Increased abundance of Bacteroidetes and Chloroflexi	Soil	Maize (*Zea mays*)	Increase in nitrogen use efficiency and higher yield	Sun et al. (2020)
Bacillus velezensis	Increase in the abundance of beneficial bacteria and reduction of Xanthobacteraceae, *Cladophialophora* and *Penicillium* pathogens	Rhizosphere	Orchid *(Anoectochilus roxburghii* e *Anoectochilus formosanus*)	Greater growth	Wei et al. (2020)
Paenibacillus mucilaginosus, and *Sinorhizobium meliloti*	Greater abundance of Firmicutes (including *Bacillus*) and Acidobacteria	Rhizosphere	Alfalfa (*Medicago sativa*)	Greater growth	Ju et al. (2020)
Rhizobium leguminosarum	Higher number of operating taxonomic units (OTUs) of *Rhizobium*, *Azospirillum* and unclassified Rhizobiales	Root	Rice (Oryza sativa)	Greater growth	Jha et al. (2020)
Serratia marcescens, and *Arthrobacter ginsengisoli*	Reduction in the relative abundance of *Bacillus* and *Fictibacillus* and increase in *Pseudomonas* and *Microbacterium*	Root	Indian mustard (*Brassica juncea*)	Higher biomass	Wang et al. (2020)
Soil inoculation with its indigenous microbiota	*Pseudomonas* and *Acinetobacter* were abundant in the leaf, while *Enterobacter* and *Luteibacter* were abundant at the root	Leaf Root	Chrysanthemum (*Dendranthema grandiflora*)	-	Pangesti et al. (2020)

6. How does the bioinoculants change the structure of the bacteriome?

Although the studies presented here show that bioinoculation affects the structure of bacterial communities in soil and plants, little is known about the mechanisms behind the changes in the abundance, structure, and activity of microbiomes. Mawarda et al. (2020) proposed four mechanisms that can drive these changes. The first is the competition for resources (Figure 2b), where the bioinoculant, with initially high population size, surpasses native taxa that compete for the same resources (Yang et al., 2017). When it becomes abundant, the introduced bioinoculant can suppress similar functional taxa or stimulate unrelated taxa multiplication (Mawarda et al., 2020). In this case, the bioinoculants will have a high chance of successfully establishing itself in the new habitat, if there are empty niches (not occupied by the native microbiota) (Mawarda et al., 2020).

For non-established bioinoculants, the results of transient and unsuccessful invasions can also induce changes in the composition of the native community (Mawarda et al., 2020). In this case, the fact that the invader is in higher numbers will guarantee its triumph over the resident taxa, which will seek the other niches (Mallon et al., 2018). Even if this invader is later eliminated from the community, it has left unoccupied niches that can make new invasions (from the same or similar invader) successful and lasting (Mallon et al., 2018). In an experiment carried out by Gadhave et al. (2018), the recovery of three *Bacillus* species inoculated in broccoli was very low; even so, the inoculant altered the indigenous community root by reducing the abundance of *Lysobacter* and *Acidovorax* and increasing the abundance of *Acinetobacter*.

The second and third mechanism proposed by Mawarda et al. (2020) involves antagonism and synergism between bioinoculants and microbiomes (Figure 2b). In the case of antagonism, the bioinoculant that targets specific pathogens can also suppress the growth and activity of non-target microbial taxa through previously described biocontrol mechanisms (topic 4). In synergism, bioinoculants that produce specific metabolites can stimulate the growth of the native microbiota. Recently, Li et al. (2019) showed that facilitating interactions with the resident community promoted and antagonistic interactions suppressed the invasion of the pathogenic bacterium *Ralstonia solanacearum* in tomatoes.

In the fourth and final proposed mechanism, Mawarda et al. (2020) explain that the bioinoculant can affect native communities by modifying the rate and composition of exudates released by plant roots (Figure 2b). Many bioinoculants boost root growth and alter their exudation pattern, which modifies the rhizospheric microbiome by enriching specific

taxa. Florio et al. (2017; 2019) showed that the inoculation of *Azospirillum lipoferum* altered the root exudation of maize and increased the abundance of denitrifying groups in the soil.

It is not yet clear whether the impact generated in the microbiome by bioinoculation persists for long periods or is transient. For Mawarda et al. (2020), the native microbiota can be resilient and recover its initial composition at variable time intervals (transient impact) (Figure 2a). However, these authors also warn about the possibility that the microbiome does not return or does not return quickly to its initial composition (non-transient impact), and this irreversible change may affect the functioning of the soil and plants (Figure 2a). If the microbiome structure does not change after inoculation, the community is said to be resistant (Figure 2a). As most studies carried out to date have evaluated the non-target effect of bioinoculants for a short period, it is difficult to conclude whether their impact on the microbiome persists for a longer time. Studies that seek out to measure the duration of this impact in different field conditions are required.

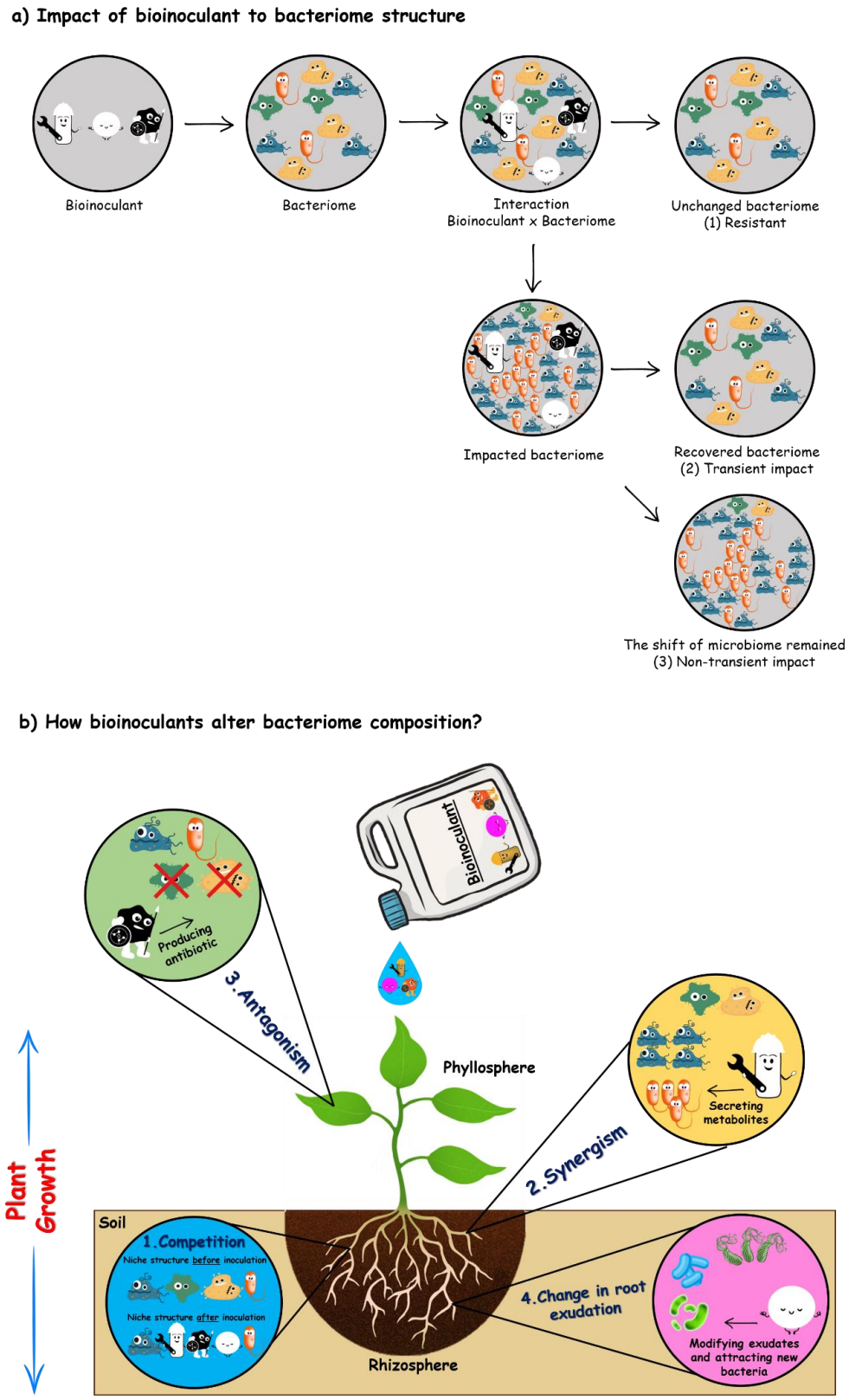


Figure 2. The indirect impact of bioinoculant on plants by changing the structure of bacteriome and possible mechanisms explaining these changes. After inoculation, the structure of the bacteriome may not change (a1) or maybe transient (a2) or permanent (a3). The interaction between the bioinoculant and the bacteriome can occur through competition for resources (b1), synergism (b2), antagonism (b3) and alteration of root exudation (b4).

7. Conclusion and future perspectives

Relating the bacteriome structure to the growth and health of plants has made it difficult to understand and recognize the indirect mechanisms of bioinoculants as promoters of plant growth. Based on the studies, it is evident that the promotion of growth by an inoculated bacterium includes, in addition to direct effects (acquisition of nutrients, production of phytohormones, and biocontrol), its interaction with the native microbiota of the soil and plants. Accordingly, changes in the taxonomic groups of the microbiome need to be further investigated along with the impact of this change on the functioning of indigenous microbial communities and plant growth. For this, multi-omic studies need to be further explored, since the microbiome's impact and resilience after bioinoculation will only be adequately evaluated when combining taxonomic and functional characteristics of the community. More research in this regard is necessary to ensure the effectiveness of bioinoculants and microbiomes' proper functioning.

8. References

Allito, B. B., Ewusi-Mensah, N., & Logah, V. (2020). Legume-Rhizobium Strain Specificity Enhances Nutrition and Nitrogen Fixation in Faba Bean (*Vicia faba* L.). *Agronomy, 10*(6), 826.

Ambrosini, A., de Souza, R., & Passaglia, L. M. P. (2016). Ecological role of bacterial inoculants and their potential impact on soil microbial diversity. *Plant and Soil, 400*(1), 193-207.

Banerjee, S., Schlaeppi, K., & van der Heijden, M. G. A. (2018). Keystone taxa as drivers of microbiome structure and functioning. *Nature Reviews Microbiology, 16*(9), 567-576.

Ben Zineb, A., Trabelsi, D., Ayachi, I., Barhoumi, F., Aroca, R., & Mhamdi, R. (2020). Inoculation with Elite Strains of Phosphate-Solubilizing Bacteria Enhances the Effectiveness of Fertilization with Rock Phosphates. *Geomicrobiology Journal, 37*(1), 22-30.

Bhatt, K., & Maheshwari, D. K. (2020). Zinc solubilizing bacteria (*Bacillus megaterium*) with multifarious plant growth-promoting activities alleviates growth in *Capsicum annuum* L. *3 Biotech, 10*(2), 36.

Borgi, M. A., Saidi, I., Moula, A., Rhimi, S., & Rhimi, M. (2020). The Attractive *Serratia plymuthica* BMA1 Strain With High Rock Phosphate-Solubilizing Activity and Its Effect on the Growth and Phosphorus Uptake by *Vicia faba* L. Plants. *Geomicrobiology Journal, 37*(5), 437-445.

Brasil. Decreto n° 4.954, de 14 de janeiro de 2004. Aprova o regulamento da Lei no 6.894, de 16 de dezembro de 1980, que dispõe sobre a inspeção e fiscalização da produção e do comércio de fertilizantes, corretivos, inoculantes ou biofertilizantes destinados à agricultura, e dá outras providências. Diário Oficial [da] República Federativa do Brasil, Brasília, DF, 15 jan. 2004.

Chaudhary, T., Dixit, M., Gera, R., Shukla, A. K., Prakash, A., Gupta, G., et al. (2020). Techniques for improving formulations of bioinoculants. *3 Biotech, 10*(5), 199.

Cheffi Azabou, M., Gharbi, Y., Medhioub, I., Ennouri, K., Barham, H., Tounsi, S., et al. (2020). The endophytic strain *Bacillus velezensis* OEE1: An efficient biocontrol agent against *Verticillium* wilt of olive and a potential plant growth promoting bacteria. *Biological Control, 142*, 104168.

Cheng, T., Yao, X.-Z., Wu, C.-Y., Zhang, W., He, W., & Dai, C.-C. (2020). Endophytic *Bacillus megaterium* triggers salicylic acid-dependent resistance and improves the rhizosphere bacterial community to mitigate rice spikelet rot disease. *Applied Soil Ecology, 156*, 103710.

Compant, S., Samad, A., Faist, H., & Sessitsch, A. (2019). A review on the plant microbiome: Ecology, functions, and emerging trends in microbial application. *Journal of Advanced Research, 19*, 29-37.

De la Fuente Cantó, C., Simonin, M., King, E., Moulin, L., Bennett, M. J., Castrillo, G., et al. (2020). An extended root phenotype: the rhizosphere, its formation and impacts on plant fitness. *The Plant Journal, 103*(3), 951-964.

Dong, L., Li, Y., Xu, J., Yang, J., Wei, G., Shen, L., et al. (2019). Biofertilizers regulate the soil microbial community and enhance *Panax ginseng* yields. *Chinese Medicine, 14*(1), 20.

Dos Santos, L. F., Souta, J. F., de Paula Soares, C., da Rocha, L. O., Santos, M. L. C., de Matos, C. G. G., et al. (2020). Insights into the structure and role of seed-borne bacteriome during maize germination. doi.org/10.1101/2020.06.02.130856.

Eisenhauer, N., Schulz, W., Scheu, S., & Jousset, A. (2013). Niche dimensionality links biodiversity and invasibility of microbial communities. *Functional Ecology, 27*(1), 282-288.

Florio, A., Bréfort, C., Gervaix, J., Bérard, A., & Le Roux, X. (2019). The responses of $NO2^-$- and N_2O-reducing bacteria to maize inoculation by the PGPR *Azospirillum lipoferum* CRT1 depend on carbon availability and determine soil gross and net N_2O production. *Soil Biology and Biochemistry, 136*, 107524.

Florio, A., Pommier, T., Gervaix, J., Bérard, A., & Le Roux, X. (2017). Soil C and N statuses determine the effect of maize inoculation by plant growth-promoting rhizobacteria on nitrifying and denitrifying communities. *Scientific Reports, 7*(1), 8411.

Fu, L., Penton, C. R., Ruan, Y., Shen, Z., Xue, C., Li, R., et al. (2017). Inducing the rhizosphere microbiome by biofertilizer application to suppress banana *Fusarium* wilt disease. *Soil Biology and Biochemistry, 104*, 39-48.

Gadhave, K. R., Devlin, P. F., Ebertz, A., Ross, A., & Gange, A. C. (2018). Soil Inoculation with *Bacillus* spp. Modifies Root Endophytic Bacterial Diversity, Evenness, and Community Composition in a Context-Specific Manner. *Microbial Ecology, 76*(3), 741-750.

Galaviz, C., Lopez, B. R., de-Bashan, L. E., Hirsch, A. M., Maymon, M., & Bashan, Y. (2018). Root growth improvement of mesquite seedlings and bacterial rhizosphere and soil community changes are induced by inoculation with plant growth-promoting bacteria and promote restoration of eroded desert soil. *Land Degradation & Development, 29*(5), 1453-1466.

Glick, B. R. (2020). Microbiomes and Endophytes. In *Beneficial Plant-Bacterial Interactions* (pp. 39-62). Cham: Springer International Publishing.

Gou, J. Y., Suo, S. Z., Shao, K. Z., Zhao, Q., Yao, D., Li, H. P., et al. (2020). Biofertilizers with beneficial rhizobacteria improved plant growth and yield in chilli (*Capsicum annuum* L.). *World Journal of Microbiology and Biotechnology, 36*(6), 86.

Gu, Y., Dong, K., Geisen, S., Yang, W., Yan, Y., Gu, D., et al. (2020). The effect of microbial inoculant origin on the rhizosphere bacterial community composition and plant growth-promotion. *Plant and Soil, 452*(1), 105-117.

Hagaggi, N. S. A., & Mohamed, A. A. A. (2020). Enhancement of *Zea mays* (L.) growth performance using indole acetic acid producing endophyte *Mixta theicola* isolated from*Solenostemmaargel*(Hayne). *South African Journal of Botany*.doi.org/10.1016/j.sajb.2020.02.034.

Hardoim, P. (2019). The Ecology of Seed Microbiota. In S. K. Verma & J. J. F. White (Eds.), *Seed Endophytes: Biology and Biotechnology* (pp. 103-125). Cham: Springer International Publishing.

He, Y., Wu, Z., Wang, W., Liu, X., & Ye, B.-C. (2019). Bacterial community and phosphorus species changes in pepper rhizosphere soils after *Pseudomonas putida* Rs-198 inoculation. *Rhizosphere, 11*, 100164.

Htwe, A. Z., Moh, S. M., Soe, K. M., Moe, K., & Yamakawa, T. (2019). Effects of Biofertilizer Produced from *Bradyrhizobium* and *Streptomyces griseoflavus* on Plant Growth, Nodulation, Nitrogen Fixation, Nutrient Uptake, and Seed Yield of Mung Bean, Cowpea, and Soybean. *Agronomy, 9*(2), 77.

Im, S. M., Yu, N. H., Joen, H. W., Kim, S. O., Park, H. W., Park, A. R., et al. (2020). Biological control of tomato bacterial wilt by oxydifficidin and difficidin-producing *Bacillus methylotrophicus* DR-08. *Pesticide Biochemistry and Physiology, 163*, 130-137.

Jha, P. N., Gomaa, A.-B., Yanni, Y. G., El-Saadany, A.-E. Y., Stedtfeld, T. M., Stedtfeld, R. D., et al. (2020). Alterations in the Endophyte-Enriched Root-Associated Microbiome of Rice Receiving Growth-Promoting Treatments of Urea Fertilizer and *Rhizobium* Biofertilizer. *Microbial Ecology, 79*(2), 367-382.

Jorge, G. L., Kisiala, A., Morrison, E., Aoki, M., Nogueira, A. P. O., & Emery, R. J. N. (2019). Endosymbiotic *Methylobacterium oryzae* mitigates the impact of limited water availability in lentil (*Lens culinaris* Medik.) by increasing plant cytokinin levels. *Environmental and Experimental Botany, 162*, 525-540.

Ju, W., Jin, X., Liu, L., Shen, G., Zhao, W., Duan, C., et al. (2020). Rhizobacteria inoculation benefits nutrient availability for phytostabilization in copper contaminated soil: Drivers from bacterial community structures in rhizosphere. *Applied Soil Ecology, 150*, 103450.

Kalia, A., Sharma, S. P., Kaur, S., & Kaur, H. (2020). Bacterial Inoculants: How Can These Microbes Sustain Soil Health and Crop Productivity? In B. Giri & A. Varma (Eds.), *Soil Health* (pp. 337-372). Cham: Springer International Publishing.

Kang, S.M., Khan, A. L., Waqas, M., Asaf, S., Lee, K.E., Park, Y.G., et al. (2019). Integrated phytohormone production by the plant growth-promoting rhizobacterium *Bacillus tequilensis* SSB07 induced thermotolerance in soybean. *Journal of Plant Interactions, 14*(1), 416-423.

Ke, X., Feng, S., Wang, J., Lu, W., Zhang, W., Chen, M., et al. (2019). Effect of inoculation with nitrogen-fixing bacterium *Pseudomonas stutzeri* A1501 on maize plant growth and the microbiome indigenous to the rhizosphere. *Systematic and Applied Microbiology, 42*(2), 248-260.

Kour, D., Rana, K. L., Yadav, A. N., Yadav, N., Kumar, M., Kumar, V., et al. (2020). Microbial biofertilizers: Bioresources and eco-friendly technologies for agricultural and environmental sustainability. *Biocatalysis and Agricultural Biotechnology, 23*, 101487.

Kour, D., Rana, K. L., Yadav, N., Yadav, A. N., Kumar, A., Meena, V. S., et al. (2019). Rhizospheric Microbiomes: Biodiversity, Mechanisms of Plant Growth Promotion, and Biotechnological Applications for Sustainable Agriculture. In A. Kumar & V. S. Meena (Eds.), *Plant Growth Promoting Rhizobacteria for Agricultural Sustainability: From Theory to Practices* (pp. 19-65). Singapore: Springer Singapore.

Laforest-Lapointe, I., & Whitaker, B. K. (2019). Decrypting the phyllosphere microbiota: progress and challenges. *American Journal of Botany, 106*(2), 171-173.

Li, M., Wei, Z., Wang, J., Jousset, A., Friman, V.-P., Xu, Y., et al. (2019). Facilitation promotes invasions in plant-associated microbial communities. *Ecology Letters, 22*(1), 149-158.

Liu, H., Brettell, L. E., & Singh, B. (2020). Linking the Phyllosphere Microbiome to Plant Health. *Trends in Plant Science, 25(9),* 841-844.

Liu, J., Xiang, Y., Zhang, Z., Ling, W., & Gao, Y. (2017). Inoculation of a phenanthrene-degrading endophytic bacterium reduces the phenanthrene level and alters the bacterial community structure in wheat. *Applied Microbiology and Biotechnology, 101*(12), 5199-5212.

