PhD Thesis in Bioscience Engineering

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The wheat microbiome and its associated *Pseudomonas*

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Disclaimer

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Si j'ai vu plus loin, c'est parce que je me suis tenu sur les épaules de géants. - Isaac Newton

Scientific communications

During my PhD, I participated in six publication projects and four conferences where I presented an oral presentation and three posters.

Published manuscripts:

Delitte M., Caulier S., Bragard C. and Desoignies N. (2021). Plant Microbiota Beyond Farming Practices: A Review. In Frontiers in Sustainable Food Systems (Vol. 5).

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Abbreviations

16S rRNA - 16S ribosomal Ribonucleic Acid
AHL - Acyl Homoserine Lactone
AMF - Arbuscular mycorrhizal fungi
ANI - Average Nucleotide Identity
BCA - Biocontrol Agent
CFU - Colony-Forming Units
CLPs - Cyclic Lipopeptides
Ct - Cycle threshold
dDDH - Digital DNA-DNA Hybridization
DAPG - 2, 4-diacetylphloroglucinol
DNA - Deoxyribonucleic Acid
EPS - Extracellular Polymeric Substances
FAO - The Food and Agriculture Organization
FHB - Fusarium Head Blight
GBDP - Genome Blast Distance Phylogeny
gapA - Glyceraldehyde-3-phosphate dehydrogenase A
GFP - Green Fluorescent Protein
glnS - Glutaminyl-tRNA synthetase
gltA - Citrate synthase
gyrB - Gyrase subunit B
HCN - Hydrogen Cyanide
HPR - Hexyl Propyl Resorcinol
ileS - Isoleucyl-tRNA synthetase
IPM - Integrated Pest Management
KEGG - Kyoto Encyclopedia of Genes and Genomes
LFC - Log-fold change
LNA - Locked Nucleic Acid
LOD - Limit of Detection
MLSA - Multi-Locus Sequence Analysis
mRNA - Messenger RNA
NCBI - National Center for Biotechnology Information
NGS - Next generation sequencing
NRPS - Nonribosomal Peptide Synthetases
nuoD - NADH dehydrogenase subunit D

ONT - Oxford NAnopore Technology
OTU - Operational Taxonomic Units
PCA - Phenazine-1-carboxylic Acid
PCN - Phenazine-1-carboxamide
PGAP - Prokaryotic Genome Annotation Pipeline
PGPR - Plant growth promoting rhizobacteria
PKS - Polyketide Synthases
qPCR - Quantitative Polymerase Chain Reaction
QS - Quorum Sensing
recA - Recombinase A
rpoB - DNA-directed RNA polymerase subunit beta
rpoD - RNA polymerase sigma factor
rRNA - Ribosomal Ribonucleic Acid
SNP - Single-Nucleotide Polymorphism
STB - Septoria Tritici Blotch
TYGS - Type Strain Genome Server
UV - Ultraviolet
Vfr - Virulence Factor Regulator
VFDB - The virulence factor database
VOC - Volatil organic compound

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Chapter 1
General introduction

1.1 Importance of cereal crops in agriculture

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Cereal crops stand as pillars of global agriculture, underpinning the food security and nutritional needs of populations worldwide. Among these, wheat, in particular, plays a crucial role due to its extensive cultivation and consumption across various continents. *Triticum aestivum*, commonly known as common wheat, serves as the primary type of wheat cultivated. However, other cereals such as durum wheat, spelt, and barley also contribute significantly to agricultural landscapes. .

Durum wheat (*Triticum durum*) is predominantly used in pasta production due to its high protein and gluten content, which gives pasta its firm texture. This species is mainly grown in countries like Italy and Canada, contributing substantially to their economies. In Europe, Italy is the largest producer of durum wheat, while in Belgium, its cultivation is limited to less than 2% of total cereal production.

Spelt (*Triticum spelta*), an ancient grain, is known for its nutty flavor and is often used in artisanal bread and health food products. Spelt is gaining popularity in Europe, especially in Germany and Switzerland, due to its perceived health benefits. In Belgium, spelt is increasingly found in organic and health-focused food markets.

Barley (*Hordeum vulgare*) is another crucial cereal, mainly used for animal feed, brewing beer, and certain health foods. Globally, major producers include Russia, Germany, and France. In Europe, barley's role is prominent in the brewing industry, with countries like Belgium

leveraging it for their renowned beer production.

These cereals differ from *Triticum aestivum* in terms of their uses, nutritional profiles, and regional significance. While common wheat is versatile and widely used, durum wheat, spelt, and barley each have specialized applications and cultural importance, particularly within European countries.

Globally, wheat is one of the largest cereal crops produced, second only to maize in terms of total production. Its adaptability to diverse climates and environments makes it a valuable crop in both developed and developing countries, highlighting its role in international food security (FAO, 2017). As a staple food, wheat provides essential nutrients, including carbohydrates, proteins, and fibers, serving as a dietary cornerstone for millions (CIMMYT, 2020). The Food and Agriculture Organization (FAO) underscores wheat's importance by regularly monitoring and reporting on its production, trade, and consumption patterns, which significantly influence global food markets and economies.

In the context of Belgian agriculture, wheat holds a prominent position, reflecting its global importance (Statbel, 2023). Belgium, with its fertile lands and favorable climate, is a notable producer of high-quality wheat, catering to both domestic needs and international markets. This underscores not only the crop's adaptability but also the expertise of Belgian farmers in optimizing yields and quality. Wheat cultivation in Belgium contributes significantly to the agricultural sector (European Commission, 2021; The Brussels Times, 2024), and supports rural economies.

The significance of wheat extends beyond its nutritional value, encompassing economic, cultural, and environmental dimensions (FAO, 2022). As the world faces challenges such as climate change and population growth, the role of wheat and other cereal crops in ensuring sustainable agricultural practices and food security becomes ever more critical. The cultivation of wheat, therefore, represents a key intersection of tradition and innovation, highlighting the ongoing need for research, sustainable practices, and policies that support the continued success of this essential crop in both global and Belgian agriculture.

1.2 Challenges in wheat cultivation

Wheat farming, a cornerstone of worldwide agriculture and essential for ensuring food security, faces a variety of obstacles that can markedly affect both its yield and quality. Among these, the prevalence of diseases such as *Zymoseptoria tritici*, responsible for Septoria tritici blotch

(STB), represents a major hurdle for farmers (Fones and Gurr, 2015). This fungal pathogen is particularly problematic due to its ability to adapt and develop resistance to fungicides, making its management increasingly difficult. The disease causes considerable yield losses annually, highlighting the urgent need for effective and sustainable control strategies.

Beyond *Z. tritici*, wheat fields are threatened by other diseases and pests, including rusts like *Puccinia striiformis* (yellow rust) and *Puccinia triticina* (brown rust), as well as Fusarium head blight caused by *Fusarium* spp. (Chai et al., 2022). These challenges necessitate the application of chemical pesticides and fungicides, which, while effective in disease control, pose significant environmental and health risks (Rani et al., 2021). The extensive use of these chemicals has been linked to biodiversity loss, soil degradation, and water contamination. Residues from pesticides can persist in the environment and enter the food chain, posing potential health risks to humans and non-target species alike. Wheat and barley alone account for 45 to 67% of pesticide use in Germany, France, The Netherlands and Denmark (watch, 2023).

The environmental impact of chemical protection strategies extends to the issue of pesticide resistance. The frequent application of fungicides has led to the evolution of resistant strains of pathogens like *Z. tritici* (Estep et al., 2015; Lucas et al., 2015; Hellin et al., 2021). This resistance undermines the efficacy of existing fungicides, necessitating higher doses or the development of new chemicals, further exacerbating environmental and health concerns.

These challenges underscore the need for a paradigm shift towards more sustainable wheat cultivation practices. Integrated Pest Management (IPM) strategies, which combine biological, cultural, physical, and chemical tools in a holistic approach, offer a promising path forward (Karlsson Green et al., 2020). IPM aims to minimize the reliance on chemical pesticides, reducing the environmental impact and the risk of resistance development. Moreover, advancements in genetic research and breeding techniques are paving the way for the development of wheat varieties with inherent resistance to diseases like STB (Brown et al., 2015; Tidd et al., 2023) or rust (Babu et al., 2020; Jamil et al., 2020), offering a long-term solution to one of the most pressing challenges in wheat cultivation, even if pathogens can also adapt themselves to overcome the plant resistance.

1.3 The shift towards sustainable agriculture

The global agricultural sector is increasingly embracing sustainable practices, a shift driven by the recognition of the urgent need to address environmental concerns, climate change, and food security. This movement towards sustainability is highlighted by comprehensive initiatives such as the European Union's Green Deal, which aims to transform the EU into a fair and prosperous society, with a modern, resource-efficient, and competitive economy where there are no net emissions of greenhouse gases by 2050 (European Commission, 2020). A key component of this ambitious plan is the "Farm to Fork" strategy, which specifically targets the agricultural sector, proposing actions to make food systems fair, healthy, and environmentally-friendly (Schebesta et al., 2020).

The EU Green Deal represents a significant commitment to sustainable agriculture, focusing on reducing dependency on chemical pesticides and fertilizers, increasing organic farming, and enhancing biodiversity. The Green Deal also encourages innovation in farming techniques, including the development and adoption of biocontrol methods and disease-resistant crops, which can help reduce the environmental and health risks associated with traditional agricultural practices. The focus on sustainability within the Green Deal underscores the importance of a holistic approach to agriculture, one that balances production needs with environmental preservation.