Lu, H., Wu, Z., Wang, W., Xu, X., & Liu, X. (2020). Rs-198 Liquid Biofertilizers Affect Microbial Community Diversity and Enzyme Activities and Promote *Vitis vinifera*L. Growth. *BioMed Research International, 2020,* 8321462.

Luziatelli, F., Ficca, A. G., Bonini, P., Muleo, R., Gatti, L., Meneghini, M., et al. (2020). A Genetic and Metabolomic Perspective on the Production of Indole-3-Acetic Acid by *Pantoea agglomerans* and Use of Their Metabolites as Biostimulants in Plant Nurseries. *Frontiers in Microbiology, 11*, 1475.

Ma, M., Jiang, X., Wang, Q., Guan, D., Li, L., Ongena, M., et al. (2018). Isolation and identification of PGPR strain and its effect on soybean growth and soil bacterial community composition. *International Journal of Agriculture and Biology, 20*(6), 1289-1297.

Mącik, M., Gryta, A., & Frąc, M. (2020). Chapter Two - Biofertilizers in agriculture: An overview on concepts, strategies and effects on soil microorganisms. In D. L. Sparks (Ed.), *Advances in Agronomy* (Vol. 162, pp. 31-87): Academic Press.

Mahmud, K., Makaju, S., Ibrahim, R., & Missaoui, A. (2020). Current Progress in Nitrogen Fixing Plants and Microbiome Research. *Plants, 9*(1), 97.

Mallon, C. A., Le Roux, X., van Doorn, G. S., Dini-Andreote, F., Poly, F., & Salles, J. F. (2018). The impact of failure: unsuccessful bacterial invasions steer the soil microbial community away from the invader's niche. *The ISME Journal, 12*(3), 728-741.

Mawarda, P. C., Le Roux, X., Dirk van Elsas, J., & Salles, J. F. (2020). Deliberate introduction of invisible invaders: A critical appraisal of the impact of microbial inoculants on soil microbial communities. *Soil Biology and Biochemistry, 148*, 107874.

Misra, M., Sachan, A., & Sachan, S. G. (2020). Current Aspects and Applications of Biofertilizers for Sustainable Agriculture. In A. N. Yadav, J. Singh, A. A. Rastegari & N. Yadav (Eds.), *Plant Microbiomes for Sustainable Agriculture* (pp. 445-473). Cham: Springer International Publishing.

Misra, S., & Chauhan, P. S. (2020). ACC deaminase-producing rhizosphere competent Bacillus spp. mitigate salt stress and promote *Zea mays* growth by modulating ethylene metabolism. *3 Biotech, 10*(3), 119.

Mohanram, S., & Kumar, P. (2019). Rhizosphere microbiome: revisiting the synergy of plant-microbe interactions. *Annals of Microbiology, 69*(4), 307-320.

Nambirajan, G., Ashok, G., Baskaran, K., & Viswanathan, C. (2020). *Bacillus* and Endomicrobiome: Biodiversity and Potential Applications in Agriculture. In A. N. Yadav, A. A. Rastegari, N. Yadav & D. Kour (Eds.), *Advances in Plant Microbiome and Sustainable Agriculture: Diversity and Biotechnological Applications* (pp. 189-205). Singapore: Springer Singapore.

Pangesti, N., Pineda, A., Hannula, S. E., & Bezemer, T. M. (2020). Soil inoculation alters the endosphere microbiome of chrysanthemum roots and leaves. *Plant and Soil*.doi.org/10.1007/s11104-020-04655-5

Pramanik, P., Goswami, A. J., Ghosh, S., & Kalita, C. (2019). An indigenous strain of potassium-solubilizing bacteria *Bacillus pseudomycoides* enhanced potassium uptake in tea plants by increasing potassium availability in the mica waste-treated soil of North-east India. *Journal of Applied Microbiology, 126*(1), 215-222.

Radhakrishnan, R., & Lee, I.-J. (2016). Gibberellins producing *Bacillus methylotrophicus* KE2 supports plant growth and enhances nutritional metabolites and food values of lettuce. *Plant Physiology and Biochemistry, 109*, 181-189.

Rahimi, S., Talebi, M., Baninasab, B., Gholami, M., Zarei, M., & Shariatmadari, H. (2020). The role of plant growth-promoting rhizobacteria (PGPR) in improving iron acquisition by altering physiological and molecular responses in quince seedlings. *Plant Physiology and Biochemistry, 115*, 406-415.

Rawat, P., Shankhdhar, D., & Shankhdhar, S. C. (2020). Plant Growth-Promoting Rhizobacteria: A Booster for Ameliorating Soil Health and Agriculture Production. In B. Giri & A. Varma (Eds.), *Soil Health* (pp. 47-68). Cham: Springer International Publishing.

Rodríguez, M., Torres, M., Blanco, L., Béjar, V., Sampedro, I., & Llamas, I. (2020). Plant growth-promoting activity and quorum quenching-mediated biocontrol of bacterial phytopathogens by *Pseudomonas segetis* strain P6. *Scientific Reports, 10*(1), 4121.

Rodriguez, P. A., Rothballer, M., Chowdhury, S. P., Nussbaumer, T., Gutjahr, C., & Falter-Braun, P. (2019). Systems Biology of Plant-Microbiome Interactions. *Molecular Plant, 12*(6), 804-821.

Saad, M. M., Eida, A. A., & Hirt, H. (2020). Tailoring plant-associated microbial inoculants in agriculture: a roadmap for successful application. *Journal of Experimental Botany, 71*(13), 3878-3901.

Sammauria, R., Kumawat, S., Kumawat, P., Singh, J., & Jatwa, T. K. (2020). Microbial inoculants: potential tool for sustainability of agricultural production systems. *Archives of Microbiology, 202*(4), 677-693.

Santos, L. F., Souta, J. F., Rocha, L. O., Soares, C. d. P., Santos, M. L. C., Grativol, C., et al. (2021). Altered bacteria community dominance reduces tolerance to resident fungus and seed to seedling growth performance in maize (*Zea mays* L. var. DKB 177). *Microbiological Research*, 243, 126643.

Sarikhani, M. R., Aliasgharzad, N., & Khoshru, B. (2020). P Solubilizing Potential of Some Plant Growth Promoting Bacteria Used as Ingredient in Phosphatic Biofertilizers with Emphasis on Growth Promotion of *Zea mays* L. *Geomicrobiology Journal, 37*(4), 327-335.

Sarwar, S., Khaliq, A., Yousra, M., Sultan, T., Ahmad, N., & Khan, M. Z. (2020). Screening of Siderophore-Producing PGPRs Isolated from Groundnut (*Arachis hypogaea* L.) Rhizosphere and

Their Influence on Iron Release in Soil. *Communications in Soil Science and Plant Analysis, 51*(12), 1680-1692.

Sessitsch, A., Pfaffenbichler, N., & Mitter, B. (2019). Microbiome Applications from Lab to Field: Facing Complexity. *Trends in Plant Science, 24*(3), 194-198.

Shahzad, R., Waqas, M., Khan, A. L., Asaf, S., Khan, M. A., Kang, S.M., et al. (2016). Seed-borne endophytic *Bacillus amyloliquefaciens* RWL-1 produces gibberellins and regulates endogenous phytohormones of *Oryza sativa*. *Plant Physiology and Biochemistry, 106*, 236-243.

Sharma, R., Bisaria, V. S., & Sharma, S. (2017). Impact of Rhizobial Inoculants on Rhizospheric Microbial Communities. In A. P. Hansen, D. K. Choudhary, P. K. Agrawal & A. Varma (Eds.), *Rhizobium Biology and Biotechnology* (pp. 1-10). Cham: Springer International Publishing.

Sharma, R., Paliwal, J. S., Chopra, P., Dogra, D., Pooniya, V., Bisaria, V. S., et al. (2017). Survival, efficacy and rhizospheric effects of bacterial inoculants on *Cajanus cajan*. *Agriculture, Ecosystems & Environment, 240*, 244-252.

Sharma, R., Pooniya, V., Bisaria, V. S., Swarnalakshmi, K., & Sharma, S. (2020). Bioinoculants play a significant role in shaping the rhizospheric microbial community: a field study with *Cajanus cajan*. *World Journal of Microbiology and Biotechnology, 36*(3), 44.

Sharma, S., Sharma, R., Gupta, R., & Bisaria, V. S. (2016). Revisiting Action of Bioinoculants: Their Impact on Rhizospheric Microbial Community Function. In D. P. Singh, H. B. Singh & R. Prabha (Eds.), *Microbial Inoculants in Sustainable Agricultural Productivity: Vol. 2: Functional Applications* (pp. 41-51). New Delhi: Springer India.

Shen, Z., Wang, B., Zhu, J., Hu, H., Tao, C., Ou, Y., et al. (2019). Lime and ammonium carbonate fumigation coupled with bio-organic fertilizer application steered banana rhizosphere to assemble a unique microbiome against Panama disease. *Microbial Biotechnology, 12*(3), 515-527.

Sivakumar, N., Sathishkumar, R., Selvakumar, G., Shyamkumar, R., & Arjunekumar, K. (2020). Phyllospheric Microbiomes: Diversity, Ecological Significance, and Biotechnological Applications. In A. N. Yadav, J. Singh, A. A. Rastegari & N. Yadav (Eds.), *Plant Microbiomes for Sustainable Agriculture* (pp. 113-172). Cham: Springer International Publishing.

Srivastava, P., Balhara, M., & Giri, B. (2020). Soil Health in India: Past History and Future Perspective. In B. Giri & A. Varma (Eds.), *Soil Health* (pp. 1-19). Cham: Springer International Publishing.

Sun, B., Gu, L., Bao, L., Zhang, S., Wei, Y., Bai, Z., et al. (2020). Application of biofertilizer containing *Bacillus subtilis* reduced the nitrogen loss in agricultural soil. *Soil Biology and Biochemistry, 148*, 107911.

Thokchom, E., Thakuria, D., Kalita, M. C., Sharma, C. K., & Talukdar, N. C. (2017). Root colonization by host-specific rhizobacteria alters indigenous root endophyte and rhizosphere soil bacterial communities and promotes the growth of mandarin orange. *European Journal of Soil Biology, 79*, 48-56.

Tian, L., Lin, X., Tian, J., Ji, L., Chen, Y., Tran, L.-S. P., et al. (2020). Research Advances of Beneficial Microbiota Associated with Crop Plants. *International Journal of Molecular Sciences, 21*(5), 1792.

Trabelsi, D., & Mhamdi, R. (2013). Microbial Inoculants and Their Impact on Soil Microbial Communities: A Review. *BioMed Research International*, 863240.doi.org/10.1155/2013/863240

Verma, M., Mishra, J., & Arora, N. K. (2019). Plant Growth-Promoting Rhizobacteria: Diversity and Applications. In R. C. Sobti, N. K. Arora & R. Kothari (Eds.), *Environmental Biotechnology: For Sustainable Future* (pp. 129-173). Singapore: Springer Singapore.

Wang, J., Li, Q., Xu, S., Zhao, W., Lei, Y., Song, C., et al. (2018). Traits-Based Integration of Multi-Species Inoculants Facilitates Shifts of Indigenous Soil Bacterial Community. *Frontiers in Microbiology, 9*, 1692.7

Wang, L., Lin, H., Dong, Y., Li, B., & He, Y. (2020). Effects of endophytes inoculation on rhizosphere and endosphere microecology of Indian mustard (*Brassica juncea*) grown in vanadium-contaminated soil and its enhancement on phytoremediation. *Chemosphere, 240*, 124891.

Wei, M., Zhang, M., Huang, G., Yuan, Y., Fu, C., & Yu, L. (2020). Coculture with two *Bacillus velezensis* strains enhances the growth of *Anoectochilus* plants via promoting nutrient assimilation and regulating rhizosphere microbial community. *Industrial Crops and Products, 154*, 112697.

Xiong, W., Guo, S., Jousset, A., Zhao, Q., Wu, H., Li, R., et al. (2017). Bio-fertilizer application induces soil suppressiveness against *Fusarium* wilt disease by reshaping the soil microbiome. *Soil Biology and Biochemistry, 114*, 238-247.

Yaghoubi Khanghahi, M., Pirdashti, H., Rahimian, H., Nematzadeh, G., & Ghajar Sepanlou, M. (2019). The role of potassium solubilizing bacteria (KSB) inoculations on grain yield, dry matter remobilization and translocation in rice (*Oryza sativa* L.). *Journal of Plant Nutrition, 42*(10), 1165-1179.

Yang, T., Wei, Z., Friman, V.-P., Xu, Y., Shen, Q., Kowalchuk, G. A., et al. (2017). Resource availability modulates biodiversity-invasion relationships by altering competitive interactions.*Environmental Microbiology,19*(8), 2984-2991.

Zaheer, A., Malik, A., Sher, A., Mansoor Qaisrani, M., Mehmood, A., Ullah Khan, S., et al. (2019). Isolation, characterization, and effect of phosphate-zinc-solubilizing bacterial strains on chickpea (*Cicer arietinum* L.) growth. *Saudi Journal of Biological Sciences, 26*(5), 1061-1067.

Zhang, Y., Gao, X., Shen, Z., Zhu, C., Jiao, Z., Li, R., et al. (2019). Pre-colonization of PGPR triggers rhizosphere microbiota succession associated with crop yield enhancement. *Plant and Soil, 439*(1), 553-567.

Zhao, Y., Zhang, M., Yang, W., Di, H. J., Ma, L., Liu, W., et al. (2019). Effects of microbial inoculants on phosphorus and potassium availability, bacterial community composition, and chilli pepper growth in a calcareous soil: a greenhouse study. *Journal of Soils and Sediments, 19*(10), 3597-3607.

Zhong, Y., Yang, Y., Liu, P., Xu, R., Rensing, C., Fu, X., et al. (2019). Genotype and rhizobium inoculation modulate the assembly of soybean rhizobacterial communities. *Plant, Cell & Environment, 42*(6), 2028-2044.

CHAPTER 5:

Unraveling the dynamics of bacteria born in maize seeds in response to microbial inoculation

Chapter 5: Manuscript in preparation

Unraveling the dynamics of bacteria born in maize seeds in response to microbial inoculation

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Resumo

Produtos à base de micro-organismos (bioinoculantes) têm atraído atenção mundial como tecnologia sustentável para a agricultura. Por muitos anos, os cientistas relacionaram os efeitos dos bioinoculantes a seus mecanismos de ação direta nas plantas (biofertilização, bioestimulação, biocontrole e mitigação do estresse abiótico) e excluíram seus efeitos sobre a comunidade nativa (bacterioma). Existem evidências de que bioinoculantes afetam o crescimento vegetal ao modular a estrutura da comunidade residente. Neste trabalho, avaliamos se a inoculação de *Herbaspirillum seropedicae* combinada com ácidos húmicos (HA) pode alterar a estrutura do bacterioma da raiz de milho. Para isso, sementes de milho (*Zea mays* var. SHS 5050) não desinfestadas e desinfestadas (em hipoclorito de sódio a 1.25% por 30 min) foram germinadas em placas com meio ágar-água e transferidas para sistema hidropônico contendo solução de cloreto de cálcio ($CaCl_2$) com os seguintes tratamentos: 1) controle (sem inoculação); 2) *H. seropedicae* (RAM10); 3) ácidos húmicos; e 4) combinação de *H. seropedicae* e ácidos húmicos. Após 5 dias, o crescimento do milho foi avaliado e as raízes submetidas à contagem de bactérias em placa e PCR em Tempo Real (qPCR), microscopia de epifluorescência e eletrônica de varredura, além de terem seu DNA extraído para sequenciamento do rRNA 16S. Na contagem em placa (UFC) e por qPCR (bactéria/ng de DNA), um maior número de bactérias totais e de *H. seropedicae* foi observado nos tratamentos inoculados com a bactéria. Os resultados da microscopia mostraram que *H. seropedicae* colonizou diferentes regiões da raiz de milho, principalmente a zona de ramificação. Resultados do sequenciamento mostraram que a desinfestação da semente e a inoculação de *H. seropedicae* (combinada ou não com ácidos húmicos) afetou a estrutura do bacterioma da raiz ao alterar sua diversidade, abundância e composição. Em conclusão, a inoculação bacteriana modulou a comunidade nativa da raiz de milho, o que pode ter influenciado seu crescimento.

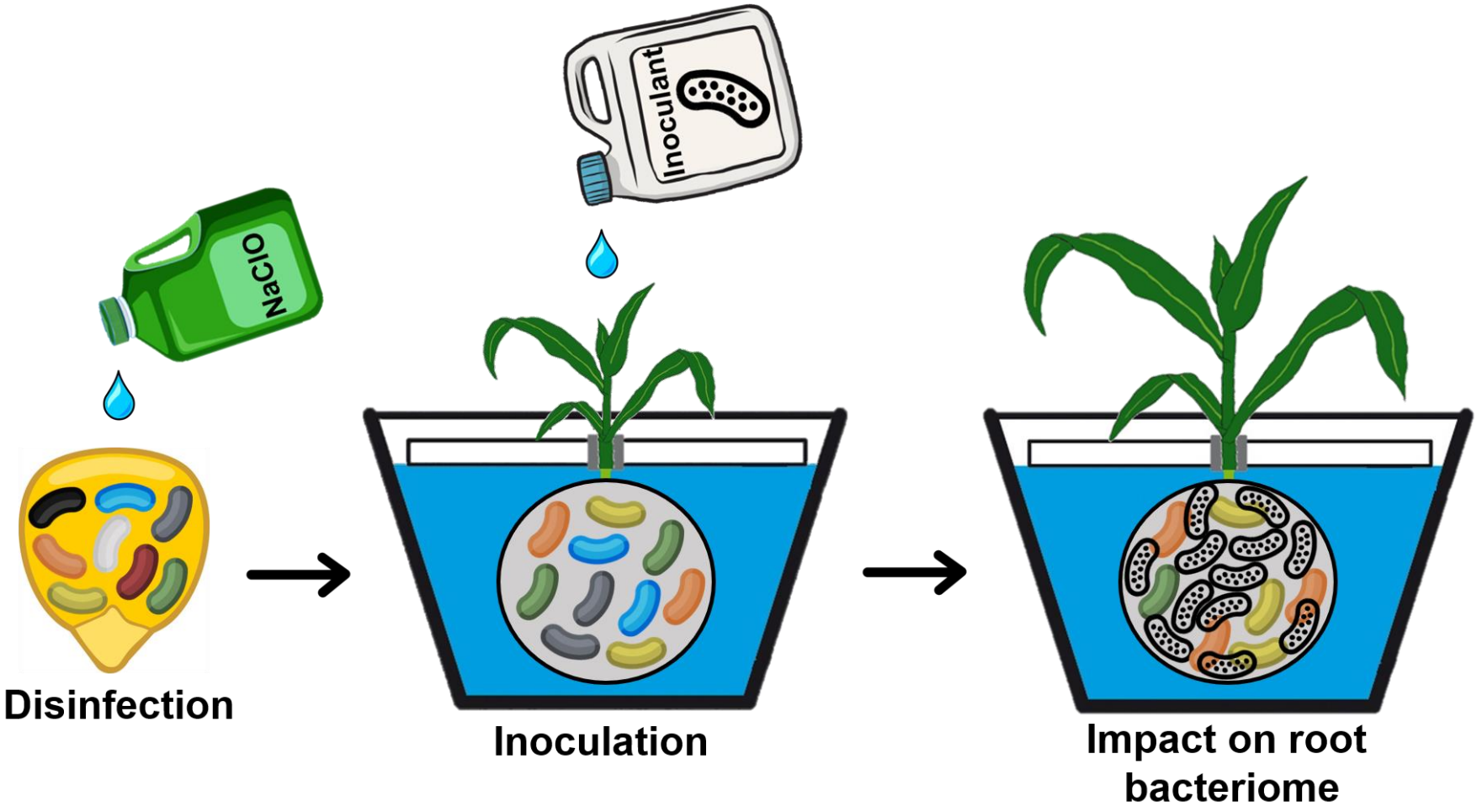
Palavras-chave: bacterioma, bioinoculante, desinfestação, *Herbaspirillum seropedicae*, ácidos húmicos, sequenciamento do rRNA 16S.

Abstract

Products based on microorganisms (bioinoculants) have attracted worldwide attention as a sustainable technology for agriculture. For many years, scientists have linked the effects of bioinoculants to their mechanisms of direct action on plants (biofertilization, biostimulation, biocontrol, and mitigation of abiotic stress) and have excluded their effects on plants by altering the structure of the native community (bacteriome). There is increasing evidence that bioinoculants affect plant growth by modulating the structure of the resident microbial community. In this work, we evaluated whether the inoculation of *Herbaspirillum seropedicae* combined with humic acids (HA) can re-shape the structure of the maize root bacteriome. For this, maize seeds (*Zea mays* var. SHS 5050) that were non-disinfected and disinfected (in sodium hypochlorite at 1.25% for 30 min) were germinated in plates with agar-water medium and transferred to a hydroponic system containing calcium chloride solution ($CaCl_2$) with the following treatments: 1) control (without inoculation); 2) *H. seropedicae* (RAM10); 3) humic acids; and 4) combination of *H. seropedicae* and humic acids. After five days, the growth of the maize was evaluated, and the roots were subjected to bacteria count in a plate and Real-Time PCR (qPCR), epifluorescence microscopy and scanning electron microscopy, in addition to having their DNA extracted for sequencing the 16S rRNA. For colony plate count (cell number per g^{-1} of the root) and by qPCR (bacterium/ng of DNA), a greater number of total bacteria and *H. seropedicae* was observed in the treatments inoculated with the bacterium. The results of the microscopy showed that *H. seropedicae* colonized different regions of the maize root, mainly the lateral root emission zone. Results of the sequencing showed that the disinfection of the seed and the inoculation of *H. seropedicae* (combined or not with humic acids) affected the structure of the root bacteriome by changing its diversity, abundance, and composition. In conclusion, bacterial inoculation modulated the native maize root community, which may have influenced its growth.

Keyword: bacteriome, bioinoculant, disinfection, *Herbaspirillum seropedicae*, humic acids, 16S rRNA sequencing.

Graphical Abstract
(Resumo Gráfico)



1. Introduction

In sustainable agriculture, products based on microorganisms, the so-called bioinoculants (Brasil, 2004), have been used to increase crop productivity (Sharma et al. 2016). In the last decade, several studies have reported the effectiveness of bioinoculants and related their effects to mechanisms of direct action on plants, such as biofertilization, biostimulation, biocontrol, and mitigation of abiotic stress (Kour et al. 2020; Sammauria et al. 2020; Tian et al. 2020). However, the indirect effect of bioinoculants on plants when changing the structure of the resident microbial community (called "microbiome" or "bacteriome" for bacterial communities) has been little explored and overlooked until recently. This effect is caused by the entry of a "foreign" microorganism into a natural microbial community (a non-pathogenic microbial invasion), which is often practised in agriculture through the use of microbial inoculants (Mallon et al. 2015a,b; Mallon et al. 2018).

When inoculated into the environment, selected bacterial strains can displace resident taxa and alter the microbial network and the functioning of the community (Mallon et al. 2015a,b; Xing et al. 2020). On the other hand, the native community can also resist the invasion, which would explain the failure of many bioinoculants in the field. This resistance is related to the diversity/composition of native communities and the availability of local resources (Mallon et al. 2015a,b; Xing et al. 2020). If the resistance of the community is overcome, the inoculated bacteria expand its population and permanently displaces resident taxa (Xing et al. 2020). Bioinoculants with low colonization efficiency or later successionally eliminated can also leave permanent marks on the bacteriome structure (Mallon et al. 2018).

The effect of microbial inoculations on the structure of native communities has been further investigated in the soil-rhizosphere system and almost unexplored in seeds and roots (rhizoplane and endophytic region) (Mawarda et al. 2020; Santos e Olivares, in press). Although neglected, the seed bacteriome has already been shown to contribute to germination, growth, and plant protection and cannot be disregarded at the time of bacterial inoculation (Dos Santos et al. 2020; Santos et al., 2021). We believe that these seed-borne bacteria can be affected by bioinoculants and also determine their success. Few studies have analyzed changes in the root bacteriome of different plant species after the application of bioinoculants (of varying composition), and some have correlated changes in community structure with the promotion of plant growth (Liu et al. 2017; Sheridan et al. 2017; Thokchom et al. 2017; Gadhave et al. 2018; Jha et al. 2020; Wang et al. 2020; Pangesti et al. 2020).

In this work, we evaluated whether the inoculation of *Herbaspirillum seropedicae* in combination with humic acids (HA) can alter the structure of the maize root bacteriome. We hypothesized that the inoculation of *H. seropedicae* combined or not with HA could alter the structure of the maize root bacteriome, whereas the inoculation of HA alone would have a lesser effect. Furthermore, we expected modulations in the diversity, abundance, and composition of the native community to reveal key taxa of the bacteriome and possibly responsible for plant growth.

2. Material and Methods

2.1. Hydroponic assay

2.1.1. Extraction of humic acids

Humic substances were extracted as described by the International Humic Substance Society, with some adaptations. Humic acids (HA) were extracted from vermicompost produced with sugar cane filter cake. For extraction, a 0.5 M NaOH solution was mixed with the vermicompost (10: 1, v/v) under an inert N_2 atmosphere. After 12 hours, the suspension was centrifuged (5,000 g) and acidified with 6 M HCl at pH 1.5 to precipitate the HA. Solubilization and acidification were repeated three times. After centrifugation (5,000 g) for 15 min, the sample was washed with water until negative for $AgNO_3$, followed by dialysis against deionized water using a 1000-Da cut membrane (Thomas Scientific, Swedesboro, NJ, USA). The dialysate was lyophilized, and the carbon content was analyzed by dry combustion (CHN analyzer Perkin Elmer series 2400, Norwalk, CT, USA). The HA powder was solubilized in KOH (1%).

2.1.2. Preparation of the bacterial inoculum

The bacterium used was *H. seropedicae* strain RAM10, derived from *H. seropedicae* ZA95, isolated from rice (Baldani et al. 1986) and linked with the *gfp* reporter gene. The pre-inoculum was prepared with the growth of 50 µL of the bacteria in a 5 mL tube containing DYGS medium for 48 h at 30 °C under an orbital shaker at 150 rpm (Baldotto et al. 2011). The viability and purity of the pre-inoculum were verified by phase-contrast and epifluorescence microscopy. Then, Erlenmeyer containing 180 mL of DYGS liquid medium were inoculated with 300 µL of the pre-inoculum and grown under the same conditions reported above for 24 h. After growth, the bacterial cells were centrifuged, resuspended in

sterile water and the optical density adjusted to approximately 1.0 to 595 nm (approximately 10^8 cells per ml).

2.1.3. Disinfection and germination of maize seeds

Maize seeds (*Zea mays* L.) of the SHS 5050 variety (Santa Helena Sementes, Brazil) were immersed in sterile distilled water for 5-h, being part of the seeds not disinfected (NDS treatment). Disinfected seeds (DS treatment) were treated with 70% alcohol for 5 min and sodium hypochlorite (NaClO; Butterfly Ecologia, Audax Company) 1.25% for 30 min. Washings in sterile distilled water were performed between the solutions (1x) and after immersion in hypochlorite (5x). Then, non-disinfected and disinfected seeds were germinated in Petri dishes (7 repetitions; 21 seeds per dish) containing agar-water medium (0.5%) and packed in BOD at 30 ºC and 12/12 h photoperiod (light/dark) for 3 and 4 days, respectively.

2.1.4. Seedling treatment with H. seropedicae and Humic acids

After germination, the maize seedlings were transferred to a hydroponic system containing 1,800 mL of $CaCl_2$ solution (2 M), with the following treatments: a) control (without inoculation); b) *H. seropedicae*; c) humic acids; d) combination of *H. seropedicae* and humic acids. The bioinoculant was produced by diluting 180 mL of the bacterial solution and/or 240 mg of humic acids in $CaCl_2$ solution at pH 6.7, with a final concentration of 35 mg of carbon L^{-1} and approximately 10^7 cells mL^{-1}. After 5-d in hydroponic cultivation, seedlings were evaluated for total length and biomass (fresh and dry), with the aid of a millimetre ruler and analytical balance, respectively. Means of four repetitions were analyzed by ANOVA, followed by the Tukey test ($p \leq 0.05$).

2.2. Maize root bacteria count

2.2.1. Plate colony-count

The count of total and inoculated bacteria was performed by colony forming units (CFU). For this, maize roots (1 g) were macerated in saline solution (NaCl; 99 mL; 8.5 g L^{-1}), subjected to serial dilution (10^{-3} to 10^{-6}), and plated (100 µL) in solid medium NB (Nutrient Broth) with and without nalidixic acid antibiotic (20 mg L^{-1}). Total bacteria were quantified in a medium without antibiotics, while *H. seropedicae*, which is naturally resistant to nalidixic acid, was grown in the medium with antibiotics. The antibiotic was dissolved in KOH (0.1

M), filtered in Millipore®, and mixed with the NB medium. The plates were incubated in BOD at 30 ºC for 5 to 6 days.

2.2.2. Real-time PCR

Total and inoculated bacteria were quantified by real-time PCR (qPCR) from the 16S rRNA. For this, the total DNA of the maize roots was extracted by the CTAB method (Cetyltrimethylammonium bromide) (Chen and Ronald, 1999; Doyle and Doyle, 1987). The qPCR used SYBR Green (Promega; 7.5 μL), DNA template (1 μL; 40 ng), water, and *primers* (10 μM; 0.5 μL of each). The *primers* 926F (AAACTCAAAKGAATTGACGG) and 1062R (CTCACRRCACGAGC TGAC) (Bacchetti De Gregoris et al. 2011) were used to quantify the total bacteria. For the quantification of *Herbaspirillum*, the *primers* HRC54F (CGGTTTTGGCTAATATCCAG) and HRC54R (AGTGTTATCCCAGGGGCTGC) (Boa Sorte, 2013) were used. The reactions were incubated in a thermocycler (Step-One-Plus; Applied Biosystems) for 5 min at 95 °C, then 40 cycles of 15 s at 95 °C and 1 min at 60 °C. The bacterial proportions were calculated based on the values of Ct (cycle threshold) and the standard curve (Staroscik, 2004). The standard curve was generated by diluting the DNA of the bacterium *Escherichia coli* ATCC 25922 and *H. seropedicae* RAM10 in series of 10^2 - 10^{-8} (20 - 2 x 10^9 ng of DNA). *E. coli* and *H. seropedicae* were grown in liquid medium NB and DYGS (180 rpm, at 30 ºC), respectively. The bacterial DNA was extracted with Wizard Genomic DNA Purification Kit (Promega).

2.3. Epifluorescence and scanning electron microscopy

In epifluorescence, microscopic observations were made in different regions of the maize root, including lateral root, elongation zone, and root cap. Whole roots were placed on glass slides with sterile distilled water and observed under an Axioplan Zeiss microscope equipped with specific filters for the detection of GFP (BP 460-490 nm; LP 510-550 nm) and AxioCam digital photography system.

For scanning electron microscopy (SEM), maize roots were cut into 1 cm segments, comprising a lateral root, elongation zone and root cap, and immediately fixed in glutaraldehyde (2.5%) and paraformaldehyde (4%) in sodium phosphate buffer. (0.05 mol L^{-1}, pH 7.0). Then, the samples were washed with the same buffer (3 times for 10 min), dehydrated in a series of ethanol (15, 30, 50, 70, 90 and 2 x 100% at 10 min) and dried in a critical point device (Bal-tec CPD 030). The segments were mounted on aluminium stubs,

metalized with ionized platinum in a sputtering coat apparatus (Bal-tec SCD 050) and visualized in SEM Zeiss EVO 40 at 15 kV.

2.4. Sequencing of maize root bacteriome

Maize roots from the hydroponic test were stored at -70 ºC. Frozen samples were macerated in liquid nitrogen to extract total DNA (from 0.2 g) using the CTAB method (Cetyltrimethylammonium bromide) (Chen and Ronald, 1999; Doyle and Doyle, 1987). The extracted DNA was quantified in NanoDrop 2000® spectrophotometer (Thermo Scientific) and the quality confirmed in agarose gel (0.8%) electrophoresis (80 V, for 70 min). The total DNA was sent to the company "NGS Soluções Genômicas" for sequencing of the 16S rRNA gene in Illumina MiSeq, with three repetitions per treatment. The samples were amplified with primers 515FB (GTGYCAGCMGCCGCGGTAA) and 806RB (GGACTACNVGGGTWTCTAAT) (Caporaso et al. 2011; 2012), with modifications to primer degeneracy done by the labs of Jed Furhman (Parada et al. 2016) and Amy Apprill (Apprill) et al. 2015). For PCR, 10 μL of PCR master mix, 0.5 μL of each primer (10 μM), 1 μL of DNA template, and 13 μL of PCR-grade water were used, totalling 25 μL of reaction. The PCR conditions were: initial denaturation at 94 ºC for 3 min, followed by 30 cycles of 94 ºC (30 s), 50 ºC (30 s) and 72 ºC (30 s) and a final extension of 72 ºC (10 min). The sequencing was performed on the Illumina MiSeq platform.

2.5. 16S profiling data analysis

The raw sequences of the Illumina system were analyzed following the recommendations of the Brazilian Microbiome Project (Pylro et al. 2014) and using the BMP Operating System (BMPOS) (Pylro et al. 2016). Briefly, the sequences were filtered, grouped into Operational Taxonomic Units (OTUs) with a 97% similarity cut (Edgar, 2013) and taxonomically classified with the QIIME software (Caporaso et al. 2010), using Greengenes reference sequences (McDonald et al. 2012).

The BIOM file was imported into the R environment using the phyloseq package (McMurdie and Holmes, 2013), and the sampling quality estimated from Good's coverage (Good, 1953). Taxonomy up to gender level was estimated in centered log-ratio (clr) transformed abundance (Gloor and Reid, 2016). Beta diversity (bacterial diversity between different samples) was compared by main coordinates analysis (PCoA) using the phyloseq package and the significance between groups visualized by Permutational multivariate

analysis of variance (Permanova) (Anderson, 2017) with the adonis function available in vegan package (Oksanen et al. 2015).

Alpha diversity (bacterial diversity within each sample) was calculated (estimate_richness function in the phyloseq package) by the species observed and by the Shannon diversity index (considering the number and abundance of species), while the richness was estimated by the Chao index (weight total and rare species). The diversity indices were submitted to analysis of variance (ANOVA) and normality confirmed by the Shapiro-Wilk W test ($p > 0.05$). Significant differences were later analyzed by post hoc Tukey's HSD test within the agricolae R-package ($p < 0.05$).

Amplicon sequence variants (ASVs) tables were set up based on the number of times that sequences differing in only one nucleotide were observed in each sample. Venn Diagrams represented shared and unique ASVs. ASVs were also used to quantify the abundance (clr-transformed abundance) of the bacterium *H. seropedicae* inoculated in treatments (disinfected and not disinfected; control, *H. seropedicae, H. seropedicae* + HA and HA) and within the phylum Proteobacteria and Betaproteobacteria class. The differences were compared using the Wilcoxon test ($p<0.05$). Spearman's correlation determined which bacterial genera were modulated by the bioinoculant, with a correlation matrix visualized by the corrplot package ($p<0.05$ was considered significant) (Wei and Simko, 2017).

3. Results

The number of total and inoculated bacteria in the maize root treated with *H. seropedicae* combined or not with HA was greater than the control and the isolated application of HA (not inoculated) (Table 1). Non-disinfecting maize seed ensured a high number of CFU in the uninoculated roots while disinfecting the seeds and later treating it with *H. seropedicae* (with and without HA) increased the total, and inoculated bacteria count (Table 1). Among the inoculated treatments, a greater number of total bacteria was observed after the isolated application of *H. seropedicae*, while its combination with HA increased the population of *H. seropedicae* (Fig 1).

The maize root bacteriome was also quantified by Real-time PCR, which revealed a high number of total bacteria when disinfecting the seed and applying the bioinoculant (*H. seropedicae* and/or HA) (Fig 1). In non-disinfected seeds, a greater number of total bacteria was obtained by combining *H. seropedicae* with HA (Fig 1). Real-time confirmed the presence of *H. seropedicae* in the inoculated treatments (Fig 1).

Table 1. Influence of disinfection and bioinoculants on the count of total bacteria and *H. seropedicae* in NB culture medium.

	Treatments			
	Total bacteria (CFU.mL^{-1})			
Item	**Control**	***H. seropedicae***	***H. seropedicae*+HA**	**HA**
DS	0.5 x 10^8	18 x 10^8	12 x 10^8	1.8 x 10^8
NDS	1.6 x 10^8	13 x 10^8	11 x 10^8	2.2 x 10^8
	H. seropedicae (CFU.mL^{-1})			
Item	**Control**	***H. seropedicae***	***H. seropedicae*+HA**	**HA**
DS	0.04 x 10^8	3.3 x 10^8	7 x 10^8	0.4 x 10^8
NDS	0.4 x 10^8	2.8 x 10^8	4 x 10^8	0.7 x 10^8

Germinated maize roots of disinfected (DS) and non-disinfected (NDS).

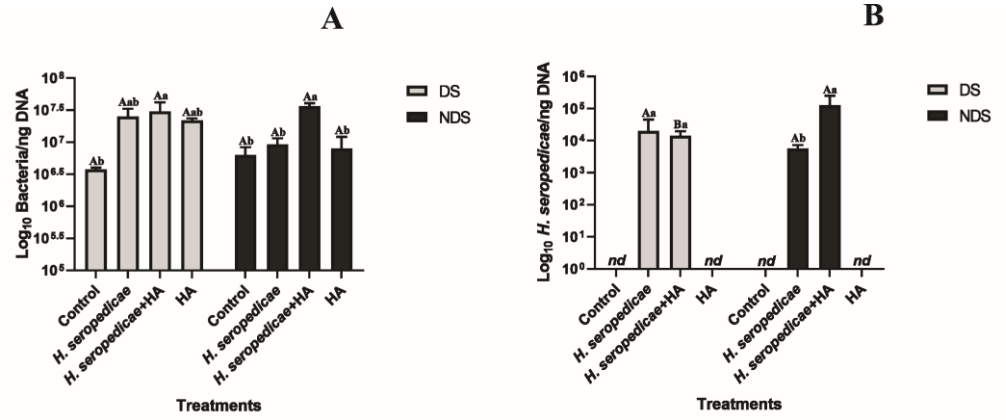


Fig 1. Quantification of total bacteria (A) and *H. seropedicae* (B) in germinated roots of non-disinfected (NDS) and disinfected (DS) seeds and treated with *H. seropedicae* and/or humic acids (HA). Different capital letters indicate significant differences for the disinfection factor (NDS and DS) and lowercase letters indicate significant differences between the bioinoculants according to the Tukey test ($p \leq 0.05$). nd: not detected.

In epifluorescence images, the bacterium *H. seropedicae* was not detected in non-inoculated treatments, including control root and treated with HA (Figs 2, 3, and 4 A1-B1, A4-B4). In the inoculated roots, *H. seropedicae* colonized the emergence region of the lateral root, elongation zone, and root cap, where it formed cell aggregations of different sizes (Figs 2, 3, and 4 A2-B2, A3-B3). The green fluorescence of the inoculated bacteria was observed in the germinated roots of disinfected and non-disinfected seeds.

Under SEM view, native bacteria isolated or in small aggregates were visualized in the uninoculated roots (Fig 5, 6, and 7 A1-B1, A4-B4). The lateral root of the inoculated maize was densely colonized by *H. seropedicae*, with the presence of biofilms in the disinfected and non-disinfected treatments (Fig 5 A2-B2, A3-B3). Cells from this region appear in SEM as curved rods typical of *Herbaspirillum*. Bacteria also colonized the maize elongation zone and root cap (Fig 6 and 7 A2-B2, A3-B3).

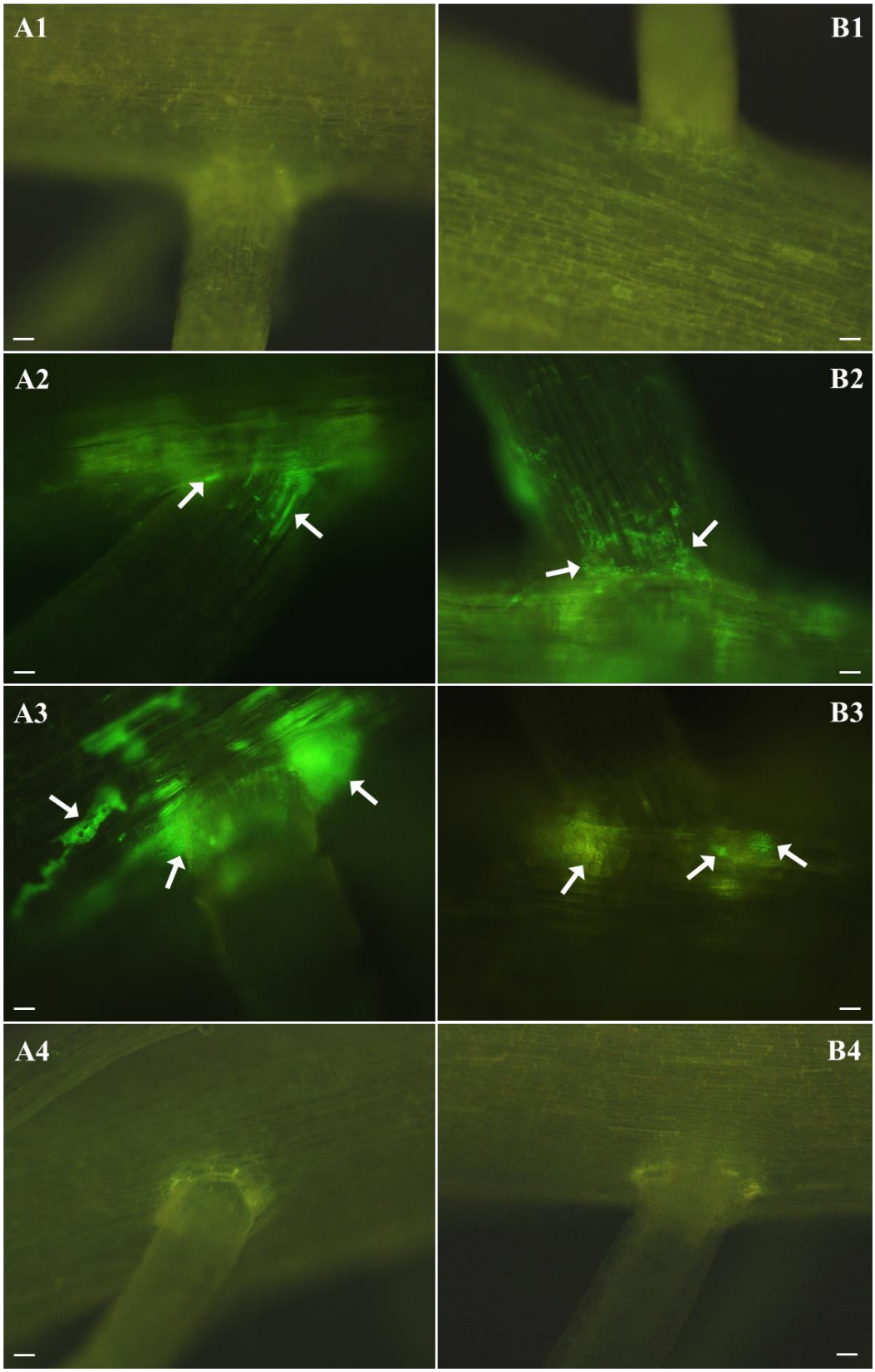


Fig 2. Colonization of *H. seropedicae* RAM10 on the lateral root of the maize visualized by epifluorescence microscopy. Germinated seedlings of disinfected (DS; A) and non-disinfected (NDS; B) seeds and treated with *H. seropedicae* and/or humic acids (HA). Treatments: Control (A1-B1); *H. seropedicae* (A2-B2); *H. seropedicae*+HA (A3-B3); HA (A4-B4). Bars represent the following scales: A1-B1-A4-B4 = 150 µm; A2-B2-A3-B3 = 250 µm.