1.4 Emerging solutions: The promise of biocontrol agents

In the quest for sustainable agriculture, biocontrol agents emerge as a promising solution to the drawbacks of traditional chemical treatments. These natural alternatives, leveraging beneficial organisms to combat pests and diseases, offer a way to protect crops while mitigating the environmental and health issues associated with synthetic pesticides and fungicides. The shift towards biocontrol agents is not just a trend but a necessary adaptation in our approach to farming, aiming for a balance between productivity and ecological stewardship (Hulot and Hiller, 2021).

Biocontrol agents, including bacteria, fungi, insects, and other microorganisms, act through various mechanisms to suppress harmful pests and diseases (O'Brien, 2017; Latz et al., 2018; Köhl et al., 2019a; Bonaterra et al., 2022). For instance, strains of *Pseudomonas* and *Bacillus* bacteria have been identified for their efficacy in combating fungal

pathogens in crops like wheat, showcasing their potential as biocontrol agents (Kildea et al., 2008; Mejri et al., 2018; Bellameche et al., 2020). These microorganisms can outcompete pathogens for nutrients and space, produce substances that inhibit or kill pests, and even stimulate the plant's own defense mechanisms.

The advantages of biocontrol agents extend beyond their effectiveness against pests and diseases. Unlike chemical treatments, biocontrol agents are typically specific to certain pests or diseases, reducing the risk of non-target effects and preserving beneficial organisms in the ecosystem (Winding et al., 2004). This specificity also means a lower likelihood of resistance development, a growing concern with traditional pesticides (Jensen et al., 2016).

However, large-scale use of biocontrol solutions is not without risks. There are concerns about the potential for non-target effects, particularly if non-native biocontrol agents are introduced into new environments (Simberloff, 2014). The ecological balance can be disrupted, leading to unintended consequences for local flora and fauna. Additionally, there is the risk of biocontrol agents evolving into new forms that could become pests themselves, behave as invasive species, or interact unpredictably with native species (Ceyhan SÖNMEZ et al, 2017).

Moreover, biocontrol strategies align with the principles of Integrated Pest Management (IPM), which advocates for a holistic approach to pest control, combining physical, biological, and chemical methods with crop management practices. Implementing biocontrol agents within IPM can enhance agricultural sustainability, ensuring long-term crop protection while minimizing the environmental impact.

The transition toward sustainable agriculture, including the adoption of biocontrol agents, is perceived with mixed feelings by farmers. While many acknowledge the long-term benefits for the environment and public health, there are concerns about the economic viability and reliability of these methods (Messing and Brodeur, 2018). Farmers often face uncertainty regarding the effectiveness of biocontrol agents under varying climatic conditions and the potential need for new equipment or training (Daw, 2009). Furthermore, the regulatory landscape can be complex and burdensome, posing additional challenges to the widespread adoption of biocontrol solutions (Ehlers, 2011).

Despite their numerous advantages, the integration of biocontrol agents into mainstream agriculture faces challenges. These include variability in effectiveness under different environmental conditions (Fedele et al., 2020), the need for precise application timing and methods (Angelopoulou et al., 2014), and regulatory hurdles. However, ongoing research and technological advancements are addressing these issues, im-

proving the reliability and usability of biocontrol solutions.

1.5 Linking microbiomes to agricultural sustainability

As agricultural practices evolve towards sustainability, understanding the intricate web of life thriving within and around plant ecosystems becomes of tremendous importance. At the heart of this web lies the plant microbiome, a complex community of bacteria, fungi, viruses, and other microorganisms that inhabit inside (Lengrand et al., 2024) or outside parts of plants, including roots, leaves, stems, and the rhizosphere (Turner et al., 2013). The plant microbiome, referred to as the phytobiome (Leach et al., 2017), is not just a mere assembly of microbes; it's a dynamic and integral component of the plant's existence, crucial for its health, growth, and productivity (Lyu et al., 2021). It encompasses the entirety of genes and genomes within these microbial communities, along with their interactions with the plant host and the environment (Vandenkoornhuyse et al., 2015). This biological consortium plays a pivotal role in nutrient uptake, disease resistance, stress tolerance, and overall plant development. The symbiotic relationships within the microbiome can significantly enhance a plant's ability to assimilate essential nutrients, combat pathogens, and withstand abiotic stresses, thereby directly influencing agricultural productivity and sustainability (Compant et al., 2019). A dysbiosis, referring to a disequilibrium in the microbiome composition, can have deleterious effects (Chen et al., 2020b).

The composition and functionality of the phytobiome are shaped by an array of biotic and abiotic factors (Haichar et al., 2021). These factors encompass a variety of environmental and biological influences, such as soil pH, salinity, type, structure, moisture, and organic matter content, along with plant exudates (Fierer, 2017), which are particularly pertinent to the microbiomes associated with belowground plant parts. Additionally, external factors like climate, the presence of pathogens, and farming practices exert influence over the microbiota of both above- and belowground plant components (Hardoim et al., 2015).

The specific plant species and its genotype play a pivotal role in the recruitment of microorganisms from the surrounding soil environment, where the morphology of roots, their exudates, and the nature of rhizodeposits significantly contribute to this process (Hartmann et al., 2009; Ladygina and Hedlund, 2010; Chaparro et al., 2014; Reinhold-Hurek et al., 2015). Moreover, other host-related aspects such as plant age, developmental stage, health, and overall fitness have been identified to influence the structure of plant bacterial communities. These

factors impact plant signaling mechanisms, including induced systemic resistance and systemic acquired resistance, as well as alter the composition of root exudates (Aleklett et al., 2015; Reinhold-Hurek et al., 2015). This intricate web of interactions underlines the complexity of the phytobiome, illustrating how both the immediate soil environment and broader ecological factors contribute to the dynamic interplay between plants and their associated microbial communities.

Agronomic strategies profoundly influence the phytobiome, a key component that shapes the health and productivity of crops (Harman et al., 2021). Traditional farming techniques, including the widespread use of fertilizers, chemical pesticides, and monoculture systems, have been identified as major disruptors of the natural microbial equilibrium. Such disruptions can result in diminished microbial diversity, disequilibrium between beneficial and pathogenic microorganisms, and a consequent decline in the microbiome's ability to provide critical ecosystem services (Frison et al., 2011). These services include enhancing soil fertility and offering a natural form of pest control, both of which are indispensable for sustainable crop production (Ebenso et al., 2022).

Sustainable agricultural practices present a constructive alternative, promoting a richer and more balanced microbiome (Yan et al., 2022). Practices such as crop rotation, organic farming, reduced tillage, and the strategic use of biocontrol agents are believed to contribute positively to the development of beneficial microbial communities. These communities play a crucial role in bolstering plant health and increasing resilience against diseases and environmental stresses, thereby supporting sustainable agricultural ecosystems (Ray et al., 2020).

Recognizing the importance of the phytobiome in agriculture leads to the exploration of innovative farming strategies that align with ecological principles. Such strategies encompass the intentional selection of crop varieties that foster beneficial microbial associations (Ryan et al., 2009), the application of microbial inoculants to support plant health (Bonaterra et al., 2022), and the adoption of agricultural practices that promote soil microbial diversity (Chen et al., 2020a; De Corato, 2020; Palojärvi et al., 2020). These measures are foundational to enhancing the functionality and health of the phytobiome, ensuring that crops can thrive in a variety of conditions.

The emphasis on nurturing and leveraging the phytobiome underscores a critical shift toward sustainable agricultural practices (Berg et al., 2014). This approach not only addresses the immediate needs of crop production but also considers the long-term health of agricultural ecosystems. By fostering a deeper understanding of the complex interactions between agricultural practices and the phytobiome, there is a clear pathway toward creating more resilient, productive, and sustain-

able farming systems.

The focus on the plant microbiome, therefore, is not just about improving crop yields but about ensuring the sustainability and vitality of our agricultural landscapes for future generations. In essence, the discussion around the phytobiome and agricultural practices is a fundamental aspect of contemporary agricultural science, highlighting the importance of ecological considerations in farming.

Chapter 2
Plant Microbiota Beyond Farming Practices: A Review

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2.1 Abstract

Plants have always grown and evolved surrounded by numerous microorganisms that inhabit their environment, later termed microbiota. To enhance food production, humankind has relied on various farming

practices such as irrigation, tilling, fertilization, and pest and disease management. Over the past few years, studies have highlighted the impacts of such practices, not only in terms of plant health or yields but also on the microbial communities associated with plants, which have been investigated through microbiome studies. Because some microorganisms exert beneficial traits that improve plant growth and health, understanding how to modulate microbial communities will help in developing smart farming and favor plant growth-promoting (PGP) microorganisms. With tremendous cost cuts in NGS technologies, metagenomic approaches are now affordable and have been widely used to investigate crop-associated microbiomes. Being able to engineer microbial communities in ways that benefit crop health and growth will help decrease the number of chemical inputs required. Against this background, this review explores the impacts of agricultural practices on soil- and plant-associated microbiomes, focusing on plant growth promoting microorganisms from a metagenomic perspective.