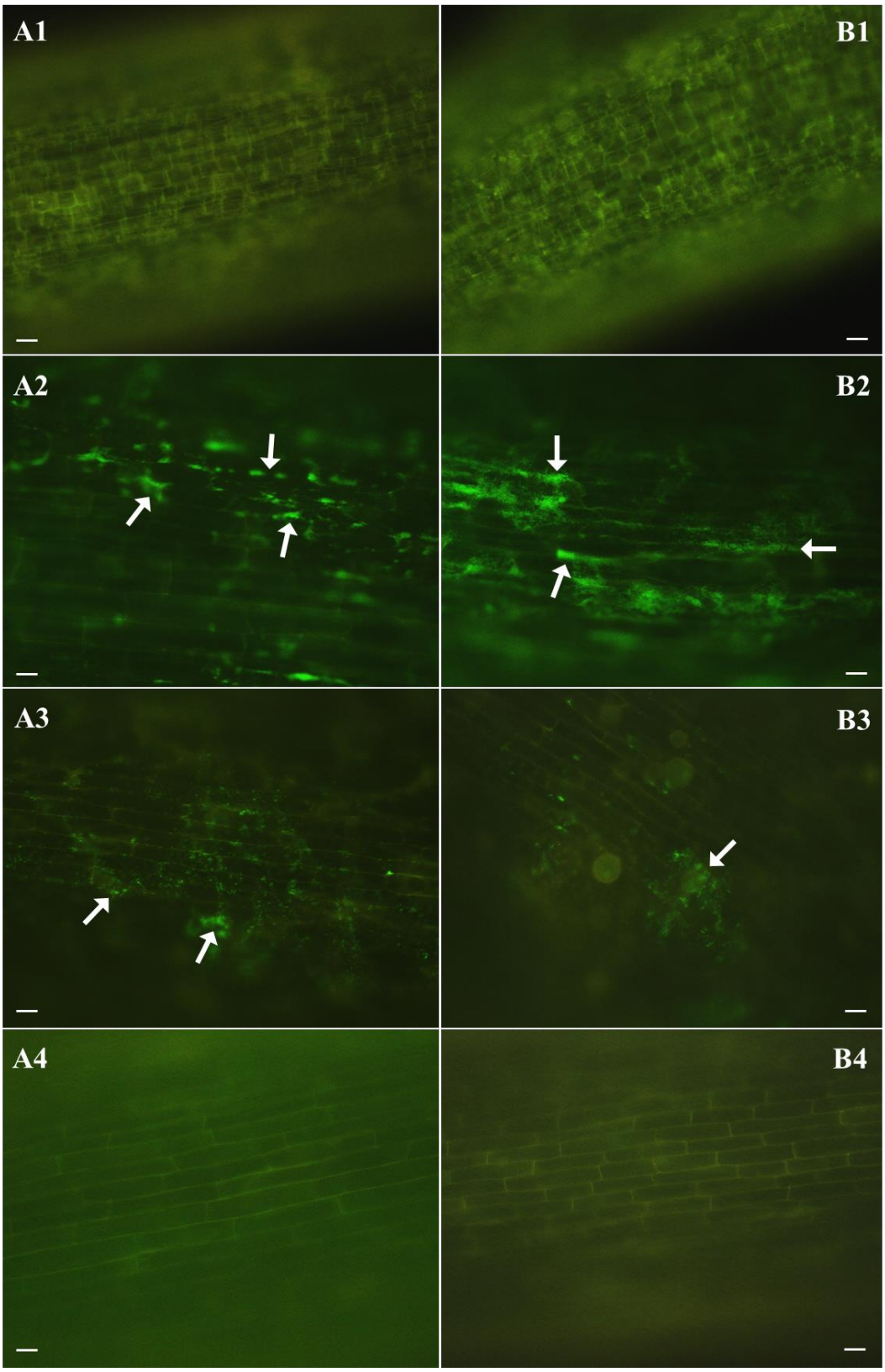


Fig 3. Colonization of *H. seropedicae* RAM10 on the zone of elongation of the maize visualized by epifluorescence microscopy. Germinated seedlings of disinfected (DS; A) and non-disinfected (NDS; B) seeds and treated with *H. seropedicae* and/or humic acids (HA). Treatments: Control (A1-B1); *H. seropedicae* (A2-B2); *H. seropedicae*+HA (A3-B3); HA (A4-B4). Bars represent the following scales: A1-B1-A4-B4 = 150 µm; A2-B2-A3-B3 = 250 µm.

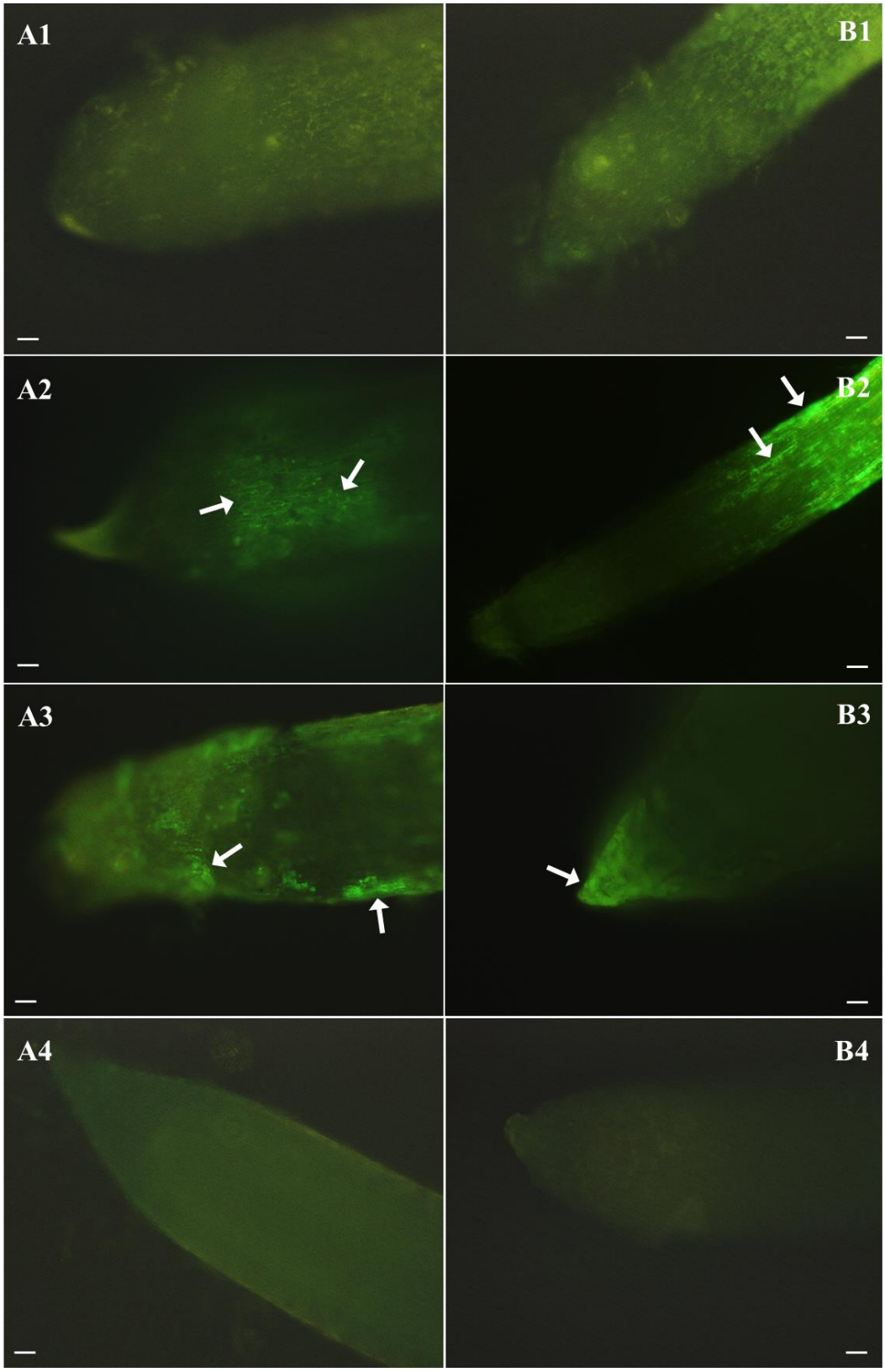


Fig 4. Colonization of *H. seropedicae* RAM10 on the root cap of the maize visualized by epifluorescence microscopy. Germinated seedlings of disinfected (DS; A) and non-disinfected (NDS; B) seeds and treated with *H. seropedicae* and/or humic acids (HA). Treatments: Control (A1-B1); *H. seropedicae* (A2-B2); *H. seropedicae*+HA (A3-B3); HA (A4-B4). Bars represent the following scales: 50 µm.

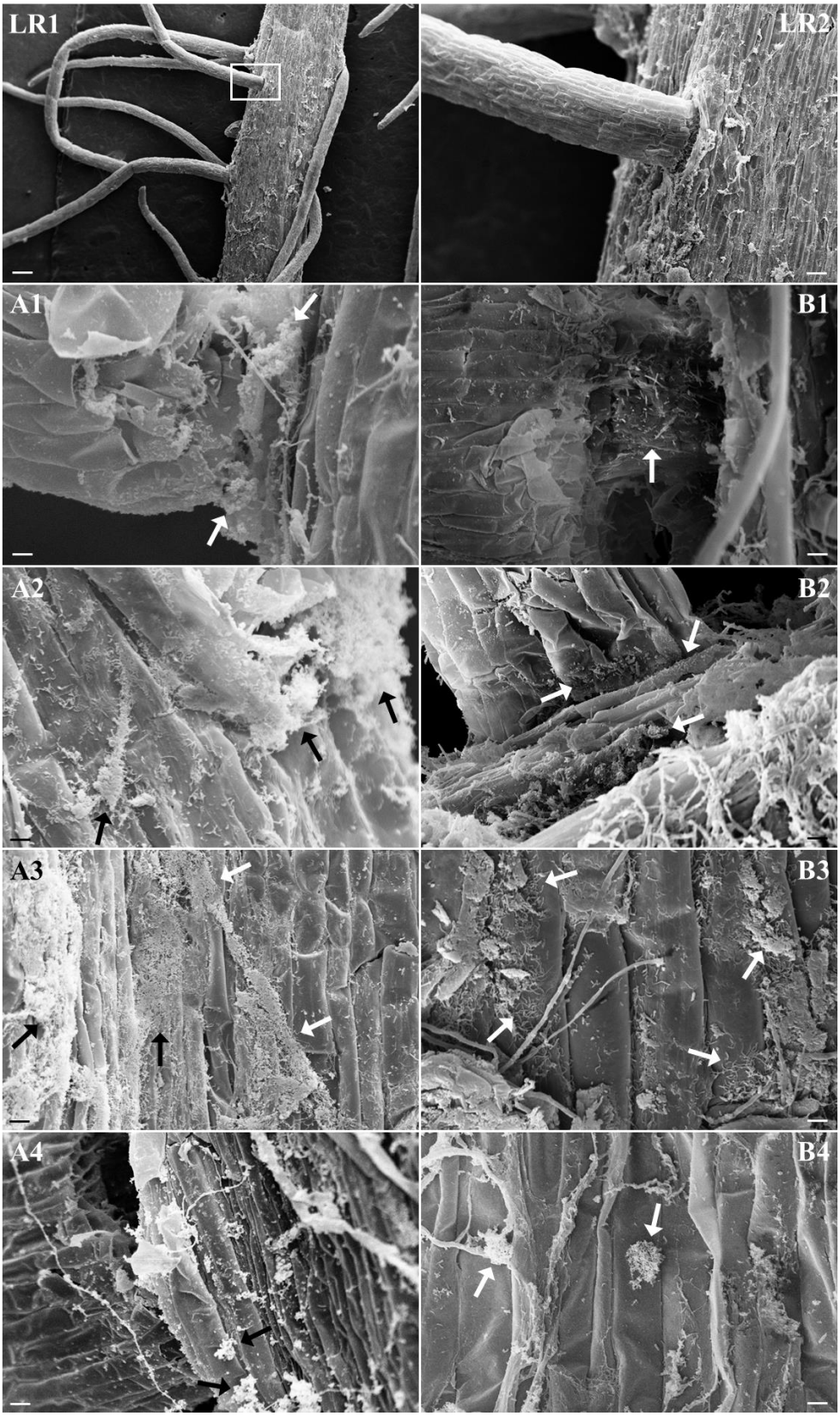


Fig 5. Bacterial colonization of maize lateral root (LR) visualized by scanning electron microscopy (SEM). Germinated seedlings of disinfected (DS; A) and non-disinfected (NDS; B) seeds and treated with *H. seropedicae* and/or humic acids (HA). Treatments: Control (A1-B1); *H. seropedicae* (A2-B2); *H. seropedicae*+HA (A3-B3); HA (A4-B4). Bars represent the following scales: panel LR1: 200 µm; LR2: 100 µm; Others: 20 µm.

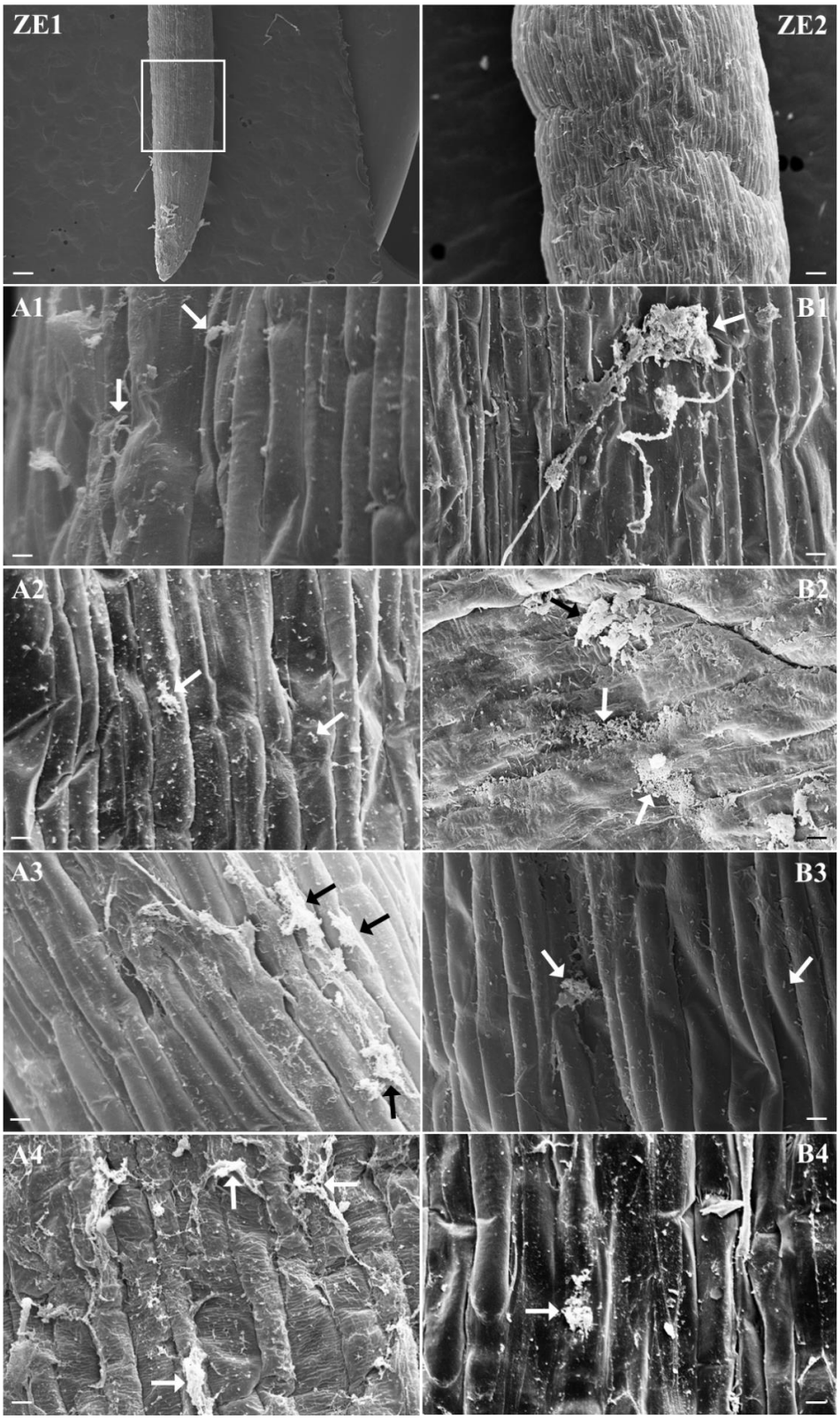


Fig 6. Bacterial colonization of maize zone of elongation (ZE) visualized by scanning electron microscopy (SEM). Germinated seedlings of disinfected (DS; A) and non-disinfected (NDS; B) seeds and treated with *H. seropedicae* and/or humic acids (HA). Treatments: Control (A1-B1); *H. seropedicae* (A2-B2); *H. seropedicae*+HA (A3-B3); HA (A4-B4). Bars represent the following scales: panel ZE1: 200 µm; ZE2: 100 µm; A1, B2, A4 and B4: 10 µm; B1, A2, A3 and B3: 20 µm.

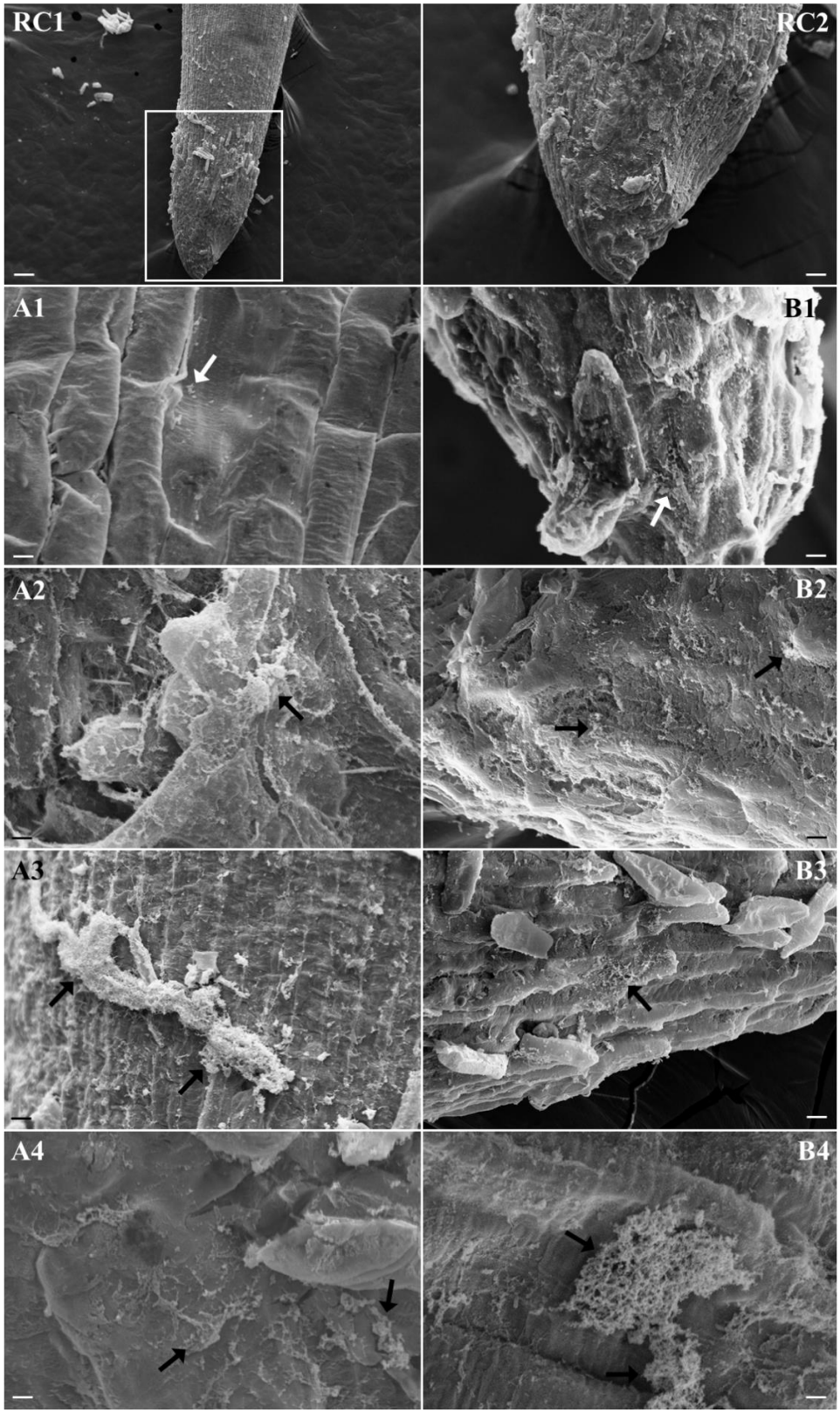


Fig 7. Bacterial colonization of maize root cap (RC) visualized by scanning electron microscopy (SEM). Germinated seedlings of disinfected (DS; A) and non-disinfected (NDS; B) seeds and treated with *H. seropedicae* and/or humic acids (HA). Treatments: Control (A1-B1); *H. seropedicae* (A2-B2); *H. seropedicae*+HA (A3-B3); HA (A4-B4). Bars represent the following scales: panel RC1: 200 µm; A1, B1, A2 and A4: 10 µm; RC2, B2, A3 and B3: 20 µm; B4: 2 µm.

The application of *H. seropedicae* with HA in disinfected and non-disinfected seeds increased the total length of the maize seedlings (Fig 8A). When the maize seeds were disinfected, the combined use of *H. seropedicae* and HA significantly increased the total dry biomass compared to the other treatments (Fig 8C). No significant differences were observed for fresh seedling biomass in NDS and DS, as well as for dry biomass in NDS (Fig 8B and 8C).

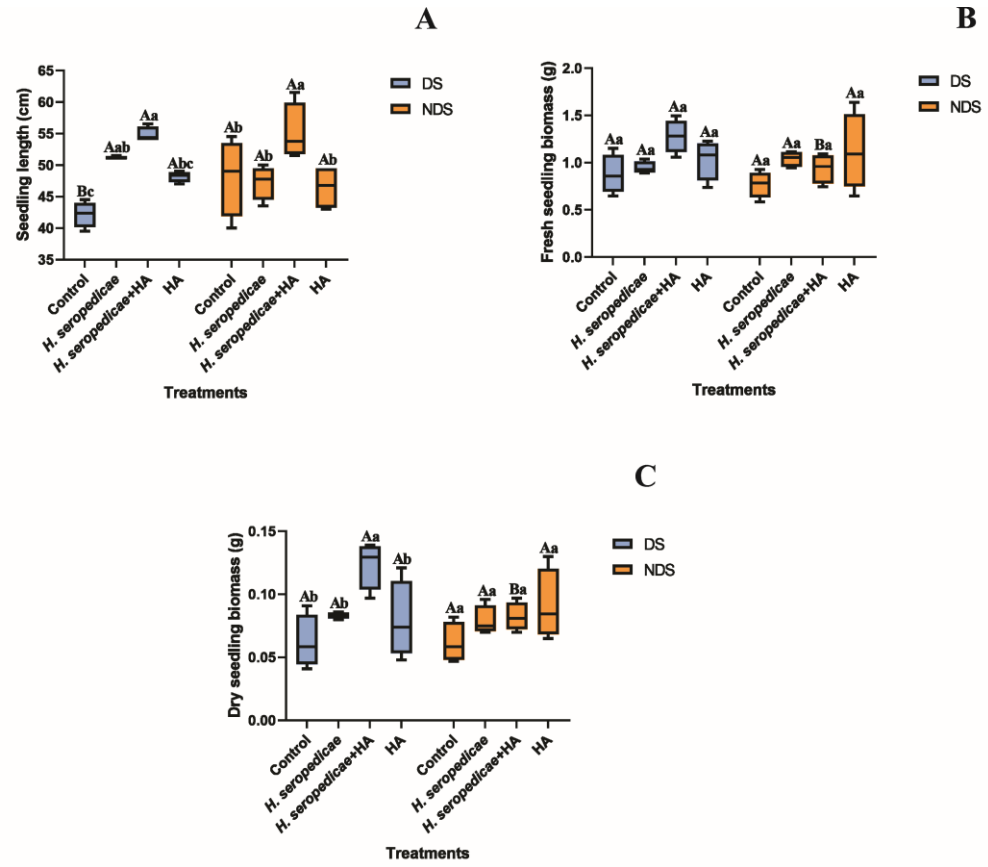


Fig 8. Growth of maize germinated of non-disinfected (NDS) and disinfected (DS) seeds and treated with *H. seropedicae* and/or humic acids (HA). Seedling length (A), fresh seedling biomass (B) and dry seedling biomass (C). Different capital letters indicate significant differences for the disinfection factor (NDS and DS) and lowercase letters indicate significant differences between the bioinoculants according to the Tukey test ($p \leq 0.05$).

The influence of seed disinfection and *H. seropedicae* and/or HA inoculation on the structure of the maize root bacterial community was analyzed by Illumina MiSeq sequencing. A total of 248,639 *reads* were obtained from 25 samples (Table 2). Good coverage in the 99-100% range indicated that the number of readings from the sequencing was sufficient to capture the bacterial diversity of the maize root (Table 2).

Table 2. Distribution of reads per sample and sequence coverage.

rn	Name	Disinfection	Treatment	Total Reads	Coverage
1	1	No	Control	5601	1.0000000
10	10	No	*H. seropedicae*+HA	10436	1.0000000
11	11	No	*H. seropedicae*+HA	10983	1.0000000
12	12	No	*H. seropedicae*+HA	15930	1.0000000
13	13	Yes	Control	7334	1.0000000
14	14	Yes	Control	7201	1.0000000
15	15	Yes	Control	6342	1.0000000
16	16	Yes	HA	5778	1.0000000
17	17	Yes	HA	6598	1.0000000
18	18	Yes	HA	6940	1.0000000
19	19	Yes	*H. seropedicae*	9658	0.9998965
2	2	No	Control	4687	1.0000000
20	20	Yes	*H. seropedicae*	11095	1.0000000
21	21	Yes	*H. seropedicae*	13639	0.9999267
22	22	Yes	*H. seropedicae*+HA	19051	1.0000000
23	23	Yes	*H. seropedicae*+HA	14125	1.0000000
24	24	Yes	*H. seropedicae*+HA	21123	1.0000000
3	3	No	Control	4452	1.0000000
32	32	*H. seropedicae*	2	27889	1.0000000
4	4	No	HA	4270	1.0000000
5	5	No	HA	5637	1.0000000
6	6	No	HA	4241	1.0000000
7	7	No	*H. seropedicae*	7930	1.0000000
8	8	No	*H. seropedicae*	7284	1.0000000
9	9	No	*H. seropedicae*	10415	1.0000000

Differences in the bacterial community of the maize root after seed disinfection and inoculation of *H. seropedicae* were visualized by principal coordinate analysis (PCoA) (Fig 9A). First, we observed the separation of treatments inoculated with *H. seropedicae* and not inoculated. Inoculated samples were grouped without the influence of disinfection. Among the non-inoculated samples, we observed the separation of disinfected and non-disinfected treatments. Permanova's analysis confirmed that disinfection ($p = 0.001$, $R2 = 0.100$) and inoculation ($p = 0.001$, $R2 = 0.467$) significantly influenced the maize root bacteriome (Table 3). Alpha diversity measures revealed that the species observed, Chao richness and Shannon diversity of the inoculated treatments (*H. seropedicae* combined or not with HA) were significantly greater than the control and the isolated application of HA (Fig 9B). The inoculation of *H. seropedicae* with HA after maize disinfection tends to increase the alpha diversity index (Fig 9B).

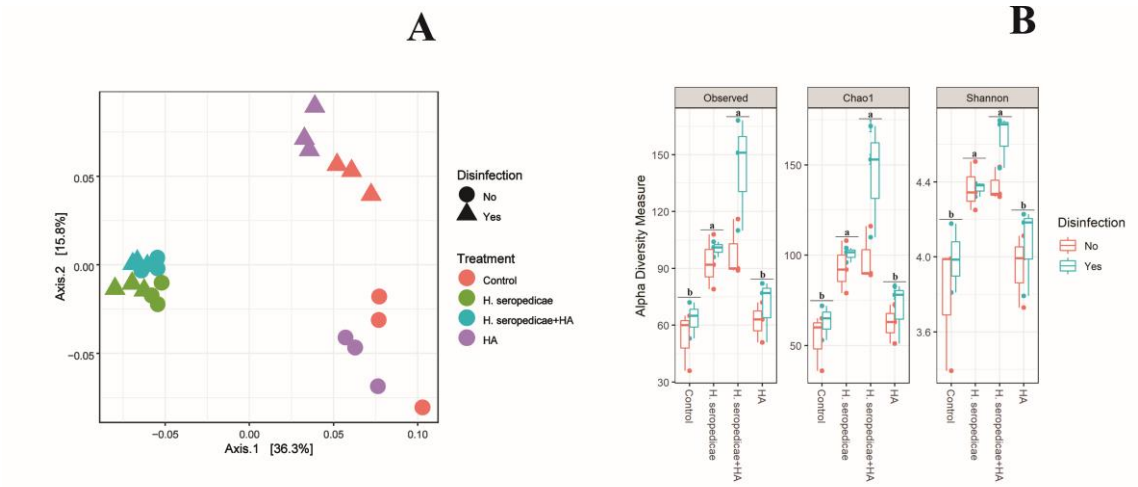


Fig 9. Beta (A) and alpha (B) diversity of the bacteriome associated with roots of non-disinfected (No) and disinfected (Yes) seeds and treated with *H. seropedicae* and/or humic acids (HA). Letters indicate significant differences for alpha diversity indices according to the Tukey test ($p \leq 0.05$).

Table 3. Permanova of the root bacteriome.

	Df[a]	SumsOfSqs[b]	MeanSqs[c]	F.Model[d]	R^2	Pr(>F)[e]
Treatment	3	0.1263022	0.0421007	8.961044	0.4672616	0.001
Disinfection	1	0.0269522	0.0269522	5.736708	0.0997110	0.001
Treatment:Disinfection	3	0.0418775	0.0139592	2.971174	0.1549279	0.001
Residuals	16	0.0751711	0.0046982	NA	0.2780995	NA
Total	23	0.2703030	NA	NA	1.0000000	NA

[a]Df: degrees of freedom
[b]Sum of Sqs: sequential sums of squares
[c]Mean Sqs: mean squares
[d]F. Model: F statistics
[e]Pr>(F): partial R-squared and P values

Disinfection and the application of bioinoculant in maize altered the root bacteriome at different taxonomic levels. These modulations were presented in a box plot with clr-transformed abundance values (Fig 10). Non-disinfected seeds maintained the abundance of the Proteobacteria phylum in the non-inoculated treatments (control and HA). However, *H. seropedicae* inoculation (with or without HA) reduced the abundance of Proteobacteria and stimulated bacteria of the phylum Bacteroidetes (Fig 10). Reduced abundance of the Proteobacteria phylum was also noticed for disinfected maize seeds inoculated with *H. seropedicae* (with and without HA). This trend was accompanied by an abundance increase of Bacteroidetes in all treatments, especially those inoculated with the bacteria (Fig 10). With disinfection, we also observed an abundance of Firmicutes and Acidobacteria in treatments that received only HA and Actinobacteria when combining *H. seropedicae* and HA (Fig 10).

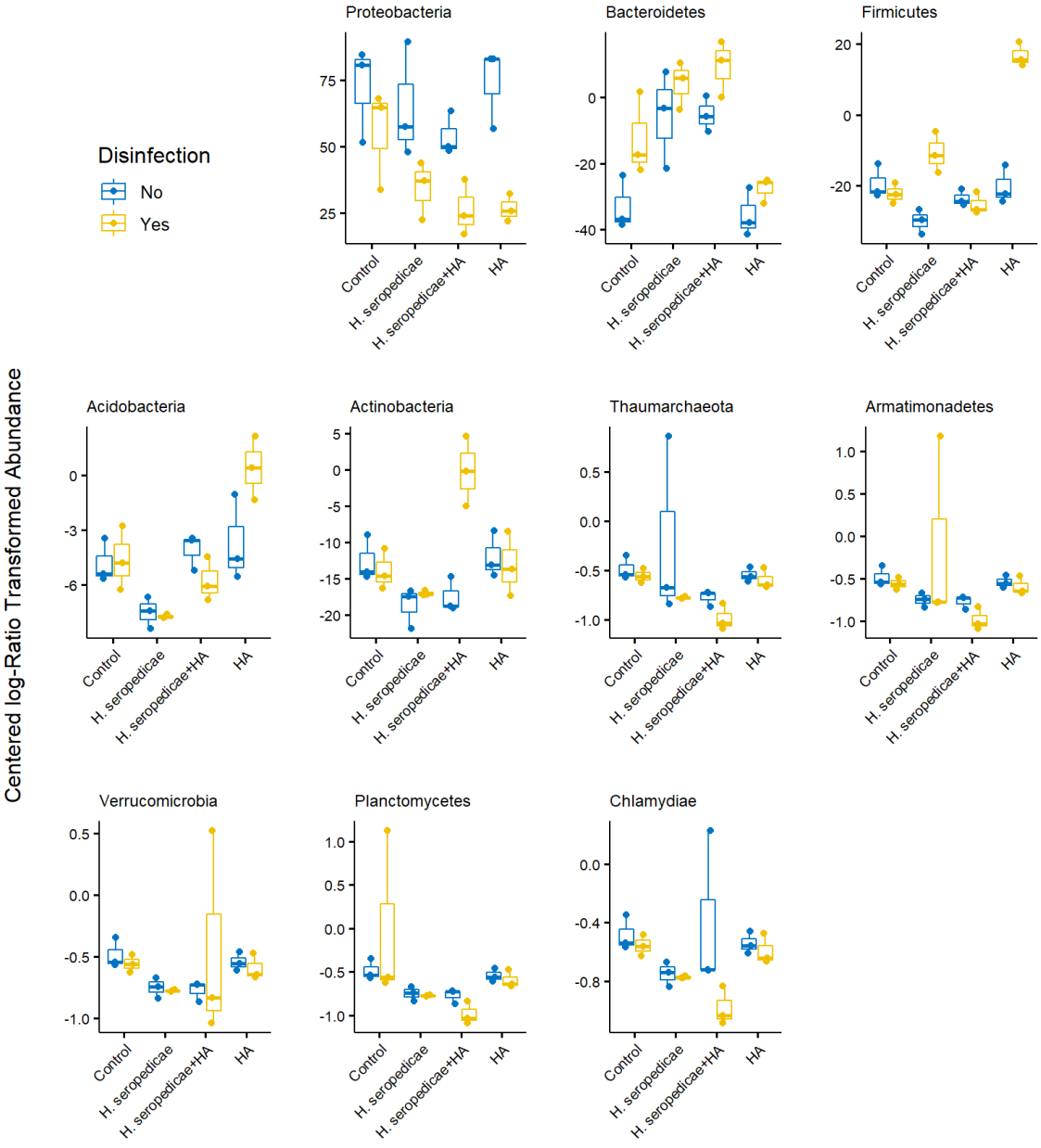


Fig 10. Centered log-ratio (clr) transformed abundance of bacterial phyla found in roots of non-disinfected (No) and disinfected (Yes) seeds and treated with *H. seropedicae* and/or humic acids (HA).

At the class level, the abundance of Alphaproteobacteria in the inoculated treatments was lower concerning the control and HA, regardless of the disinfection method (Fig 11). On the other hand, Betaproteobacteria and Gammaproteobacteria were more abundant when *H. seropedicae* (combined or not with HA) was inoculated in non-disinfected and disinfected maize, respectively (Fig 11). Some bacteria classes had their abundance linked to maize disinfection and inoculation of specific treatments, such as Bacilli and Acidobacteria_Gp1 in the presence of HA and Flavobacteriia in the presence of *H. seropedicae* (Fig 11). The classes

Sphingobacteriia, Actinobacteria, and Cytophagia were stimulated by the combined use of *H. seropedicae* with HA (Fig 11).

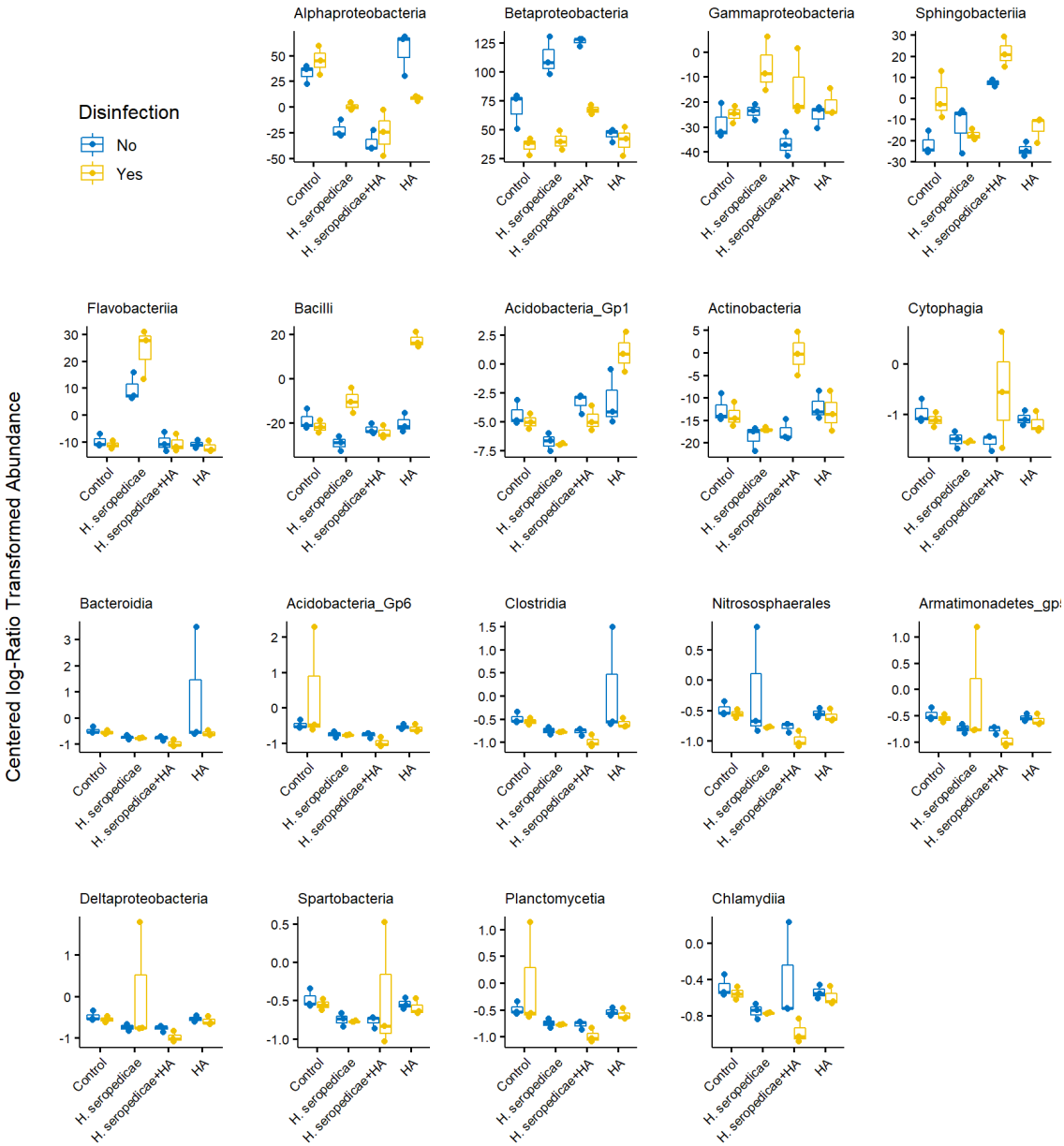


Fig 11. Centered log-ratio (clr) transformed abundance of bacterial classes found in roots of non-disinfected (No) and disinfected (Yes) seeds and treated with *H. seropedicae* and/or humic acids (HA).

In the composition of the bacteriome at the order level, seed disinfection reduced the abundance of Burkholderiales in all treatments, while the orders Pseudomonadales and Rhodocyclales were enriched after the inoculation of *H. seropedicae* (Fig 12). For Rhodospirillales and Sphingomonadales order, the abundance was higher in control and reduced in the presence of *H. seropedicae* and/or HA (Fig 12). The abundance of other orders

has been attributed to the inoculation of *H. seropedicae* (Caulobacterales, Xanthomonadales, Flavobacteriales, and Rhizobiales), HA (Caulobacterales, Bacillales, Rhizobiales, and Terriglobus) and a combination of both (Sphingobacteriales, Rhizobiales, Actinomycetales and Seeds and Cytopathines and Cyanthes and other seeds and/or not disinfected (Fig 12).