Keywords: crops, plants, farming practices, agricultural practices, cropping practices, PGPR, PGPM, microbes, microorganism, NGS, metagenomic, smart farming, microbiome engineering, microbiota

2.2 Introduction

For ten millennia, humankind has continuously reshaped its environment for the purpose of food production. With the green revolution, farmers began to intentionally reshape their microbial niches through the massive use of chemical inputs such as pesticides. The intensification of farming practices dramatically unbalanced crop-associated microbial communities. The emergence in the 1980s of Plant Growth-Promoting Rhizobacteria (PGPR) was designed to take account of microbial communities and their beneficial traits for crops as a whole (Kloepper et al., 1980). Since then, numerous beneficial microorganisms have been identified and broadly characterized.

Most PGPR or biocontrol agents are associated with rhizosphere and root-endosphere microbiota that may be considered derivatives of surrounding bulk soil microbiota. Understanding the fate of such microbiota is fundamental to developing smart farming practices, although a tremendous amount of work is required to determine how to achieve this. Rhizosphere microbial communities are modulated by various abiotic and biotic factors. There are numerous underlying mechanisms explaining the composition, structure, and fate of below-ground microbiota, such as the rhizosphere effect mediated by bipartite interactions (Hartmann et al., 2008; Mendes et al., 2013). Some bacteria can be vertically transmitted from the seed to the next generation and thrive

in the early stages of root development (Hardoim et al., 2012; Liu et al., 2012). Above-ground microbial community assembly is also strongly host- and compartment-dependent, suggesting an important relationship between a plant and its epiphytic microbiota, which can originate from seed, soil, and air (Hardoim et al., 2015; Vorholt, 2012). Despite the tremendous progress that has been made in the study of crop beneficial microbes, there has been no successful development of field-effective bio-based plant protection products. This highlights the need to consider the fate of such microorganisms in the agroecosystems to which they have been introduced and the biological functionalities effectively provided by a crop-associated microbiome.

A microbiome was initially defined as the genome of microbial communities inhabiting specific ecological niches and interacting through distinct and specific functions (Whipps et al., 1988). Recently, Berg et al. (2020a) expanded this definition, enlarging the concept of the microbiome to include all microbiota, including prokaryote and eukaryote microorganisms, their habitats, and their "theatre of activity" mediated by microbial structures, metabolites, and nucleic elements.

Addressing the diversity, composition, and structure of a microbiome will provide deeper insights into the numerous microbial functionalities supporting plant health and growth (Compant et al., 2019; Lemanceau et al., 2017). Such metagenomic studies have only been achievable following the emergence of next-generation sequencing technologies (NGS). NGS also contributes to the development of other omics technologies (transcriptomic and proteomic), enabling the description of biological functions carried out by microbiota. In the coming years, considerable progress in the understanding of microbiomes will be achieved through a combination of NGS technologies and descriptive approaches that equate more closely to biological facts.

One can expect to decrease the need for chemical inputs by modulating microbial communities in a way that benefits crop health and growth. Against this background, the present work reviews, from a metagenomic perspective, the impacts of cropping practices on soil and plant microbiomes with a focus on microorganisms promoting plant growth.

2.3 Impact of tillage practices on soil microbiota

For centuries, tilling has been helping farmers to prepare land for crops. Ploughing assists in incorporating crop residues, preparing the seedbed,

alleviating soil compaction in topsoil layers and decreasing weed, pest, and soil-borne plant pathogen load (Hobbs et al., 2008; Tilman et al., 2002). Although plowing is known to increase soil fertility and yield in the short term, it leads to major soil structure disturbance. The destruction of soil macro-aggregates and networks of pores results in severe soil erosion and ecological-niche homogenization. Conservation or reduced tilling as well as no-till practices emerged in the 1930s to address the detrimental effects of conventional tillage. With massive improvements in NGS technologies, tilling practices are now increasingly investigated from a microbial perspective. Indeed, most studies describe plowing as one of the major drivers of soil microbiome diversity along with pedological context and farming practices such as organic management or cover crops (Alahmad et al., 2019; Babin et al., 2019; Degrune et al., 2019; Hartmann et al., 2015; Wang et al., 2017). Tilling regimes impact soil and plant microbial communities in terms of their diversity, structure, and composition.

From a metagenomic perspective, the impact of tilling on soil microbial diversity is usually investigated through α- or β-diversity indexes describing respective differences within and between communities. Conventional tillage has low impact on fungal α-diversity while increasing prokaryotic α-diversity and has a significant impact on prokaryotic β-diversity favoring opportunistic commensal and copiotroph microbes (Babin et al., 2019; Banerjee et al., 2019; Degrune et al., 2017; Hartman et al., 2018; Piazza et al., 2019; Sommermann et al., 2018; Srour et al., 2020). Tilling facilitates fast organic matter decomposition resulting in a sudden nutrient release that is homogeneously distributed in tilled soil columns, thus increasing the abundance of r-strategists or fast-growing microorganisms (Degrune et al., 2017; Schmidt et al., 2018). A fall in α-diversity under conservation tillage practices is common and is attributable to a reduction in evenness as much as in richness (Degrune et al., 2016; Piazza et al., 2019; Tyler, 2019). Nevertheless, conservation tillage and no-till practices favor slower organic matter degradation and the establishment of less diverse but more oligotrophic, complex, and stable microbial communities (Degrune et al., 2017; Song et al., 2017; Srour et al., 2020; Tyler, 2019; Wang et al., 2017). Babin et al. (2019) consistently observed a higher abundance of predicted genes involved in oligotrophic lifestyles under low tillage conditions. On a long-term basis, decreasing tilling intensity promotes a higher abundance of microbes degrading more complex organic compounds, which enhances soil fertility (Karlen et al., 1994; Souza et al., 2013). Wang et al. (2017) reported concordant results indicating higher soil organic carbon and nitrogen leading to higher nitrogen and carbon plant accumulation under reduced tillage regimes. Low soil disturbance farming systems appear to increase soil nutrient content and stability as well as the number of oligotrophic and structured soil microbiota (Srour et al., 2020; Wang et al., 2020).

The impact of tilling on the structure of microbial communities is often evidenced through β-diversity analyses; however, network analyses are required to investigate its complexity in greater depth. The exploration of such networks requires specific metrics describing their size (nodes and edges), the degree of co-occurrence/exclusion of interacting Operational Taxonomic Units (OTUs), network cohesion (density), centrality, and modularity (Schmidt et al., 2019). Hartman et al. (2018) found that plowing structures soil bacterial communities by increasing network density but also diminishes their size, modularity, and stability. Higher density indicates a larger proportion of interacting prokaryotic OTUs, whether through co-occurrence or co-exclusion relationships. This is consistent with the increases in prokaryotic α-diversity discussed above. A lower modularity index suggests conventional tilling practices break down the structure of soil prokaryotic communities (Newman, 2006). The effects of tilling on networks of fungal communities remain unclear but negatively impact the structure of fungal microbiota. Banerjee et al. (2019) identified a significant negative correlation between plowing intensity and fungal network connectivity. They demonstrated that fungal networks were larger and more densely connected under no-till while tilling disrupted hyphal networks.

In addition to the impacts of soil inversion on the complexity and stability of soil microbial networks, tilling has been shown to noticeably affect the composition of microbial communities (Hartman et al., 2018; Nelkner et al., 2019; Wang et al., 2017). Historically, one of the reasons farmers used to plow land was to decrease soil-borne pathogen load (Tilman et al., 2002). In fact, by burying crop residues, moldboard ploughing helps to control residue-borne or moist-dependent plant pathogens. Reduced tilling or no-tilling keeps crop residues in upper soil horizons which favors plant pathogens belonging to *Fusarium* species (Hartman et al., 2018; Sommermann et al., 2018). However, the absence of soil disturbance promotes other fungi forming hyphal networks such as plant beneficial arbuscular mycorrhizal fungi (AMF) (Degrune et al., 2017; Srour et al., 2020). Consistent with this, Lienhard et al. (2014) suggest Actinobacteria exhibiting a mycelia-like growth habit are more sensitive to soil work.

Thus, taxonomical data generated through metagenomic approaches should be considered cautiously depending on taxonomical level and specific biological and/or ecological functions. While both conventional and conservation tillage increase the abundance of Actinobacteria (Babin et al., 2019; Hartman et al., 2018), Cania et al. (2020) demonstrated that low tilling intensity favors those harboring a higher potential to produce exo- and lipo-polysaccharides. These biomolecules are crucial in soil macro-aggregate formation and stability, and therefore play crucial roles in preventing severe erosion events and ecological-niche homogenization. Favoring heterogeneous soil microbial niches with overlapping phyloge-

netic groups and redundant ecological functions contributes to the creation of more resilient and sustainable agroecosystems (Schmidt et al., 2019; Srour et al., 2020). The sustainability of such agroecosystems also relies on disease suppressive traits favored under no-till conditions as described by Srour et al. (2020). However, among α-Proteobacteria, Sphingomonads associated with disease suppressive traits are favored in tilth soils while the abundance of Rhizobiales, encompassing symbiotic nitrogen-fixing bacteria, increases under conservation tilling (Babin et al., 2019; Degrune et al., 2017; Souza et al., 2013; Wang et al., 2016c). These findings illustrate the need to consider lower taxonomical levels when investigating soil microbiomes. Ultimately, these studies are paving the way for informed soil work decision-making that will help in recruiting specific microbial guilds and building healthier soils.

2.4 Impact of soil cover on soil microbiota

In addition to the adaptation of the tilling regime, growing cover crops, also known as catch crops, is another efficient way to prevent soil erosion and increase long-term soil fertility. Abdalla et al. (2019) recently reviewed the impacts of cover crops on the leaching of soil nutrients and crop productivity. They concluded that seeding catch crops significantly decreases nitrogen leaching and increases soil organic carbon sequestration and grain yields when favoring mixed legume-non-legume cover crops.

Indeed, mixing cover crops seems to favor more abundant and specialized microbiomes (Finney et al., 2017). Overall, although the α-diversity of soil microbial communities is not impacted, specific microbial guilds seem to be recruited when catch crops are implemented in crop rotation (Cloutier et al., 2020; Kim et al., 2020). Cloutier et al. (2020) identified significant changes in soil fungal microbiome with more abundant and distinct arbuscular mycorrhizal fungal communities under cover crop mixtures. Their findings corroborate previous work that shows cover crop mixture and some specific cover crop species (oat and cereal rye) increase AMF abundance in bulk soils (Finney et al., 2017). By contrast, Detheridge et al. (2016) observed lower levels of root endophytic fungi such as AMF under clover cover crops. Because clovers are leguminous crops, the authors argued that this finding might be due to the release of bacterially-fixed nitrogen as they identified a negative correlation between high soil nitrate levels and root-associated fungal populations such as AMF. The importance of considering the identity and diversity of cover crops when attempting to benefit from fungal microbiome management has been pointed out by Cloutier et al. (2020). A recent study highlights a positive effect of cover crop diversity on bacterial microbiota evenness. However, Garland et al. (2021) showed

this effect to be negligible when considering environmental factors. The low impact of cover crop diversity on microbial biodiversity might be attributed to the occurrence of sampling when one crop is implemented in the field. The authors suggest that diversifying crop systems in a space by intercropping might have a significant impact on overall microbial diversity. By contrast, this study evidences the proportion of time spent using cover crops to be a determinant of taxa-specific and soil microbial diversity.

Soil as much as plant bacterial microbiomes are impacted by cover crops when implemented and destroyed (Fernandez et al., 2016; Finney et al., 2017). As expected, cover crop burial brings fresh organic matter into the soil and increases bacterial diversity and abundance. Some oligotrophic microbes among Acidobacteria and Verrucomicrobia phyla are promoted along with fast-growing bacteria involved in rapid organic matter decomposition among Actinobacteria and Firmicutes (Pascault et al., 2013; Ramirez et al., 2012). Cover crops also contribute to an increased functional redundancy and complementarity in soil prokaryotic communities. The increased functional redundancy derives from the larger soil heterogeneity and niche partitioning provided by the implementation and burial of catch crops (Alahmad et al., 2019). Remarkably, Nivelle et al. (2016) observed that conventional tilling by disrupting macroaggregates diminishes the beneficial impact of cover crops and impairs microbial functional diversity. Alahmad et al. (2019) demonstrated that bacterial communities favored under cover crop regimes are specifically involved in the metabolism of numerous carboxylic acids. Accordingly, Nivelle et al. (2016) report faster catabolism of carbohydrate and phenolic compounds within microbial communities associated with cover crops. Both studies support the hypothesis advocated by Alahmad et al. (2019) of a functional complementarity within cover crop-associated bacterial microbiomes.

Beyond the beneficial traits harbored by cover crop recruited soil microbiota, Romdhane et al. (2019) considered how to select an appropriate way to terminate cover crops. According to Cloutier et al. (2020), Alahmad et al. (2019), and Garland et al. (2021), farmers could eventually resort to cover crop diversity and duration to induce shifts in both bacterial and fungal microbiomes in order to reduce fertilization needs while maintaining yields. There is still much work to be done with regard to the low number of studies investigating the impact of cover crops from a metagenomic perspective.

2.5 Fertilization & Amendments

Traditionally employed in agriculture, fertilization and amendments have an important impact on soil and plant microbiomes. By modulating the availability of nitrogen – or other minerals –, carbon, or through modification of the soil structure, these inputs affect the soil and plant life. On the other hand, microbiomes can increase the bioavailability of soilborne nutrients. Most nutrients (N, P, S) are results from organic matter degradation and have to be mineralized by telluric bacteria or fungi in order to be available for plants (Van Der Heijden et al., 2008). In natural conditions, microbial mineralization is the key driver of plant growth (Schimel and Bennett, 2004). Plant exudates are known to shape the microbiome and enhance nutrient microbial conversion and bioavailability for plants (Jacoby et al., 2017). Through this molecular dialogue, plants established a 'microbial nutrient supply chain'. This relation can be unbalanced by fertilization, especially chemical inputs.

2.5.1 Fertilization

It has been established that nitrogen supply can impact disease development. Indeed, high concentrations of nitrogen, usually in crops, are often positively correlated with an increase in the susceptibility of plants to diseases (Moore, 1952).

One notable study led by Fierer et al. (2012) investigated soil communities across nitrogen gradients using genomic as well as physiological tools. In terms of diversity, both sites receiving the highest levels of nitrogen differed from others (intermediate and low levels). The authors evidenced a shift to copiotrophic bacterial communities. This shift was confirmed by metagenomic analyses, with high-rate reproducing copiotrophic bacteria exhibiting an increase in DNA, RNA, and protein metabolism and a decrease in urea decomposition, suggesting a diminishing reliance on organic forms of nitrogen. These observations may have an impact on specific organisms such as plant pathogens. Wei et al. (2015) suggested that the invasion of plant roots by pathogens decreases when there is overlap between the resident communities and invading pathogens due to intensified competition for resources. However, when the results of Wei et al. (2015) and Fierer et al. (2012) are compared, it can be inferred that N-fertilization, by limiting resource competition, enhances phytopathological disorders. Berg and Koskella (2018) confirmed that N also decreases the protective ability of phyllosphere microbiota. After evidencing that a phyllosphere microbiome could prevent leaf colonization by *Pseudomonas syringae*, the authors demonstrated that the use of a fertilizer significantly decreased this pro-

tective effect.

Another study found that N-fertilization drastically reduced phagotrophic protists in different soil types. These protists are microbial predators and could play a role in the modulation of soil microbial communities (Zhao et al., 2020).

Nitrogen fertilization can also have an impact on the phosphorus cycle. Dai et al. (2020b) demonstrated that the long-term use of N-fertilization decreased microbial P-solubilizing and mineralizing capacity by modulating microbial communities while P fertilization favored immobilization by microorganisms by altering the functional profiles of soil microbiota. Another study focusing on phosphorus inputs alone also found that acid phosphatase activity was reduced along with the solubility of mineral P (Pantigoso et al., 2018). Similar to the findings of Dai et al. (2020b) for N-inputs, the authors suggested that P fertilization decreases the P-solubilizing abilities of soil microbiomes.

Few studies have been conducted on K fertilization alone. Although Pan et al. (2014) showed that K fertilization shapes the soil communities but not the functions in grasslands, no other study was found to be relevant in this context. Studies on NPK fertilization in combination revealed the same trends in soil microbiomes (Chen et al., 2020a; Pan et al., 2014). Based on such findings, Zhang et al. (2017) suggested that the affected pH rather than the nutrients was responsible for these shifts in microbial communities.

2.5.2 Organic Amendments

Bonanomi et al. (2018) reported that the suppressive effects of organic amendments have been exhibited in 78 plant pathogens since the 1940s. Although the authors recognized that the results were often inconsistent and difficult to adapt in prediction tools, the comprehension of chemical actions such as glucosinolates in suppressiveness (Larkin and Griffin, 2007) or the link between amendment, suppressiveness, and bacterial communities (He et al., 2012) paves the way to a better management of beneficial microbes through amendments. Nevertheless, numerous studies concerning amendments and microbiomes focus on a single pathology or a single crop (Cesarano et al., 2017; Inderbitzin et al., 2018) or describe microbiomes in different conditions (Bonanomi et al., 2016), without successfully identifying guidelines for microbiome management. A notable point of view on this issue was presented by Bonanomi et al. (2010) in a meta-analysis of 252 papers. The authors primarily explored the characteristics of organic soil amendments linked to a suppressive effect in soilborne diseases. They found that multiple characteristics can apparently be discarded and that six parameters are particularly useful

for predicting suppressiveness prediction. These parameters, both enzymatic and microbial, are the FDA (flurorescein diacetate), enzymatic activity, substrate respiration, microbial biomass, total culturable bacteria, and populations of *Pseudomonads* and *Trichoderma* spp.

Another potentially notable mechanism is the determination of the feeding preferences of microbes. Bonanomi et al. (2018) compiled an analysis of recent studies based on ^{13}C cross-polarized magic angle spinning nuclear magnetic resonance (C-NMR) and evidenced several differences in substrate preferences. Although these data provide valuable insights, the authors insist on the need for an in-depth study in collaboration with laboratories worldwide. Although studies demonstrate the positive effect of a combination of organic amendment and beneficial microorganisms (Latha et al., 2011; Shen et al., 2015), these current solutions cannot be generalized.