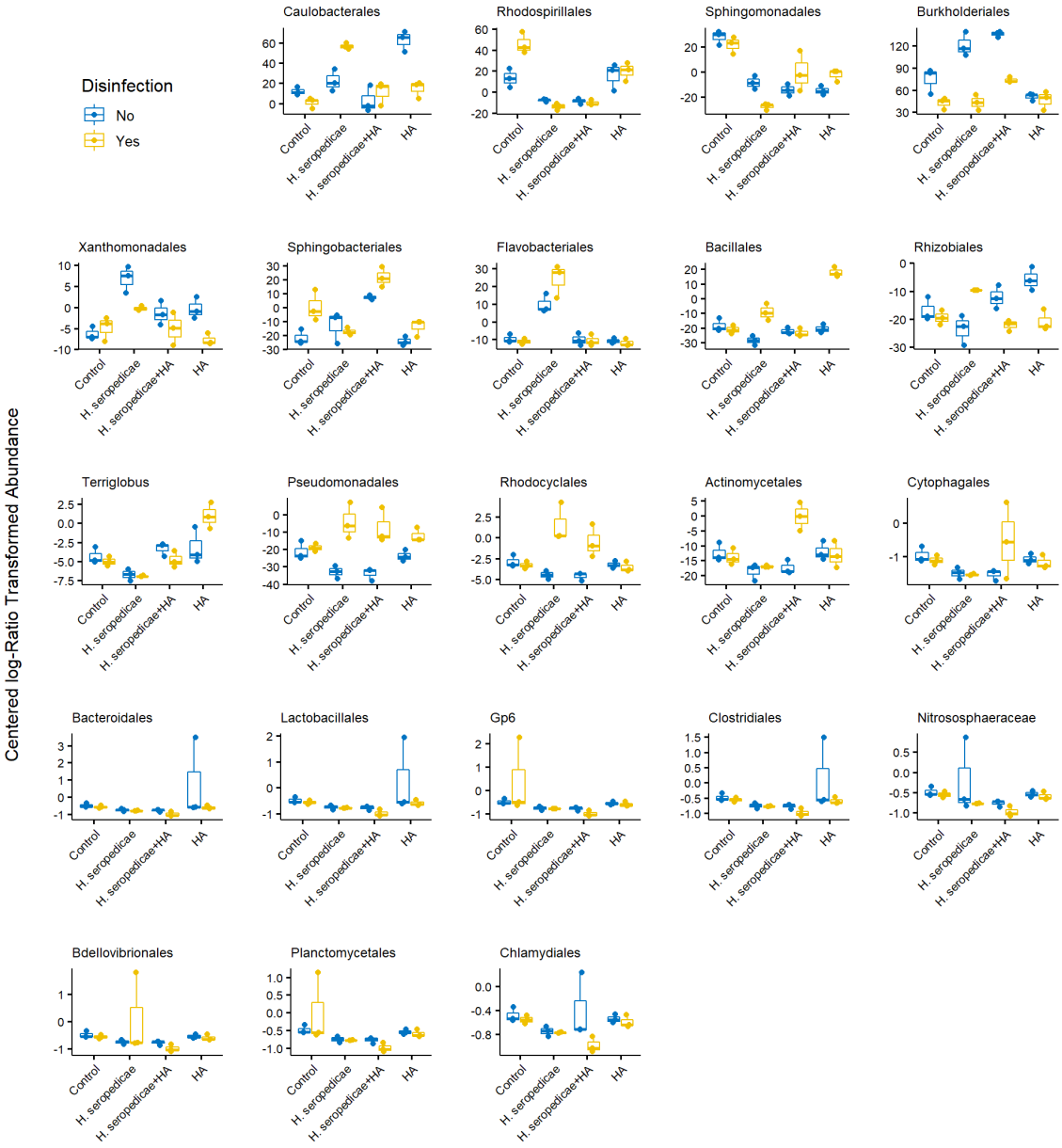
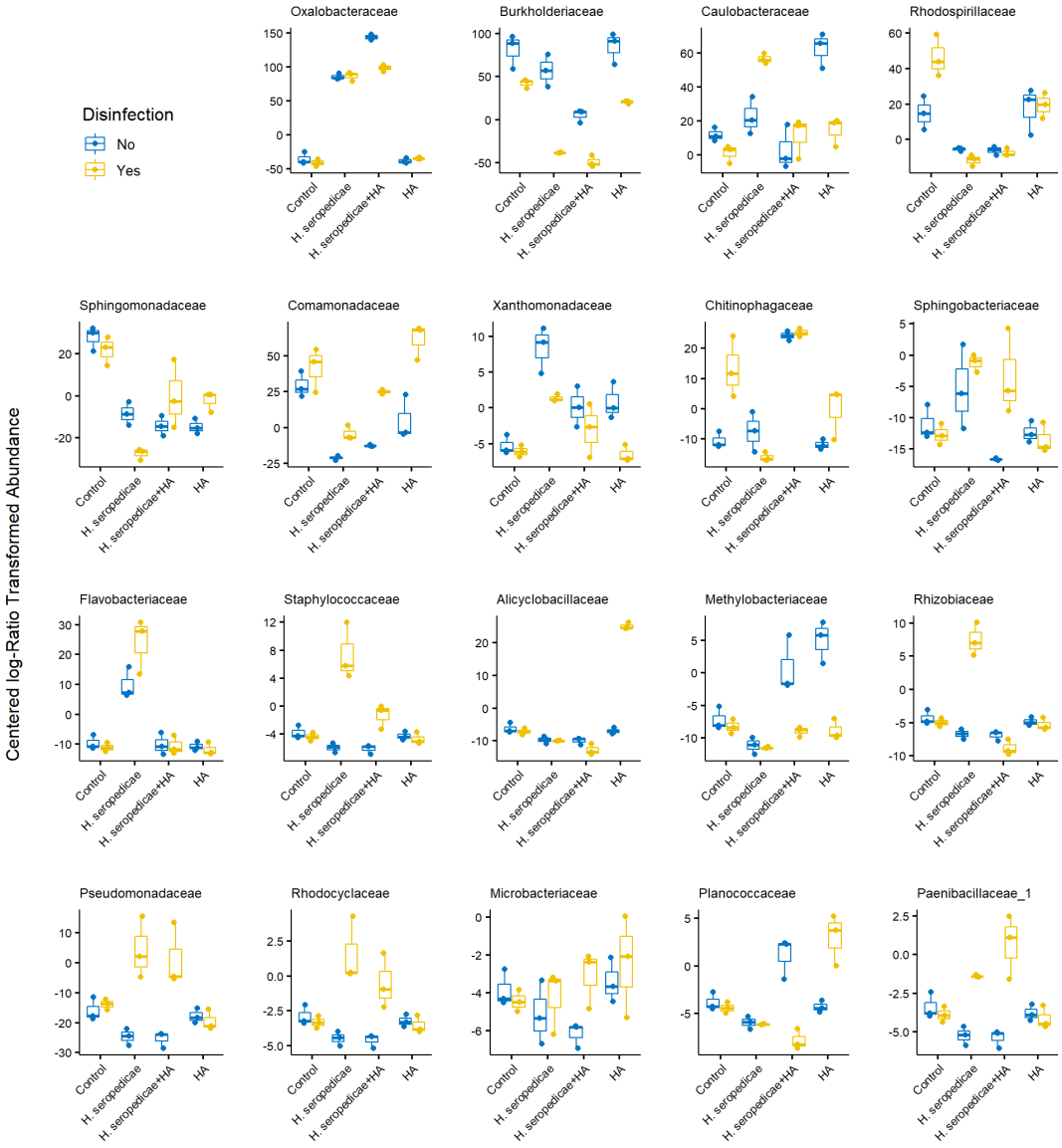


Fig 12. Centered log-ratio (clr) transformed abundance of bacterial orders found in roots of non-disinfected (No) and disinfected (Yes) seeds and treated with *H. seropedicae* and/or humic acids (HA).

Among the families identified by sequencing, Burkholderiaceae was reduced by disinfecting maize with hypochlorite, while inoculation of *H. seropedicae* with or without HA stimulated the abundance of Oxalobacteraceae, Pseudomonadaceae, Rhodocyclaceae, and

Paenibacillaceae_1, mainly in the disinfected treatment (Fig 13). The abundance of the other families varied between the control treatments (Rhodospirillaceae, Sphingomonadaceae, Comamonadaceae, Moraxellaceae, and Sinobacteraceae), *H. seropedicae* (Caulobacteraceae, Xanthomonadaceae, Sphingobacteriaceae, Flavobacteriaceae, Staphylococcaceae, Rhizobiaceae, Bradyrhizobiaceae, and Bacillaceae_1), HA (Caulobacteraceae, Comamonadaceae, Alicyclobacillaceae, Methylobacteriaceae, Microbacteriaceae, Planococcaceae, and Acetobacteraceae) e *H. seropedicae*+HA (Chitinophagaceae, Methylobacteriaceae, Microbacteriaceae, Planococcaceae, Intrasporangiaceae, Micrococcaceae, Brucellaceae, and Cytophagaceae), with most of the taxa stimulated after maize disinfection (Fig 13).



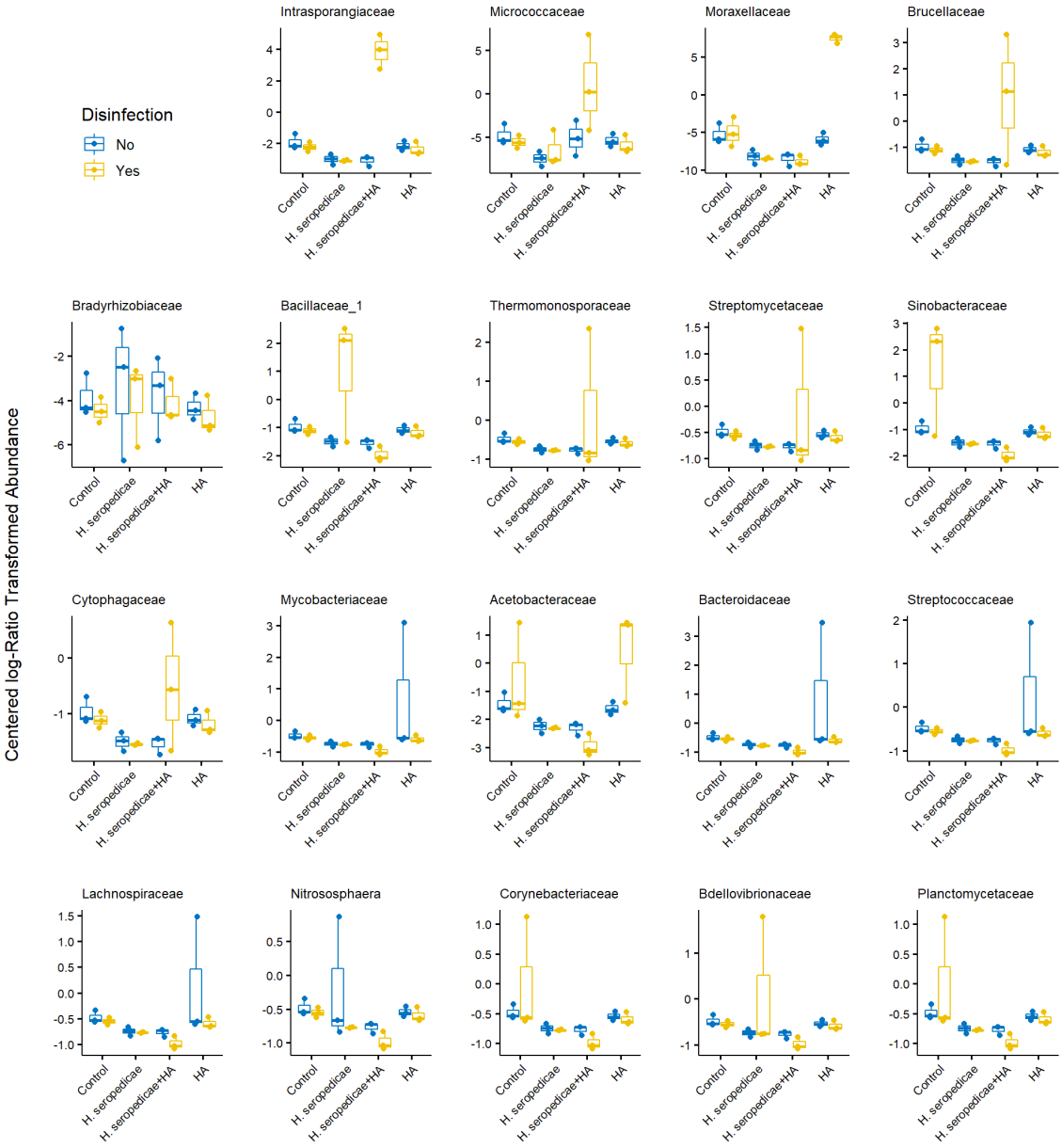
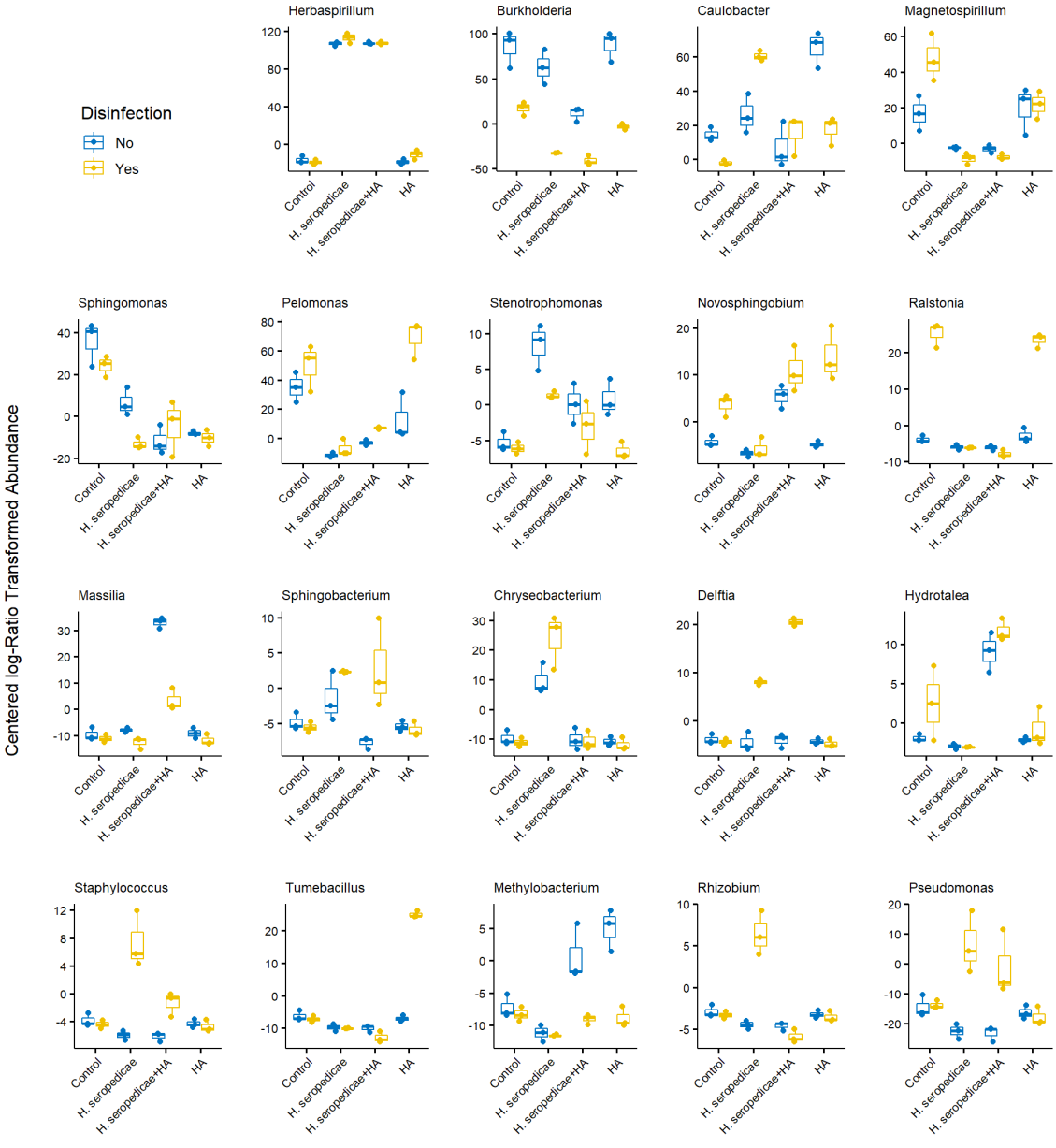


Fig 13. Centered log-ratio (clr) transformed abundance of bacterial families found in roots of non-disinfected (No) and disinfected (Yes) seeds and treated with *H. seropedicae* and/or humic acids (HA).

The analysis of the bacterial genera of the maize root revealed, as expected, a greater abundance of *Herbaspirillum* in the inoculated treatments and a reduction of the *Burkholderia* genus when seeds were disinfected (Fig 14). The single application of *H. seropedicae* or combined with humic acids also reduced the abundance of *Burkholderia* and other genera, such as *Magnetospirillum*, *Sphingomonas*, *Pelomonas*, and *Ralstonia* (Fig 14). The genera *Caulobacter*, *Stenotrophomonas*, *Sphingobacterium*, *Chryseobacterium*, *Delftia*, *Staphylococcus*, *Rhizobium*, *Pseudomonas*, *Shinella*, *Paenibacillus*, *Pedobacter*, *Sediminibacterium*, *Bosea*, and *Bacillus* were enriched in treatments inoculated only with the

bacteria. At the same time, *Novosphingobium*, *Massilia*, *Sphingobacterium*, *Delftia*, *Hydrotalea*, *Methylobacterium*, *Pseudomonas*, *Shinella*, *Leucobacter*, *Sporosarcina*, *Paenibacillus*, *Pedobacter*, *Intrasporangium*, *Arthrobacter*, *Ochrobactrum*, *Azospirillum*, *Naxibacter*, and *Micrococcus* were more abundant when combining the bacteria with HA (Fig 14). The isolated application of HA stimulated the abundance of *Caulobacter*, *Pelomonas*, *Novosphingobium*, *Tumebacillus*, *Methylobacterium*, *Sporosarcina*, *Acinetobacter*, and *Enhydrobacter* (Fig 14). Most of the cited taxa became abundant in the maize root after disinfecting the seeds and inoculating the bacteria *H. seropedicae* (Fig 14).



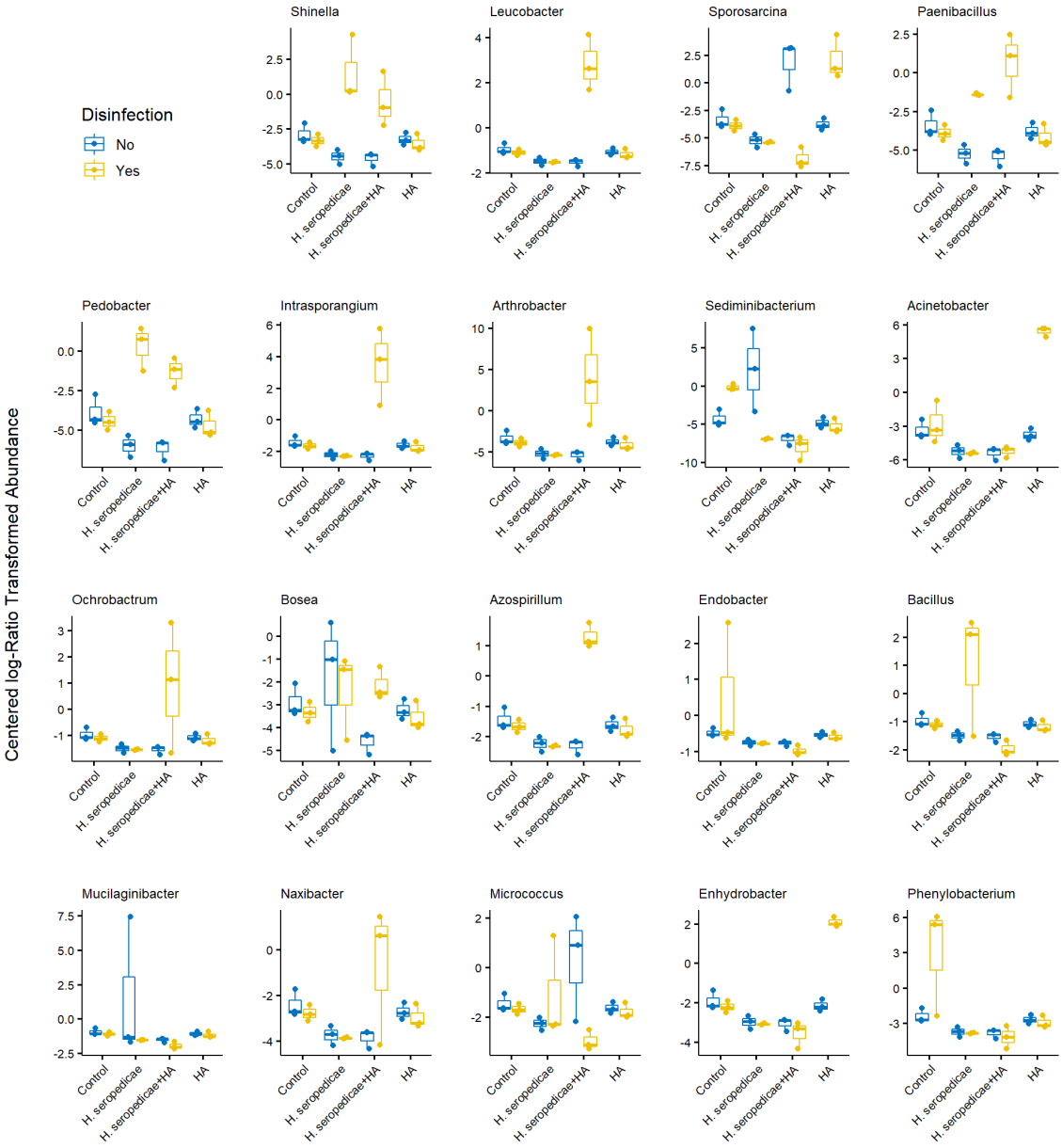


Fig 14. Centered log-ratio (clr) transformed abundance of bacterial genera found in roots of non-disinfected (No) and disinfected (Yes) seeds and treated with *H. seropedicae* and/or humic acids (HA).