Fertilization and organic amendments shape the soil microbiome, principally through nutrient availabilities, but also through pH or modulation of other soil parameters. Although chemical fertilization seems to make the soil microbiome "dependent" on these nutrients, favoring copiotrophic bacteria, and decreases the solubilizing and mineralizing abilities of bacteria of N- and P- cycles, organic amendments offer more possibilities. In this respect, Ling et al. (2016) found that long-term organic amendments support stronger functional potential and more interactions within soil communities than chemical fertilization, most likely due to better soil stability and a good buffering capacity.

2.6 Plant genotype and microbiome

Several factors can influence the composition of plant microbiomes, including genotypes, plant developmental stage, and plant health (Berg et al., 2015). Characteristic root exudates are usually considered the causative factor underlying the recruitment of specific microbial communities and are influenced by plant genotype. The recruitment of the plant-associated microbiome can vary in terms of structure and functionality and depends to a great extent on the physical properties of soil and nutrient availability (Berg and Smalla, 2009).

Numerous studies have linked microbial diversity with a reduction in the incidence of disease (Keesing et al., 2010; Kopecky et al., 2019). A low potato common scab is observed, even in favorable conditions, when high bacterial diversity is present in the soil (Latz et al., 2012; van Elsas et al., 2012). Higher soil microbiome diversity offers better odds of finding a higher abundance of rare species able to bring specific protective functions against pathogens (Latz et al., 2012). As reported by Mendes

et al. (2018), the exclusive and abundant presence of a bacterial taxon is a poorer indicator of disease suppression than the relative abundance of bacterial taxa.

The breeding of cultivars resistant to pathogens is a well-known practice in the control of diseases. In some cases, the genetic background of the plant may not be the only driver of such resistance. Wei et al. (2019) demonstrated that the apparent resistance of cotton cultivar to Verticillium wilt is partially due to the plant microbiome. An abundance of beneficial microbes in the cotton rhizosphere offers complementary protection against this soil-borne pathogen. Similar findings were reported for cucumber resistance to Fusarium wilt (Yao and Wu, 2010) and tomato resistance to *Ralstonia solanacearum* (Kwak et al., 2018).

The relative abundance of well-known beneficial rhizospheric and root endospheric microbial groups can vary significantly between resistant and susceptible cultivars (Wei et al., 2019).

However, plant genotype is not the major driver of the early establishment of a rhizospheric microbiome. Several studies indicate that soil type rather than cultivar determines the composition of the rhizospheric microbiome (Chen et al., 2019; Van Overbeek and Van Elsas, 2008; Xu et al., 2009). This feature has been specifically evidenced for fungi (Nallanchakravarthula et al., 2014), bacteria (Schlemper et al., 2017), and arbuscular mycorrhizal fungi (Santos-González et al., 2011).

Plant genotype becomes an obvious determinant of root-associated microbiomes as plants mature (Inceoğlu et al., 2010; Schlemper et al., 2017). For a particular growth stage, different cultivars can have different dynamics in their exudates release dynamics (Micallef et al., 2009; Mönchgesang et al., 2016; Sasse et al., 2018), thereby affecting rhizosphere microbial communities in a particular way.

The effect of the host genotype on microbial populations is much more important in the endosphere (Urbina et al., 2018). This is not surprising as co-evolution processes have selected populations well adapted to this niche. In the very early stages of a plant's life, i.e., around germination, the microbial community of the spermosphere is composed of microbes originating from inside and outside the seed as well as microbes recruited from soil during imbibition (Lemanceau et al., 2017). At this point, the main factor affecting the colonizer communities of the spermosphere is the seed genotype (Adam et al., 2018; Sahadevan et al., 2019). Microbial communities associated with germinating seeds can have a direct impact on the promotion of plant growth, nitrogen fixation, and disease control (Rahman et al., 2018; Sabu et al., 2019; Walitang et al., 2017). With the transformation of rootlets in the spermosphere into roots creating the rhizosphere, the plant genotype becomes of minor importance in community shaping compared to soil type.

A recent study on tomato root endospheric fungi evidenced a clear difference in the phytohormone profiles between two cultivars harboring different endophytic communities (Manzotti et al., 2020). More work is needed to determine whether the hormonal profile determines the composition of endophytic communities' (as with root exudates) or whether it is a consequence of mycobiome composition. For bacteria, it has been shown that most endophytic bacteria can interfere with plant hormonal systems (Jasim et al., 2015, 2016).

Plant genotype can also play a critical role under mixed-cropping conditions. It has been shown that two varieties of pea can influence each other in terms of root-associated bacterial and fungal populations (Horner et al., 2019). In this particular study, the root bacterial community of one cultivar remains stable (similar to single-crop community) in response to mixed-cropping whereas the bacterial community of the second cultivar shifts toward the first. Conversely, the root fungal community of the second cultivar remains stable when mixed-cropped whereas the fungal community of the first cultivar shifts toward the second.

Polyculture has been identified as a way to enhance rhizospheric fungal diversity (LeBlanc et al., 2015), which can be modulated by the identity of plant species (LeBlanc et al., 2017) and by environmental parameters that can also have a significant influence (Schlatter et al., 2015).

Differential shifts in bacterial and fungal communities can be attributed to microbial interactions, a change in soil attributes or a change in root exudates resulting from competition between intervarieties, plant communication, or a better mineralization of organic matter enhancing nutrient availability (Hinsinger et al., 2011; Reiss and Drinkwater, 2018).

2.7 Biostimulants and microbiomes

Biostimulants can be defined as substances or microorganisms that stimulate natural processes to enhance tolerance to abiotic stress, crop quality, and nutrient uptake and efficiency (du Jardin, 2015). The effect of biostimulants can be attributable to the direct effects of carbon or nitrogen metabolism (Calvo et al., 2014) but also indirectly through the modulation of plant microbiomes through the enhancement of microbial activity (Colla et al., 2015).

Biostimulants are relatively new products and their effects on microbiomes are not well known, partly due to the great diversity of biostimulant origins. In this paper, we focus on biostimulants obtained from

protein hydrolysates from seaweed, and microbial biostimulants.

2.7.1 Biostimulants based on protein hydrolysates

Tejada et al. (2011) tested four biostimulants on degraded soils. They identified an alteration in microbial community structure and higher microbial activity, which facilitates better plant development on degraded soils. The biostimulant with the best effect in terms of microbial activity and plant development was the one derived from rice bran extract. The authors hypothesized that the effect of this biostimulant was due to its richness in little peptides (¡ 3kDa), easily assimilable by microorganisms. Other studies focused on the phyllosphere microbiome. Luziatelli et al. (2016) found that a biostimulant from protein hydrolysates modulated the leaf microbiome on lettuce. Notably, microbes isolated from lettuce leaves treated with biostimulant indicated the presence of bacteria enabling phosphorous solubilization or producing phytohormones (IAA). Moreover, the biostimulant favored the presence of *Bacillus* species exhibiting an inhibitory activity against leaf lettuce pathogens *Erwinia amylovora* and *Fusarium oxysporum*. The biostimulant could thus shape the phyllosphere, promoting plant growth.

Regarding protein hydrolysate (PH) biostimulants, Colla et al. (2017) concluded that this kind of biostimulant can help provide better resilience to biotic and abiotic stress by modulating the microbiome. Given that PH biostimulants can only select beneficial microorganisms present in the rhizo- or phyllosphere, the authors proposed their use in synergy with beneficial microbes.

2.7.2 Biostimulants derived from seaweed

Most investigations of seaweed-derived biostimulants have been conducted under the scope of the functional diversity of the microbiome, through enzyme activity research rather than an analysis of the microbial community diversity. Ji et al. (2017) reported that the P solubility was higher after a seaweed biostimulant application but without affecting the microbial communities. The positive action of seaweed biostimulants indicated greater activity of hydrogenase (Onet et al., 2019), invertase, urease, proteinase, and phosphatase (Wang et al., 2016b). These enzymes, involved in the carbon, nitrogen, and phosphor cycles, explain the better nutrition status of the plants.

2.7.3 Microbial biostimulants

Despite the fact that microbial biostimulants are relatively common in agriculture, usually through PGPR products or vermicomposts, their effect on microbiomes is poorly documented. Berg et al. (2020b) studied the effect of microbial biostimulants on soil and root microbial communities. Although no significant differences were found in the diversity of these communities, except for the fungi, the tested biostimulants did not increase the yield.

Mahnert et al. (2018) studied the impact of vermicompost on leaf and environment microbiomes under controlled conditions. It appeared that the biostimulant reshaped the microbiomes of the leaves, with an increase in Bacteroidetes and other phyla such as Verrucomicrobia, Acidobacteria, and Thaumarchaeota. Other groups containing beneficial microorganisms also increased. The authors demonstrated that the effect of the biostimulant on microbiome composition could be predicted with an accuracy of 87%. To the best of our knowledge, no other significant research has been conducted on this theme. The two research papers yield different results, maybe lightening the impact of field conditions on plant biostimulation, and that a stronger impact of plant biostimulant products are observed under controlled conditions or synthetic substrates.