In the Venn diagram, we observed a total of 40 bacterial ASVs in non-disinfected samples, of which 17 were shared between some treatments, and 23 were exclusive for one treatment (Fig 15). In disinfected samples, we observed the overlap of 31 ASVs against 35 unique ones, totaling 66 ASVs (Fig 15). Despite the variations between treatments, five genera integrated the bacterial core of germinated roots from non-disinfected seeds, including *Caulobacter*, *Magnetospirillum*, *Sphingomonas*, *Pelomonas*, and *Burkholderia* (Tables 4 e 5). The bacteriome core composition was changed in disinfected seeds, with the replacement of the *Burkholderia* genus by *Novosphingobium* and members of the Chitinophagaceae family

(Tables 4 and 5). The treatments inoculated with *H. seropedicae* had the number of shared ASVs increased from 5 (genera *Herbaspirillum*, *Chryseobacterium*, and *Delftia*; families Sphingobacteriaceae and Oxalobacteraceae) to 10 (genera *Chryseobacterium*, *Delftia*, *Massilia*, *Sphingobacterium*, *Staphylococcus*, *Stenotrophomonas*, *Shinella*, *Paenibacillus*, *Pedobacter*, and *Bosea*) after seed disinfection (Fig 15; Tables 4 and 5). Interestingly, the combined use of *H. seropedicae* with HA in disinfected seeds increased from 8 (genres *Novosphingobium*, *Hydrotalea*, *Sporosarcina*, *Micrococcus*, *Bradyrhizobium*, *Oxalobacter*, and *Neochlamydia*; family Chitinophagaceae) to 15 (genera *Leucobacter*, *Intrasporangium*, *Arthrobacter*, *Methylobacterium*, *Ochrobactrum*, *Azospirillum*, *Naxibacter*, *Actinomadura*, *Sphingobium*, *Streptomyces*, *Flavihumibacter*, and *Hymenobacter*; Oxalobacteraceae and Intrasporangiaceae families; Spartobacteria class) the number of unique ASVs for treatment (Fig 15; Tables 4 and 5).

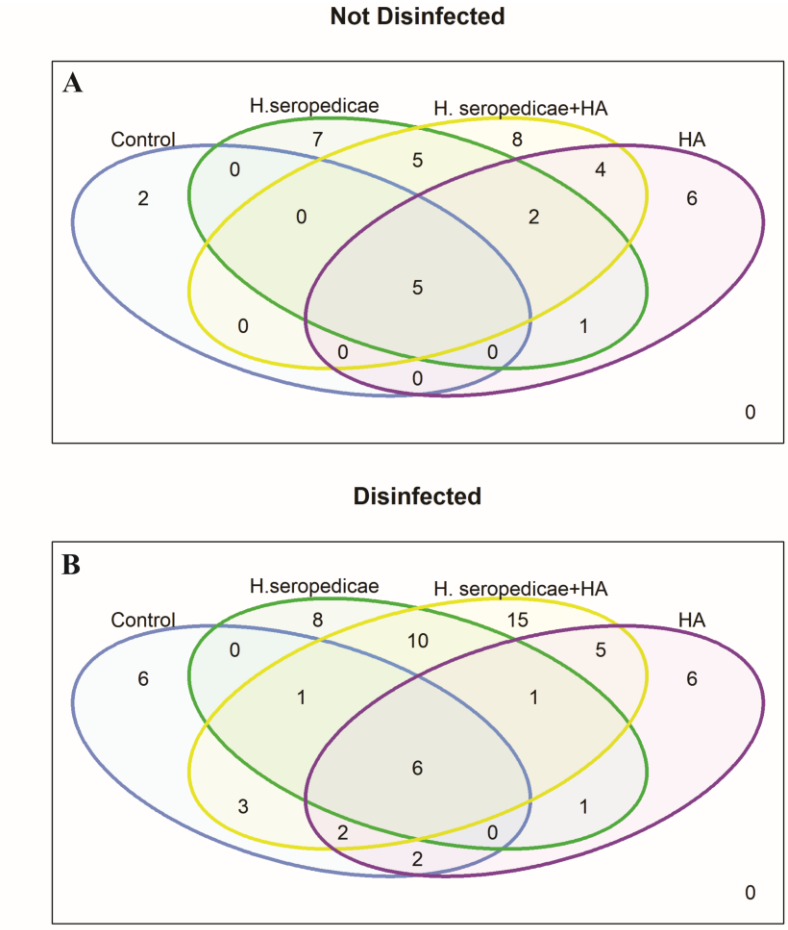


Fig 15. Venn diagram with shared ASVs between roots of non-disinfected and disinfected seeds and treated with *H. seropedicae* and/or humic acids (HA).

Table 4. Individual and shared ASVs between non-disinfected treatments.

Phylum	**Class**	**Order**	**Family**	**Genus**	**Control**	***H. seropedicae***	***H. seropedicae*+HA**	**HA**
Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	*Herbaspirillum*	0	4808	5262	0
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	*Caulobacter*	1320	1280	611	3230
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	*Magnetospirillum*	1644	328	436	1631
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Sphingomonas*	2821	1195	511	654
Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Pelomonas*	2103	69	314	1275
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	*Stenotrophomonas*	0	722	449	311
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Chitinophagaceae	*NA*	0	0	878	0
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Novosphingobium*	0	0	426	0
Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	*Ralstonia*	0	0	0	33
Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	*Massilia*	0	179	1600	77
Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	*Burkholderia*	4813	2405	901	4651
Bacteroidetes	Flavobacteria	Flavobacteriales	Flavobacteriaceae	*Chryseobacterium*	0	909	104	0
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Chitinophagaceae	*Hydrotalea*	0	0	284	0
Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylobacteriaceae	*Methylobacterium*	0	0	297	616
Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Delftia*	0	42	22	0
Firmicutes	Bacilli	Bacillales	Planococcaceae	*Sporosarcina*	0	0	197	0
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	*Sphingobacterium*	0	194	0	0
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	*NA*	0	90	10	0
Acidobacteria	Acidobacteria_Gp1	Terriglobus	NA	*NA*	0	0	55	76
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	*Mucilaginibacter*	0	86	0	0
Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	*NA*	0	20	226	0
Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae	*Micrococcus*	0	0	50	0
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Chitinophagaceae	*Sediminibacterium*	0	257	0	0
Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	*Bosea*	0	65	0	0
Proteobacteria	Gammaproteobacteria	NA	NA	*NA*	0	61	0	0
Proteobacteria	Alphaproteobacteria	NA	NA	*NA*	0	0	45	1
Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	*Bradyrhizobium*	0	0	37	0
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*NA*	0	0	3	105
Actinobacteria	Actinobacteria	Actinomycetales	Mycobacteriaceae	*Mycobacterium*	0	0	0	17
Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	*Bacteroides*	0	0	0	25
Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	*Curtobacterium*	0	0	0	7
Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	*Lactococcus*	0	0	0	5
Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	*Leifsonia*	0	6	0	0
Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*NA*	9	0	0	0
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	*NA*	0	0	0	3
Thaumarchaeota	Nitrososphaerales	Nitrososphaeraceae	Nitrososphaera	*NA*	0	2	0	0
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Sphingosinicella*	10	0	0	0
Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	*Oxalobacter*	0	0	1	0
Proteobacteria	Betaproteobacteria	NA	NA	*NA*	0	2	0	3
Chlamydiae	Chlamydiia	Chlamydiales	Parachlamydiaceae	*Neochlamydia*	0	0	1	0

Table 5. Individual and shared ASVs between disinfected treatments.

Phylum	**Class**	**Order**	**Family**	**Genus**	**Control**	***H. seropedicae***	***H. seropedicae*+HA**	**HA**
Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	*Herbaspirillum*	0	5925	5436	213
Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	*Burkholderia*	1393	0	0	992
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	*Caulobacter*	466	1730	725	1399
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	*Magnetospirillum*	2897	93	171	1297
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Sphingomonas*	1872	583	616	505
Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Pelomonas*	2688	132	153	3412
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Chitinophagaceae	*NA*	940	7	713	461
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Novosphingobium*	467	16	454	856
Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	*Ralstonia*	1267	0	0	920
Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	*Massilia*	0	64	449	0
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	*Sphingobacterium*	0	458	310	0
Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Delftia*	0	433	490	0
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Chitinophagaceae	*Hydrotalea*	183	0	307	56
Firmicutes	Bacilli	Bacillales	Staphylococcaceae	*Staphylococcus*	0	456	133	0
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	*Stenotrophomonas*	0	414	276	0
Firmicutes	Bacilli	Bacillales	Alicyclobacillaceae	*Tumebacillus*	0	0	0	1513
Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	*Chryseobacterium*	0	863	284	0
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*NA*	33	0	106	0
Acidobacteria	Acidobacteria_Gp1	Terriglobus	NA	*NA*	0	0	86	256
Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	*Rhizobium*	0	283	0	0
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	90	657	524	0
Proteobacteria	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae	*Shinella*	0	215	114	0
Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	*Leucobacter*	0	0	81	0
Firmicutes	Bacilli	Bacillales	Planococcaceae	*Sporosarcina*	0	0	0	224
Firmicutes	Bacilli	Bacillales	Paenibacillaceae_1	*Paenibacillus*	0	82	189	0
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	*Pedobacter*	0	139	129	0
Actinobacteria	Actinobacteria	Actinomycetales	Intrasporangiaceae	*Intrasporangium*	0	0	124	0
Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae	*Arthrobacter*	0	0	170	0
Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*NA*	0	0	106	3
Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylobacteriaceae	*Methylobacterium*	0	0	95	0
Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	*NA*	0	0	56	0
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	*Acinetobacter*	12	0	11	371
Actinobacteria	Actinobacteria	Actinomycetales	Intrasporangiaceae	*NA*	0	0	30	0
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Chitinophagaceae	*Sediminibacterium*	186	0	6	0
Proteobacteria	Alphaproteobacteria	Rhizobiales	Brucellaceae	*Ochrobactrum*	0	0	31	0
Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	*Bosea*	0	29	64	0
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	*Azospirillum*	0	0	103	0
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	*Endobacter*	11	0	0	0
Firmicutes	Bacilli	Bacillales	Bacillaceae_1	*Bacillus*	0	48	0	0

Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	*Naxibacter*	0	0	39	0
Proteobacteria	Alphaproteobacteria	NA	NA	*NA*	0	0	39	4
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	*Enhydrobacter*	0	0	1	131
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	*Phenylobacterium*	158	0	4	0
Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	*Kaistia*	0	40	0	0
Firmicutes	Bacilli	Bacillales	Planococcaceae	*NA*	0	0	0	42
Actinobacteria	Actinobacteria	Actinomycetales	Thermomonosporaceae	*Actinomadura*	0	0	15	0
Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae	*Micrococcus*	0	18	0	0
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Sphingobium*	0	0	54	0
Actinobacteria	Actinobacteria	Actinomycetales	Streptomycetaceae	*Streptomyces*	0	0	6	0
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Chitinophagaceae	*Flavihumibacter*	0	0	8	0
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	*NA*	36	0	0	0
Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	*Leifsonia*	0	17	0	32
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	*NA*	9	0	0	0
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	*Gluconacetobacter*	0	0	0	13
Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	*Hymenobacter*	0	0	9	0
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	*NA*	0	0	0	15
Acidobacteria	Acidobacteria_Gp6	Gp6	NA	*NA*	8	0	0	0
Actinobacteria	Actinobacteria	Actinomycetales	Corynebacteriaceae	*Corynebacterium*	2	0	0	0
Armatimonadetes	Armatimonadetes_gp5	NA	NA	*NA*	0	3	0	0
Proteobacteria	Deltaproteobacteria	Bdellovibrionales	Bdellovibrionaceae	*Vampirovibrio*	0	6	0	0
Verrucomicrobia	Spartobacteria	NA	NA	*NA*	0	0	2	0
Planctomycetes	Planctomycetia	Planctomycetales	Planctomycetaceae	*Gemmata*	2	0	0	0
Proteobacteria	Betaproteobacteria	NA	NA	*NA*	0	0	1	3
Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	*Oxalobacter*	0	5	0	0
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Blastomonas*	0	0	0	2
Proteobacteria	Alphaproteobacteria	Rhizobiales	NA	*NA*	0	4	0	0

ASVs present in the inoculated *H. seropedicae* bacteria was compared with the treatments (Fig 16A). The taxonomy of the corresponding ASVs has been modified and now displays an “Inocular” after each level. Therefore, the caption “Proteobacteria (Inoculate)” represents an ASV present in the inoculated *H. seropedicae* bacterium. First, the comparison between disinfected versus uninfected seeds within each treatment considering the abundance of inoculated *H. seropedicae* was not significant (Fig 16B). As expected, the average abundance of *H. seropedicae* inoculated between treatments was higher in treatments that received the bacteria (Fig 16B). The abundance of members of the Phylum Proteobacteria (all, other than inoculate) and Proteobacteria Inoculate between the treatments *H. seropedicae*+HA and *H. seropedicae* (grouped by disinfection) did not differ significantly (Fig 16C). However, within the inoculated treatments, the Proteobacteria Inoculate phylum was more abundant than the Proteobacteria (other than inoculate) phylum in the two disinfection conditions (Fig 16C). Within the Betaproteaobacteria class, the abundance of inoculated bacteria was greater in the disinfected and non-disinfected treatment (Fig 16D). In Figure 16D, we also see the reduction of members of the class Betaproteaobacteria (other than inoculate) after the disinfection of maize seeds.

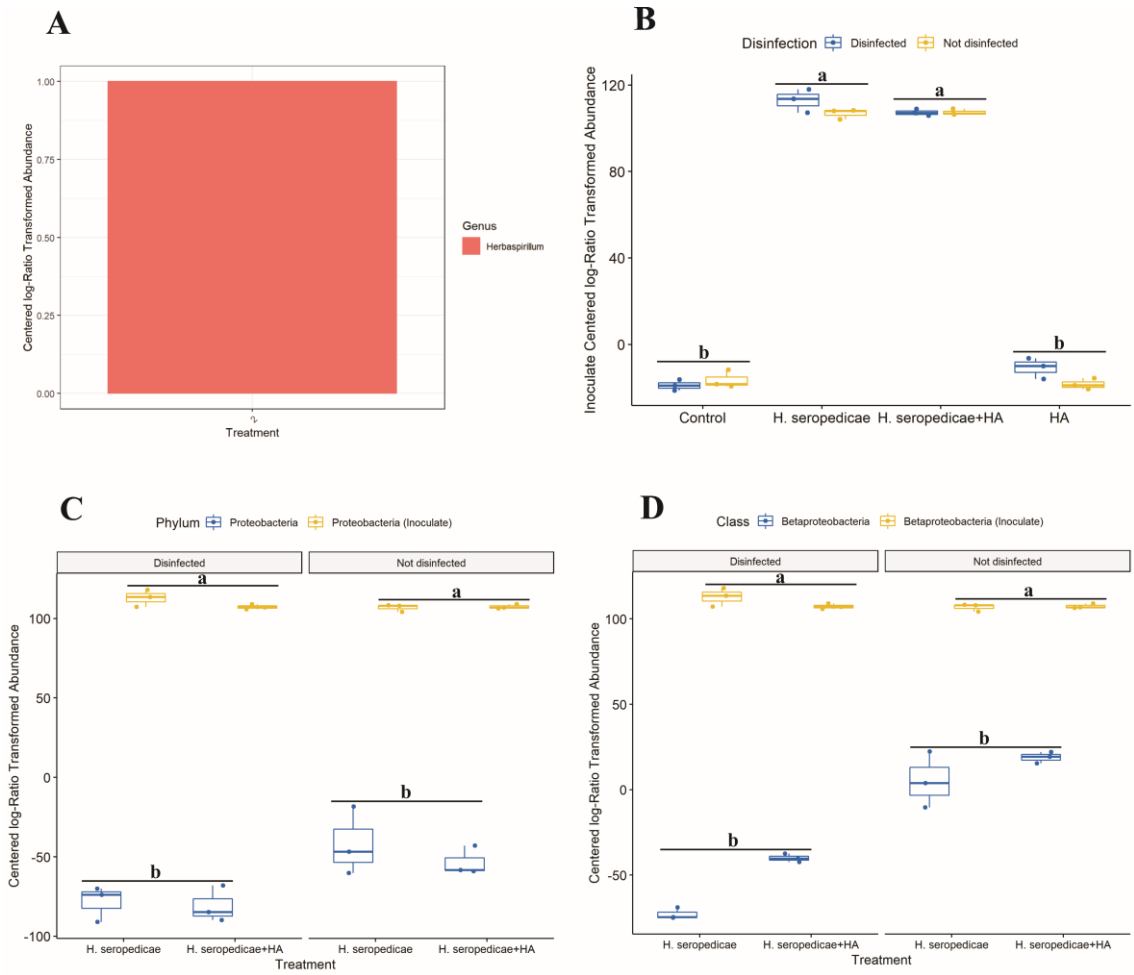


Fig 16. Abundance (ASVs; A) of the bacterium *H. seropedicae* inoculated in treatments (disinfected and not disinfected; control, *H. seropedicae*, *H. seropedicae*+HA and HA; B) and within the phylum Proteobacteria (C) and the class Betaproteobacteria (D). The differences were compared using the Wilcoxon test ($p < 0.05$).

As observed in the taxonomy of the bacterial community (Fig 14), the inoculation of *H. seropedicae* reduced the abundance of *Burkholderia* in the maize root, which led us to test the interrelationships within the bacteriome after the arrival of an inoculated bacterium. Correlation of Spearman with the 20 most abundant bacterial genera at the root revealed 15 genera negatively correlated with the inoculated *H. seropedicae* bacterium, among them *Burkholderia*, *Vampirovibrio*, *Curtobacterium*, *Mycobacterium*, *Bacteroides*, *Lactococcus*, *Gluconacetobacter*, *Endobacter*, *Sphingomonas*, *Magnetospirillum*, *Ralstonia*, *Actinomadura*, *Streptomyces*, *Flavihumibacter*, and *Pelomonas* (Fig 17). Some taxa negatively correlated with *H. seropedicae* showed a positive correlation with *Burkholderia*, such as the genera *Vampirovibrio*, *Curtobacterium*, *Mycobacterium*, *Bacteroides*, *Lactococcus*, *Gluconacetobacter*, *Endobacter*, *Sphingomonas*, and *Magnetospirillum* (Fig 17).

Burkholderia correlated negatively with the inoculated bacteria and the genera *Novosphingobium* and *Delftia* (Fig 17).

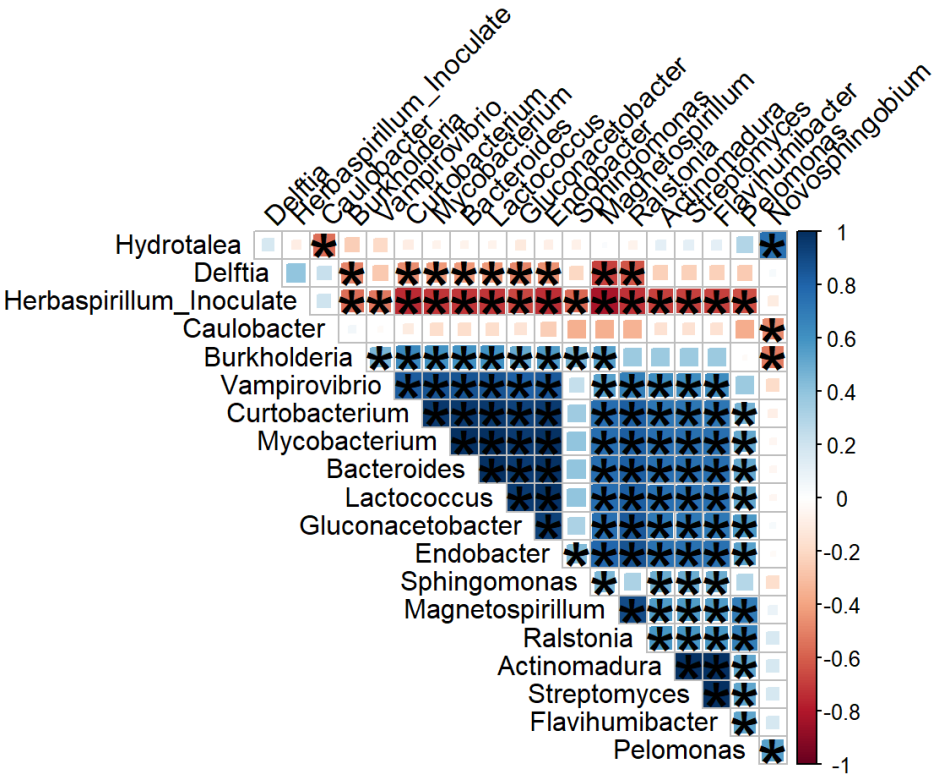


Fig 17. Spearman's rank correlation matrix of the top 20 most abundant bacterial genera across in the presence of the *H. seropedicae* Inoculate. The colours in the scale bar denote the nature of the correlation, where 1 indicates a perfect positive correlation (dark blue) and -1 indicates perfect negative correlation (dark red) between two taxa. The presence of the asterisk (*) indicates a correlation with p-value $p < 0.05$.

4. Discussion

In this study, we demonstrated the effect of inoculation of *H. seropedicae* combined or not with HA on root colonization, plant growth, and the modulation of the root bacteriome of maize.

First, *H. seropedicae* successfully colonized the surface and interior of maize roots grown in a hydroponic system, as observed in the counting results (in plate and qPCR) and microscopy (scanning electronics and epifluorescence). This endophytic diazotrophic bacterium, as its name suggests, is capable of efficiently colonizing the internal tissues of the host plant. For this, it uses cracks formed by the emergence of the root as the main infection point, which explains a large number of bacteria in this region (Matteoli et al. 2020). Once inside the plant, bacteria spread, and colonize different tissues (Matteoli et al. 2020). According to Monteiro et al. (2008), *H. seropedicae* was able to colonize the surface and internal tissues of the maize root 30 min and 24 h after inoculation. The application of HA may also have contributed to the colonization and population increase of *H. seropedicae* by inducing physiological and anatomical changes in the maize root (Olivares et al. 2017).

Counting and microscopy results suggest that *H. seropedicae* was able to compete with members of the native community and establish itself at the root. Once established, *H. seropedicae* combined with HA promoted the growth of maize by significantly increasing seedling length and total dry biomass. In the literature, we find several explanations for this result, but they are studies that consider only the direct effect of the bacteria on the host plant through biofertilization, biostimulation, and biocontrol (Matteoli et al. 2020); or studies that relate this growth to the positive effects of HA (Olivares et al. 2017). These findings partially justify the effects of bioinoculants, but they do not answer whether the introduction of a bacterium as an inoculant disturbs the structure of the native bacterial community and whether this disturbance is related to plant growth.

In the present study, we demonstrated that the disinfection of the seeds with sodium hypochlorite and the inoculation of *H. seropedicae* caused changes in the diversity, composition, and abundance of the community residing in the maize root. The result of these changes resulted in significant groupings for beta diversity and increases in alpha diversity indices, which were positively related to disinfection and inoculation. The effect of seed disinfection over the increase on diversity can be attributed to the niches vacancy at the root (by removing part of the resident community) and their occupation by other taxa of the bacteriome (Hardoim, 2019). Inoculation, on the other hand, contributed by integrating *H.*

seropedicae in the resident community or by stimulating native taxa. Collectively, disinfection and inoculation increased the diversity of the bacteriome. Similar results were observed by Dos Santos et al. (2020a) when disinfecting maize seeds of the SHS 5050 variety and having the root diversity increased under axenic conditions. It is worth mentioning that, in addition to the inoculated bacteria, there was no entry of external species into our controlled experimental system, that is, the increased diversity is attributed purely to the stimulation of taxa of the maize bacteriome itself.

Compositional changes in the maize root community were based on reductions and increases in various bacterial taxa. The genus *Herbaspirillum* was the most abundant after five days of inoculation, which indicates that *H. seropedicae* invaded the plant, overcame the bacteriome pressures, gained access to available resources, and expanded the size of its population while displacing members of the resident community (Mallon et al. 2018). Members of the genus *Burkholderia* (phylum Proteobacteria), which initially comprised a dominant portion of the bacteriome, were reduced by disinfecting the seeds and introducing *H. seropedicae*. Non-disinfected-inoculated, disinfected-non-inoculated, and disinfected-inoculated treatments showed declines in *Burkholderia* abundance. This decline proves the sensitivity of these bacteria to hypochlorite, as demonstrated by Dos Santos et al. (2020a), and suggests a preference for niche and similar resources between *H. seropedicae* and members of the *Burkholderia* genus. Nevertheless, since the initial population of *H. seropedicae* was high, it was competitively superior in the invasion process (Mallon et al. 2018). Spearman's correlations confirmed negative interactions between inoculant and *Burkholderia*.

Taxons with low initial abundance in the community had displayed consistent increase after the inoculation of *H. seropedicae* (combined or not with HA). It is the case of members of the phylum Bacteroidetes and many genera reported here. Most of the taxa were enriched at the root after seed disinfection, suggesting emerging empty niches by the action of the hypochlorite facilitated the expansion of the inoculated bacteria and some members of the resident community. In general, we believe that the introduction of *H. seropedicae* overcame members of the bacteriome that compete for the same substrate (such as *Burkholderia*), allowing rare (non-target) taxa to explore better niches and resources not used by *H. seropedicae* (Mallon et al. 2018). As a result, the inoculated bacteria reorganized the structure of the maize root microbial community.

The analysis of ASVs present in the inoculated *H. seropedicae* bacterium revealed that their abundance did not differ between the disinfected and non-disinfected treatments.

However, the impact of inoculation was greater in communities that had members removed by hypochlorite (disinfected seeds), which resulted in major changes in the composition of the bacteriome. It is believed that the supply of niches and resources not explored by the bacteriome of disinfected seeds facilitated the invasion of *H. seropedicae*. In the case of non-disinfected treatment, the inoculation also displaced the resident community, but the impact was less, which can be attributed to the resistance of members of the *Burkholderia* genus.

According to Mawarda et al. (2020), four mechanisms can explain how bioinoculants change the resident community. The first mechanism is the competition for resources, cited in this study as a possible explanation for the reduction of the *Burkholderia* genus after the inoculation of *H. seropedicae*. The existence of empty niches also contributes to the invader's success, which possibly happened when we disinfected the maize seeds. The second and third mechanisms involve antagonism and synergism, which may also justify some results of this study. The antagonism can explain the suppression of non-target bacterial taxa by the inoculant when trying to control some pathogens, while in synergism the inoculant can stimulate native taxa through the metabolites it produces. The fourth and final mechanism attributes to the inoculant the function of modifying the rate and composition of exudates released by the roots of the plants, favouring certain taxa. This mechanism may justify the high number of bacteria stimulated by the combined use of *H. seropedicae* with HA. HA, in addition to inducing the emission of lateral roots, increase the exudation of organic acids, which can be used as a carbon source to support the growth of inoculated bacteria and native maize taxa (Canellas et al. 2008; Da Silva Lima et al. 2014; Olivares et al. 2017).

We concluded that the inoculation of *H. seropedicae* combined or not with HA altered the structure of the maize root bacteriome when interacting with key members of the community, such as *Burkholderia*. We also found that the disinfection of seeds with sodium hypochlorite seems to release niches in the root and contribute to the establishment of the inoculant and native taxa of maize. We believe that the interactions described here may be related to the promotion of plant growth and need to be explored. Understanding these interactions means attributing seed-borne bacteria and bioinoculants their respective roles in the growth and protection of crops. Also, knowing the response of the native community to an inoculated bacterium can increase the effectiveness of many microbial applications.

5. References

Anderson, M.J., 2017. Permutational Multivariate Analysis of Variance (PERMANOVA), Wiley StatsRef: Statistics Reference Online, pp. 1-15.

Apprill, A., McNally, S., Parsons, R.J., Weber, L., 2015. Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. Aquatic Microbial Ecology 75, 129-137.

Bacchetti De Gregoris, T., Aldred, N., Clare, A.S., Burgess, J.G., 2011. Improvement of phylum- and class-specific primers for real-time PCR quantification of bacterial taxa. Journal of Microbiological Methods 86, 351-356.

Baldani, J.I., Baldani, V.L.D., Seldin, L., Döbereiner, J., 1986. Characterization of *Herbaspirillum seropedicae* gen. nov., sp. nov., a Root-Associated Nitrogen-Fixing Bacterium. International Journal of Systematic and Evolutionary Microbiology 36, 86-93.

Baldotto, L.E.B., Olivares, F.L., Bressan-Smith, R., 2011. Structural interaction between GFP-labeled diazotrophic endophytic bacterium *Herbaspirillum seropedicae* RAM10 and pineapple plantlets 'Vitória'. Brazilian Journal of Microbiology. Brazilian Journal of Microbiology 42, 114-125.

Boa Sorte, P.M.F. Utilização de diferentes técnicas para monitorar a colonização e estabelecimento de bactérias diazotróficas endofíticas em plantas de cana-de-açúcar. 2013. 70 f. Tese (Doutorado em Agronomia, Ciência do Solo) - Instituto de Agronomia, Departamento de Solos, Universidade Federal Rural do Rio de Janeiro, Seropédica - RJ, 2013.

Brasil. Decreto n° 4.954, de 14 de janeiro de 2004. Aprova o regulamento da Lei no 6.894, de 16 de dezembro de 1980, que dispõe sobre a inspeção e fiscalização da produção e do comércio de fertilizantes, corretivos, inoculantes ou biofertilizantes destinados à agricultura, e dá outras providências. Diário Oficial [da] República Federativa do Brasil, Brasília, DF, 15 jan. 2004.

Canellas, L.P., Teixeira Junior, L.R.L., Dobbss, L.B., Silva, C.A., Medici, L.O., Zandonadi, D.B., Façanha, A.R., 2008. Humic acids crossinteractions with root and organic acids. Annals of Applied Biology 153, 157-166.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Peña, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R., 2010. QIIME allows analysis of high-throughput community sequencing data. Nature Methods 7, 335-336.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Huntley, J., Fierer, N., Owens, S.M., Betley, J., Fraser, L., Bauer, M., Gormley, N., Gilbert, J.A., Smith, G., Knight, R., 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. The ISME Journal 6, 1621-1624.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Lozupone, C.A., Turnbaugh, P.J., Fierer, N., Knight, R., 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc Natl Acad Sci U S A 108 Suppl 1, 4516-4522.

Chen, D.H., Ronald, P.C., 1999. A Rapid DNA Minipreparation Method Suitable for AFLP and Other PCR Applications. Plant Molecular Biology Reporter 17, 53-57.

Da Silva Lima, L., Olivares, F.L., Rodrigues de Oliveira, R., Vega, M.R.G., Aguiar, N.O., Canellas, L.P., 2014. Root exudate profiling of maize seedlings inoculated with *Herbaspirillum seropedicae* and humic acids. Chemical and Biological Technologies in Agriculture 1, 23.

Dos Santos, L.F., Souta, J.F., de Paula Soares, C., da Rocha, L.O., Santos, M.L.C., de Matos, C.G.G., Roesch, L.F.W., Olivares, F.L., 2020a. Insights into the structure and role of seed-borne bacteriome during maize germination. doi.org/10.1101/2020.06.02.130856.

Doyle, J.J., Doyle, J.L., 1987. Arapid isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19, 11-15.

Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C., Knight, R., 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27, 2194-2200.

Gadhave, K.R., Devlin, P.F., Ebertz, A., Ross, A., Gange, A.C., 2018. Soil Inoculation with Bacillus spp. Modifies Root Endophytic Bacterial Diversity, Evenness, and Community Composition in a Context-Specific Manner. Microbial Ecology 76, 741-750.

Gloor, G.B., Reid, G., 2016. Compositional analysis: a valid approach to analyze microbiome high-throughput sequencing data. Canadian Journal of Microbiology 62, 692-703.

Hardoim, P.R., Hardoim, C.C.P., van Overbeek, L.S., van Elsas, J.D., 2012. Dynamics of Seed-Borne Rice Endophytes on Early Plant Growth Stages. Plos One 7, e30438.

Jha, P.N., Gomaa, A.-B., Yanni, Y.G., El-Saadany, A.-E.Y., Stedtfeld, T.M., Stedtfeld, R.D., Gantner, S., Chai, B., Cole, J., Hashsham, S.A., Dazzo, F.B., 2020. Alterations in the Endophyte-Enriched Root-Associated Microbiome of Rice Receiving Growth-Promoting Treatments of Urea Fertilizer and Rhizobium Biofertilizer. Microbial Ecology 79, 367-382.

Kour, D., Rana, K.L., Yadav, A.N., Yadav, N., Kumar, M., Kumar, V., Vyas, P., Dhaliwal, H.S., Saxena, A.K., 2020. Microbial biofertilizers: Bioresources and eco-friendly technologies for agricultural and environmental sustainability. Biocatalysis and Agricultural Biotechnology 23, 101487.

Liu, J., Xiang, Y., Zhang, Z., Ling, W., Gao, Y., 2017. Inoculation of a phenanthrene-degrading endophytic bacterium reduces the phenanthrene level and alters the bacterial community structure in wheat. Applied Microbiology and Biotechnology 101, 5199-5212.

Mallon, C.A., Elsas, J.D.V., Salles, J.F., 2015a. Microbial invasions: the process, patterns, and mechanisms. Trends in Microbiology 23, 719-729.

Mallon, C.A., Le Roux, X., van Doorn, G.S., Dini-Andreote, F., Poly, F., Salles, J.F., 2018. The impact of failure: unsuccessful bacterial invasions steer the soil microbial community away from the invader’s niche. The ISME Journal 12, 728-741.

Mallon, C.A., Poly, F., Le Roux, X., Marring, I., van Elsas, J.D., Salles, J.F., 2015b. Resource pulses can alleviate the biodiversity-invasion relationship in soil microbial communities. Ecology 96, 915-926.

Matteoli, F.P., Olivares, F.L., Venancio, T.M., Oliveira da Rocha, L., Souza da Silva Irineu, L.E., Canellas, L.P., 2020. Chapter 23 - *Herbaspirillum*, In: Amaresan, N., Senthil Kumar, M., Annapurna, K., Kumar, K., Sankaranarayanan, A. (Eds.), Beneficial Microbes in Agro-Ecology. Academic Press, pp. 493-508.

Mawarda, P.C., Le Roux, X., Dirk van Elsas, J., Salles, J.F., 2020. Deliberate introduction of invisible invaders: A critical appraisal of the impact of microbial inoculants on soil microbial communities. Soil Biology and Biochemistry 148, 107874.

McDonald, D., Price, M.N., Goodrich, J., Nawrocki, E.P., DeSantis, T.Z., Probst, A., Andersen, G.L., Knight, R., Hugenholtz, P., 2012. An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. The ISME Journal 6, 610-618.

McMurdie, P.J., Holmes, S., 2013. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. Plos One 8, e61217.

Monteiro, R.A., Schmidt, M.A., Baura, V.A.d., Balsanelli, E., Wassem, R., Yates, M.G., Randi, M.A.F., Pedrosa, F.O., Souza, E.M.d., 2008. Early colonization pattern of maize (*Zea mays* L. Poales, Poaceae) roots by *Herbaspirillum seropedicae* (Burkholderiales, Oxalobacteraceae) %J Genetics and Molecular Biology. Genetics and Molecular Biology 31, 932-937.

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Wagner, H., 2015. Vegan: community ecology package. R package vegan, vers. 2.2-1.

Olivares, F.L., Busato, J.G., de Paula, A.M., da Silva Lima, L., Aguiar, N.O., Canellas, L.P., 2017. Plant growth promoting bacteria and humic substances: crop promotion and mechanisms of action. Chemical and Biological Technologies in Agriculture 4, 30.

Pangesti, N., Pineda, A., Hannula, S.E., Bezemer, T.M., 2020. Soil inoculation alters the endosphere microbiome of chrysanthemum roots and leaves. Plant and Soil 455, 107-119.

Parada, A.E., Needham, D.M., Fuhrman, J.A., 2016. Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. Environmental Microbiology 18, 1403-1414.

Pylro, V.S., Morais, D.K., de Oliveira, F.S., Dos Santos, F.G., Lemos, L.N., Oliveira, G., Roesch, L.F., 2016. BMPOS: a Flexible and User-Friendly Tool Sets for Microbiome Studies. Microbial Ecology 72, 443-447.

Pylro, V.S., Roesch, L.F., Morais, D.K., Clark, I.M., Hirsch, P.R., Tótola, M.R., 2014. Data analysis for 16S microbial profiling from different benchtop sequencing platforms. Journal of Microbiological Methods 107, 30-37.

Sammauria, R., Kumawat, S., Kumawat, P., Singh, J., Jatwa, T.K., 2020. Microbial inoculants: potential tool for sustainability of agricultural production systems. Archives of Microbiology 202, 677-693.

Santos, L.F., Olivares, F.L., 2020. Microbial Inoculants in Agriculture and its Effects on Plant Microbiome. In: Dubey, R., Kumar, P. (Eds.), Rhizosphere Engineering: need of the future. Elsevier. In press.

Santos, L.F., Souta, J.F., Rocha, L.O., Soares, C.d.P., Santos, M.L.C., Grativol, C., Roesch, L. F.W., Olivares, F.L., 2021. Altered bacteria community dominance reduces tolerance to resident fungus and seed to seedling growth performance in maize (*Zea mays* L. var. DKB 177). Microbiological Research 243, 126643.

Sharma, S., Sharma, R., Gupta, R., Bisaria, V.S., 2016. Revisiting Action of Bioinoculants: Their Impact on Rhizospheric Microbial Community Function, In: Singh, D.P., Singh, H.B., Prabha, R. (Eds.), Microbial Inoculants in Sustainable Agricultural Productivity: Vol. 2: Functional Applications. Springer India, New Delhi, pp. 41-51.

Sheridan, C., Depuydt, P., De Ro, M., Petit, C., Van Gysegem, E., Delaere, P., Dixon, M., Stasiak, M., Aciksöz, S.B., Frossard, E., Paradiso, R., De Pascale, S., Ventorino, V., De Meyer, T., Sas, B., Geelen, D., 2017. Microbial Community Dynamics and Response to Plant Growth-Promoting Microorganisms in the Rhizosphere of Four Common Food Crops Cultivated in Hydroponics. Microbial Ecology 73, 378-393.

Staroscik, A., 2004. Calculator for determining the number of copies of a template. URI Genomics & Sequencing Center. https://cels.uri.edu/gsc/cndna.html.

Thokchom, E., Thakuria, D., Kalita, M.C., Sharma, C.K., Talukdar, N.C., 2017. Root colonization by host-specific rhizobacteria alters indigenous root endophyte and rhizosphere soil bacterial communities and promotes the growth of mandarin orange. European Journal of Soil Biology 79, 48-56.

Tian, L., Lin, X., Tian, J., Ji, L., Chen, Y., Tran, L.-S.P., Tian, C., 2020. Research Advances of Beneficial Microbiota Associated with Crop Plants. International Journal of Molecular Sciences 21, 1792.

Wang, L., Lin, H., Dong, Y., Li, B., He, Y., 2020. Effects of endophytes inoculation on rhizosphere and endosphere microecology of Indian mustard (*Brassica juncea*) grown in vanadium-contaminated soil and its enhancement on phytoremediation. Chemosphere 240, 124891.

Wei, T., Simko, V., 2017. R Package "Corrplot": Visualization of a Correlation Matrix. Retrieved from. https://github.com/taiyun/corrplot.

Xing, J., Sun, S., Wang, H., Brookes, P.C., Xu, J., 2020. Response of soil native microbial community to *Eschericia coli* O157:H7 invasion. Environmental Pollution 261, 114225.

CONCLUSÕES GERAIS

Conclusões Gerais

Assim como as plantas, os microbiomas se originam das sementes. Após muitas décadas de esquecimento, a semente finalmente foi reconhecida como fonte essencial de inóculos para o microbioma vegetal. No entanto, não estava claro quem eram os micro-organismos transferidos verticalmente e qual sua influência para o desempenho das culturas.

Os resultados deste trabalho comprovam que a semente de milho é fonte de micro-organismos para a nova planta, com perfil bacteriano distinto entre as variedades SHS 5050 e DKB 177. Durante a germinação do milho, ocorre uma sucessão ecológica secundária para a microbiota nativa, o que resulta em mudanças na abundância de táxons na raiz.

Pela primeira vez, um estudo mostrou protocolos de desinfestação já estabelecidos alterando a comunidade inicial da semente de milho e da raiz emergida. Esse distúrbio no bacterioma da semente tem consequências para a germinação e o crescimento do milho, além de aumentar sua suscetibilidade à patógenos fúngicos transmitidos por sementes.

Uma vez que alterações no bacterioma da semente impactam o desempenho das plantas, a prática de inocular micro-organismos exógenos também pode induzir mudanças na estrutura da comunidade nativa e afetar a produtividade das culturas. No presente trabalho, essa hipótese foi confirmada após a inoculação de *H. seropedicae* e/ou ácidos húmicos alterar a diversidade e a composição do bacterioma do milho. Neste caso, inoculações em sementes desinfestadas intensificam o deslocamento da comunidade e promovem o crescimento vegetal.

Conclui-se que bactérias transmitidas por sementes podem contribuir para o desenvolvimento de tecnologias sustentáveis. Mais precisamente, é possível alterar a estrutura do bacterioma pela inoculação de micro-organismos exógenos e beneficiar táxons específicos da comunidade ou isolar membros-chave do bacterioma para a formulação de novos bioinoculantes.