2.8 Impact of irrigation/water on microbiome

Irrigation can have an impact on microbial communities and plant microbiomes through the frequency of irrigation or the quality of irrigation water. Due to climatic changes, the irrigated area is predicted to increase to 62% from 2020 to 2070, with an impact on soil and therefore microbial communities (Döll, 2002). Without irrigation, soils will alternate between drying and rewetting periods. In these conditions, more active microbes are known to be affected more by these drying-rewetting stresses (Van Gestel et al., 1993). For an active microbial rhizosphere, drying and rewetting periods could be damaging. Fierer et al. (2003) focused on the impact of this alternation on soil bacterial community structure. They found that microbial communities in annual grasslands were less affected in terms of biodiversity by these events than forest (oak) soils. Nonetheless, Wu and Brookes (2005) reported a 44% decrease in microbial biomass in a single dry-rewet cycle in grassland. It is unclear whether these impacts on microbial structure are direct effects of the dry-rewet cycle or indirect effects through the perturbation of physical or biochemical soil processes, such as the C cycle (Schimel, 2018).

Long-term monoculture irrigation has been relatively poorly studied. Nevertheless, Mavrodi et al. (2018) studied the effect of long-term irrigation on wheat. They reported that beneficial phenazine-producing (Phz^+) *Pseudomonas* spp. were less abundant or detectable in irrigated fields or in higher rainfall areas. Irrigation should alter rhizodeposition and soil properties, disturbing microbiomes. Mavrodi et al. (2018) found that irrigation had a slight effect on the diversity of the wheat rhizosphere microbiome. However, some taxa displayed strong positive and negative responses to irrigation such as Bacteroidetes and Proteobacteria. Some genera, previously identified as phytopathogen antagonists such as *Chryseobacter* spp., *Pedobacter* spp. or *Brevundimonas* spp., were among the bacteria with the highest relative increase in abundance under irrigation.

The quality of irrigation water also seems to have an important impact on plant microbiomes. Cui et al. (2019), from the perspective of water management, tested different water qualities. They found that reclaimed water and piggery wastewater use increased the abundance of Bacteroidetes while decreasing Acidobacteria abundance. Although PGPR were logically more abundant in the rhizosphere microbiome, their response to the different water qualities (distilled, reclaimed, piggery wastewater) was quite variable. Finally, no increase in (phyto)pathogenic bacteria was evidenced after irrigation with reclaimed water or piggery wastewater.

Gu et al. (2019) also evidenced a modulation of the spinach microbiome according to the quality of irrigation water. Although they did not find any increase in foodborne pathogens, they evidenced an increase in potential opportunistic (phyto)pathogens. These two publications highlight the importance of the quality of irrigation water survey for plant and consumer health.

Some authors have studied the resilience of soil microbial communities after irrigation with water of different quality. It appears that a soil microbiome – and to the same extent – plant microbiome is not resistant to irrigation with treated wastewater. Differences have been observed between irrigation with freshwater and treated wastewater. Nevertheless, during the rainy season, the baseline state of microbiomes is recovered, evidencing the resilience of soil and plant microbiomes in the long-term (Frenk et al., 2014). Frenk et al. (2018) showed that under conditions of high mineral and organic carbon activities, bacterial communities can change drastically, exhibiting proteobacterial dominance. These changing communities displayed less resistance to environmental stress such as heat disturbance as they have less diversity than soils with low resource availability. However, the authors evidenced a functional resilience after the end of the stress, probably due to the high growth rates of certain groups such as Bacteroidetes or Proteobacteria.

In conclusion, if irrigation and quality of irrigation have a relative impact on diversity, the impact on the biomass of different groups can be important. If populations are resilient in the long-term, thanks to microbial seed banks (bacteria in dormancy) (Lennon and Jones, 2011), the impact of irrigation and quality of irrigation has to be considered in the short term, during one agricultural season. In this respect, observations have been nuanced. Some studies evidenced the positive role of irrigation on PGPR (Mavrodi et al., 2018) while others obtained variable (Cui et al., 2019) or negative results (Phz^+ not present in irrigated soils, Mavrodi et al. (2018)). Ultimately, even if wastewater did not seem so harmful when applied in the short term, repeated applications of this kind of wastewater have to be studied for a longer period. According to the observations of Frenk et al. (2018), the use of high availability resources irrigation water in the longer term could probably and durably reshape agricultural soil microbial communities. Conversely, plant microbiome management will probably be a future tool employed to better exploit limited water resources (de Vries and Schulte-Uebbing, 2019).

2.9 Crop protection

The application of pesticides in fields influences microbial populations inside aerial and below-ground plant parts, as well as in the soil. The effects can be due to the applied molecule itself, but also the degradation products of the molecule. Degradation can occur through multiple processes; degradation by microorganisms, hydrolysis, photolysis, sorption and binding to organic and soil components, plant uptake, and volatilization (Srivastava et al., 2020). If microorganisms are able to survive in the environment contaminated by the molecule, they can then metabolize and degrade the pesticides (Wołejko et al., 2020). Therefore, microorganisms can play a significant role in plant tolerance to herbicides (Tétard-Jones and Edwards, 2016).

Depending on the chemical, the active ingredient can be a racemic mixture or enantiomer-enriched solution. Sometimes only one of the two enantiomers has a desired effect, with the other having an indirect effect on non-target organisms (Asad et al., 2017). When a pesticide is applied, it usually leads to the eradication of groups of microorganisms sensitive to the active ingredient. Niches are consequently freed and colonized by microbes previously of minor abundance in the community (Chen and Edwards, 2001) or thriving as a result of the release from competition (Nettles et al., 2016; Roesti et al., 2005). It is also possible that treatment has no significant effect on some parts of the community, such as an effect on the fungal rhizosphere community but not on bacterial communities (Nettles et al., 2016). If a microbial population is well adapted to the pesticide, the treatment can induce a short-term

increase in microbial carbon, indicating increasing biodegradation (Astaykina et al., 2020).

In the presence of pollution, microorganisms can enhance their adaptation to the prevailing conditions by altering their metabolism (Rangasamy et al., 2018; Sun et al., 2004). Therefore, groups of microorganisms can take advantage of an active ingredient in the environment (Webber et al., 2015). For example, Newman et al. (2016) showed a shift in a bacterial community towards a tolerant community after long-term glyphosate adaptation. With the destruction of certain groups of microbes involved in the degradation of another molecule, the stability of this second product can increase (White et al., 2010). A long-lasting product in the environment can be important in ensuing long-term protection for the crop. This can also increase the likelihood of unintended effects on non-target microbiomes (Nettles et al., 2016). Plants are also capable of exuding pesticides absorbed on their aerial parts with their roots, in addition to endogenous exudates (Dinelli et al., 2007). All these mechanisms can influence the microbiota. Regarding the effects of a pesticide application, as reviewed by Wołejko et al. (2020), the effects of fungicide, insecticide, or herbicide on microbial communities vary greatly according to the molecule used and the microbial group studied. Although most studies agree on the lack of impacts on alpha diversity in the rhizosphere (Lupwayi et al., 2009, 2004; Nettles et al., 2016), effects on microbiome functionality (Fournier et al., 2020) or structure (Nettles et al., 2016) have been reported with shifts in relative abundance and community composition. In the phyllosphere, microbial diversity can even increase after a foliar treatment (Katsoula et al., 2020). Seed treatments can have a more pronounced and dynamic impact on microbial diversity. As shown by Li et al. (2018), the richness of bacteria and fungi species at seedling stage decreased with a neonicotinoid seed coating. With a decreasing concentration of neonicotinoids when reviving, the growth of microorganisms was stimulated. Overall, general microbiomes recovered at the end of the cultural season.

2.10 Discussion

From this work, a major trend appears to dictate the process followed when investigating microbial communities from a metagenomic perspective. DNA extractions are almost exclusively performed using a FastDNA Soil SPIN Kit (MP Biomedicals, USA) or PowerSoil DNA Kit (MoBio, Qiagen, USA). Consideration of the extraction procedures implemented remains relevant and may induce bias when comparing one study to another (Kennedy et al., 2014).

When studying prokaryotes, most authors target the 16S RNA gene,

focusing on the V4 region or a wider region including V4. Overlapping between V2-V3 regions is also commonly used. The diversity of eukaryotic communities is often investigated via the sequencing of an internal transcriber region (ITS) or 28S ribosomal unit. Ideally, several genetic markers should be considered (Sommermann et al., 2018).

Currently, sequencing usually relies on Illumina technology when 454-pyrosequencing tends to become more anecdotal. When Illumina technology is used, a large majority of researchers make use of the Miseq platform and, more recently, the Hiseq platform. Third-generation technologies such as PacBio or Ion Torrent continue to be marginal when investigating soil and plant microbiomes but are expected to become a gold standard in metagenomic approaches (Lee et al., 2016; van Dijk et al., 2018).

Most of the studies presented in this paper used barcoded rRNA sequences. Although this approach enables researchers to assess the impact on microbial composition and diversity, it is notable that when shotgun or enzymatic analyses are applied, authors gain better insight into the networks, relationships inside the communities, and the functional aspects of the microbiome.

The interpretation of meta-data produced by next-generation sequencing technologies depends mostly on the data management methodologies implemented. Several best practices are required, such as choosing an adapted normalization strategy (Knight et al., 2018; Schlatter et al., 2017). The normalization process should be selected to fit the size and organization of the datasets, as suggested by Weiss et al. (2017). Sufficient technical replicates should be performed in order to quantify sequencing error rates within an assay and between assays (Nguyen et al., 2015; Schloss et al., 2016).

In almost every paper reviewed, the composition and diversity of microbial communities are affected by the agronomic parameters investigated. Networks and functionalities are nearly always impacted, although these attributes have been poorly studied. Of 54 papers, only 14 studies describe networks, 14 consider biological functionalities, and only three address both aspects simultaneously.

A deeper understanding of crop-associated microbiomes and their functionalities requires a more holistic approach that combines data not only from omics technologies. Investigating the fate of microbial communities requires a four-dimensional perspective that examines microbiomes in terms of their diversity, structure, composition, and biological functions. Functionalizing microbial communities not only through prediction tools but also through quantification technologies such as qPCR or enzymatic assays will provide relevant insights that illustrate how those microbial communities and the services they provide are affected

by farming practices.

Before studying the impacts of any agricultural parameter on microbiota, environmental features should be systematically considered with greater concern when interpreting the results of such studies. Organic Carbon content, Nitrogen content, pH, soil structure, soil classification, and moisture content are several parameters that vary from field to field and significantly impact microbiomes. Moreover, some authors highlight the indirect activity of certain practices through pH change (e.g., Zhang et al. 2017). For instance, in 2006, Fierer and Jackson (2006) found that soil pH is the main parameter influencing microbiome structure. This has since been confirmed by numerous studies (Lauber et al., 2009; Rousk et al., 2010; Geyer et al., 2014; Qi et al., 2018; O'Brien et al., 2019; Schlatter et al., 2020; Tan et al., 2020).

Soil pH determines the chemical forms of elements in the soil, therefore affecting their bioavailability for plants. This would also be an indirect limiting factor for microbial life, as suggested by Zhalnina et al. (2015). In addition to nutrient availability, pH also exerts effects on catabolic activities, soil structure, and biomass activities (Wakelin et al., 2008). Low pH can have direct toxic effects on microbe cells. Some organisms, better adapted to acidophilic conditions, possess a special membrane structure, proton pumps, or special transporters (Lehtovirta-Morley et al., 2016). The relationship between soil pH and microbial diversity can also be explained by the wide range of optimal pH tolerance for a community in contrast with the rather narrow pH optima for individual species (Fernández-Calviño and Bååth, 2010). Therefore, a shift in pH will affect the survival of some but not all microbials (Santoyo et al., 2017). Numerous studies also link pH with microbial activities, such as phosphate solubilization or ammonia oxidation (Hu et al., 2013; Nautiyal et al., 2000; Sharma et al., 2013). Indeed, pH is thought to drive the community composition of ammonia-oxidizing organisms by modifying the ammonia to ammonium ratio (Gubry-Rangin et al., 2011; Stempfhuber et al., 2015).

A recent study demonstrated the influence of pH and depth on microbiomes in an agricultural soil configuration (Schlatter et al., 2020). The authors showed that pH decreases from the surface to a 10 cm depth as does bacterial richness and diversity. Notably, they observed that bacterial richness and diversity did not recover with increasing pH at depths below 10 cm. These results suggest that pH is the main factor affecting diversity at near-surface depths, while other factors (dispersal, nutrient availability) become prevalent at greater soil depths. This lower microbial diversity at around 10 cm deep could reduce the functional redundancy and resilience of the communities in the seed zone (Shade et al., 2012).

Land use history is also an important parameter to consider when investigating soil-associated microbiota. Although the underlying mechanisms are not well understood, it is known that plants can recruit specific root microbiomes through root exudates, enabling them to select beneficial microbial traits. Time after time, the soil microbiome is enriched in certain specific taxa, as suggested by the concept of a soil-borne legacy (Bakker et al., 2018). Recruited microbiomes produce bioactive metabolites or useful resources for plants. Once the crops are harvested, molecular signals and other plant-beneficial compounds might remain in the soil and benefit the next generation. This phenomenon was proposed by Lapsansky et al. (2016) and is called the soil memory effect.

Considering the duration of assays is therefore of tremendous importance as is the implementation of cover crops, intercropping, and crop rotation composition, all of which are among the numerous parameters to examine when studying crop-associated microbiomes. The long-term studies reviewed in this work reported a certain resilience or results different from those in the short term (Mavrodi et al., 2018), probably due to microbial seed banks (Lennon and Jones, 2011).

In this review, we investigated the impacts of farming practices on soil and plant microbiomes. Within the heterogeneity of the reviewed studies, as previously noted, supplementary approaches to omics facilitated a strong comprehension of underlying mechanisms. Levy et al. (2018) reviewed the different advantages and limitations of -omics techniques. They conclude that no -omics approaches provide the necessary causality and argue that, more than amplicon-based studies, a functional metagenomic approach is needed and can be supplemented by synthetic communities or reverse genomics approaches. Vorholt et al. (2017) also advise using synthetic communities for a better understanding of microbiomes. They argue that the factors shaping microbial communities in soil matrices are not well understood because of the complexity of environmental samples. For a better understanding of plant-microbiome interactions, they recommend the use of multispecies synthetic communities.

If the impact of agricultural practices on microbiomes exists, we wonder whether the microbiome could be farmed as "collaborative crops". "Seeding" practices through microbial or consortia inoculation, or a selection of varieties promoting positive microbiomes, are likely to be the future of microbiome management (Compant et al., 2019). These inoculations of strains or microbiome engineering in plants can be obtained in different ways. They can occur through host-mediated and multi-generation microbiome selection, inoculation into bulk soil, rhizosphere, seeds or seedlings, atomization into tissues such as stems, leaves, and flowers, and direct injection into tissues or wounds. Some recent relevant studies involving the aforementioned techniques are re-

viewed below. Given the lability of existing microbiomes, and the fact that soil memory is more active in a low nutrient environment and thus less adapted to conventional agricultural soils (Lapsansky et al., 2016), microbiome engineering consisting of community inoculation and host-mediated "microbiome maintenance" in single (Orozco-Mosqueda et al., 2018) or associated crops (Horner et al., 2019) could be a useful tool for future pest integrated management.

2.11 Author Contributions

MD, SC, and ND contributed equally to the work. CB reviewed the paper.

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Conflict of Interest Statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Chapter 3
Phyllospheric Epiphyte Wheat Microbiomes

Berg et al. (2020a) recently revisited the original definition of "microbiome" as proposed by (Whipps et al., 1988), emphasizing its relevance, decades after its initial introduction in 1988. The microbiome is described as a specific microbial community within a well-defined habitat, encompassing not only the microorganisms but also their interactions and the resulting ecological niches (Debode et al., 2024). This concept highlights the microbiome's dynamic nature, integrated within larger ecosystems and crucial for the health and functioning of eukaryotic hosts. The term "microbiota" is distinguished as the collection of microorganisms across various kingdoms, along with their activities and genetic elements within their environmental context (Fig. 3-1).

3.1 Understanding epiphytic wheat microbiomes

The phytobiome, an intricate ecosystem of bacteria, archaea, fungi, oomycetes, viruses, and others, is fundamental to plant health, playing a pivotal role in nutrient cycling, growth enhancement, and disease resistance as reviewed by Zhang et al. (2021b). This complex microbial community, essential for ecosystem functionality, varies across different plant compartments (Turner et al., 2013), namely the rhizosphere, phyllosphere, and endosphere (Bulgarelli et al., 2012; Lundberg et al., 2012; Lengrand et al., 2024). The phyllosphere encompasses all above-ground parts of plants, such as leaves, stems, flowers, and fruits, while the rhizosphere corresponds to the soil zone close to the plant roots where roots and microorganisms can interact through exudates and metabo-

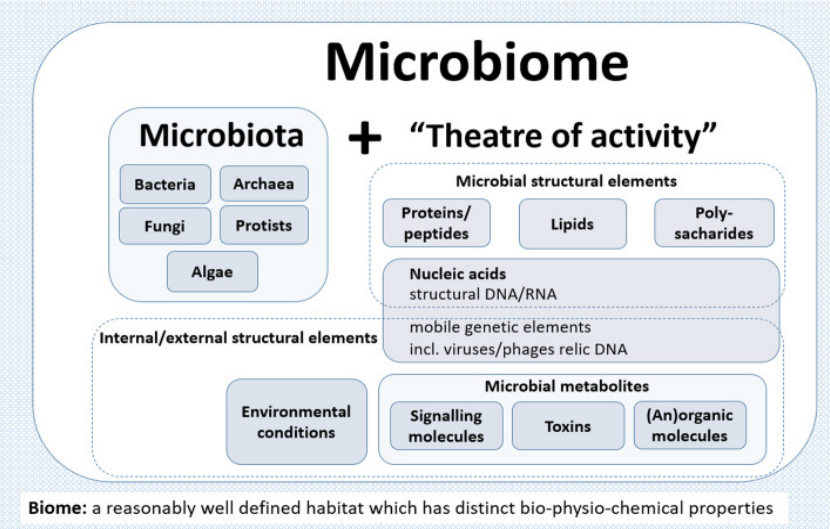

Figure 3-1: Components of the microbiome.
Microbiome encompasses the microbiota (the community of microorganisms) alongside their functional environment, including structural elements, metabolites/signal molecules, and the ambient environmental conditions. From Berg et al. (2020a).

lites (Hartmann et al., 2008). The endosphere describes the interior of plant tissues where microbes live. Each compartment's unique microbial assembly interacts with the plant and its environment in multifaceted ways, crucially influencing plant health and productivity. Altogether, they are referred to as the plant holobiont, comprising the plant and its associated microbiota, which interact with each other and determine holobiont functioning and plant performance (Mitter et al., 2016). Considering the specific case of cereals, other plant compartments (Fig. 3-2) have been delineated to study their specific microbiome (Quiza et al., 2023).

The formation of these microbiomes is a dynamic, multifactorial process shaped by plant genetics, environmental conditions, soil characteristics, and agricultural interventions. Plants secrete compounds attracting specific microbes, forming distinctive communities. The provision of stable metabolites by plant hosts reduces the microbes' need to synthesize various compounds, indicating a mutualistic relationship where microbial functions are optimized for plant benefit (Cole et al., 2017; Liu et al., 2018). As an example, a recent study reveals that depletion in bacterial genes for amino acid metabolism offers a competitive advantage for *Pseudomonas simiae* WCS417 under conditions of abundant plant-exuded nutrients (Yu et al., 2021).

The rich array of sources contributing to microbiome formation includes seeds, which pass endophytic microbes to offspring, soil, the

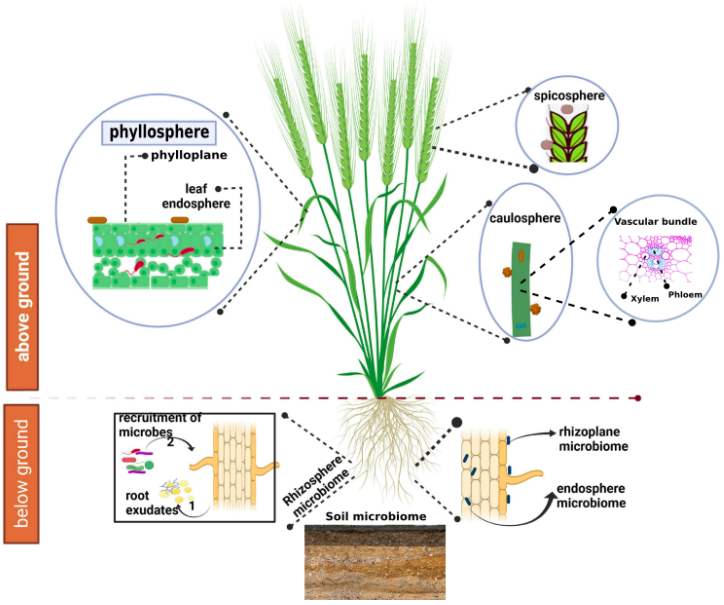

Figure 3-2: Microbiomes associated with cereal plant compartments. *These different microbiomes form the plant holobiont. Microbes from the soil can be recruited through root exudates and colonize the rhizoplane, the root surface. Some may enter the root and colonize root endosphere, along with seed-inherited microbes. They can also use vascular bundles to reach the above ground parts of the plant by transiting trough the caulosphere, the compartment comprising the inside and outside of the stem. They can reach the phyllosphere encompassing the phylloplane, the leaf surface, and the leaf endosphere. In the phyllosphere, they meet other microorganisms that can be sourced from air, water, dust, insects and other plants. Finally, when cereal heads are developing microbes can also colonize them by the same processes than for the phyllosphere. Adapted from Chen et al. (2022).*

primary reservoir of microbial diversity, air, water, insects, and other plants, all facilitating microbial transfer and community complexity. Despite all these factors, the concept of plant core microbiota is increasingly used. It consists of members of the microbial community that are persistent and ubiquitous in almost all the communities associated with a particular host (Toju et al., 2018). The core microbiota contains key microbial taxa that carry genes with functions that are essential for host fitness. Many members of the core microbiota of different plant species, such as *Arabidopsis thaliana*, barley, citrus, grapevine, rice, soybean, sugarcane, and wheat, are common at least at the genus level (for example, *Agrobacterium*, *Cladosporium*, *Coniothyrium*, *Erwinia*, *Fusarium*, *Methylobacterium*, *Pseudomonas*, *Sphingomonas*, and *Resinicium*) (Hamonts et al., 2018; Xu et al., 2018; Simonin et al., 2020; Nadarajah and Abdul Rahman, 2023). This suggests that co-occurring members of the core microbiota are selectively recruited and enriched in parallel and are well-adapted to life on and/or inside plant tissues. Microbial families

belonging to Rhizobiales and Pseudomonadales were shown to be part of the core microbiota of several plants, with 5 to 17% of mean relative abundance (Garrido-Oter et al., 2018), but also represent a universal core plant microbiome (Trivedi et al., 2020).

Microbiomes are of tremendous importance for plant health, as shown by a study where a naturally resistant *A. thaliana* grown in sterile conditions became susceptible to *Botrytis cinerea* infection, while the same sterile plants to whom the natural microbiome was brought back remained resistant (Ritpitakphong et al., 2016). This indicates that in this case, the resistant phenotype was due to the natural phyllospheric microbiome. The foliar microbiome also aids in stress tolerance, enabling wheat to better withstand environmental challenges like drought (Azarbad et al., 2022), extreme temperatures (Bernard et al., 2022; Tian et al., 2022) or flooding (Francioli et al., 2022). Several other studies showed that microbiomes can bring a natural protection against diseases: stripe rust (Dai et al., 2020a) and Fusarium head blight (Chen et al., 2018) of wheat or powdery mildew and ramularia of barley (Gravouil et al., 2016) but also against insects (Li et al., 2023). Identifying and characterizing the microbial actors is the first step in understanding how the microbiomes can protect plants from pathogens (Peñuelas and Terradas, 2014). For instance, the wheat foliar microbiome includes keystone taxa, such as Proteobacteria, Actinobacteria, and Firmicutes, which are vital for plant growth (Sivakumar et al., 2020), disease suppression (Kumar et al., 2021), and nutrient cycling (Wang et al., 2023). However, despite advances in sequencing and analysis, as network analyses (Matchado et al., 2021), delineating the wheat phyllosphere core microbiome remains challenging (Kavamura et al., 2021), due to the myriad of influencing factors, including environmental (biotic (Ellis, 2017) and abiotic factors (Sapkota et al., 2017)), plant-specific (niches, genotype, developmental stage (Chen et al., 2022)), and anthropogenic elements like agricultural practices.

3.2 Foliar treatments in wheat culture

In Belgium, wheat plays a pivotal role in agriculture. Maintaining its health and productivity is of paramount importance. To this end, a variety of foliar phytosanitary treatments are employed to protect wheat crops from pests and diseases, ensuring the stability of yield and quality. These treatments can be broadly categorized into chemical pesticides and biocontrol agents, each with its specific intended effects and applications.

Chemical-based pesticides are widely used in Belgian wheat culture for their efficacy in controlling a wide range of foliar diseases and pests.

Commonly applied chemical treatments include fungicides to combat fungal diseases like Septoria tritici blotch (STB), rusts, and Fusarium head blight (FHB). These fungicides are critical in managing pathogens that can severely impact yield and grain quality. Insecticides are also deployed to address pest issues such as aphids and cereal leaf beetles, which not only damage the crop directly but can also act as vectors for disease transmission. Herbicides play a role in weed management, ensuring that wheat crops are not out-competed for resources. The application of these chemicals is timed and targeted based on disease and pest forecasts, with the aim of maximizing their effectiveness while minimizing the need for repeated applications. The application of such compounds, typically fungicides, will have a direct effect on fungi populations on the leaves but could also have unintended effects on beneficial plant-associated fungi in other compartments, such as in the soil. Table 3-1 illustrates the estimated amounts of chemical fungicide used in Belgium in 2021, extrapolated from sales data obtained from Corder asbl (2023).

Table 3-1: Estimated quantities of chemical fungicides used in Belgium in 2021 to protect wheat

Class/group	Molecule	Mode of action	Estimated quantity [kg]	Estimated quantity [%]
Triazoles	prothioconazole	DMI (demethylation inhibitors), affects the membrane ergosterol biosynthesis	16.607	19,9
	tebuconazole		7.915	9,5
	mefentrifluconazole		6.721	8,1
	bromuconazole		2.012	2,4
	epoxiconazole		768	0,9
	metconazole		706	0,8
	tetraconazole		301	0,4
	difenoconazole		131	0,2
	cyproconazole		128	0,2
	propiconazole		48	0,1
Mineral	sulfur	disrupts the metabolic processes in fungi	13.909	16,7
SDHI	fluxapyroxad	affects energy production by inhibiting succinate dehydrogenase enzyme in the fungal respiratory chain	2.710	3,3
	bixafen		2.570	3,1
	benzovindiflupyr		2.358	2,8
	isopyrazam		600	0,7
	boscalid		269	0,3
Strobilurins	pyraclostrobin	blocks the electron transport chain, which inhibits energy production in mitochondrial respiration	3.681	4,4
	azoxystrobin		1.374	1,6
	fluoxastrobin		1.029	1,2
	trifloxystrobine		462	0,6
Imidazole	prochloraz	inhibits the biosynthesis of ergosterol	6.268	7,5
Dithiocarbamate	mancozebe	disrupting fungal metabolism	4.852	5,8
	thirame		102	0,1
Morpholine	fenpropimorphe	inhibits the biosynthesis of ergosterol, an essential component of the fungal cell membrane	619	0,7
	spiroxamine		2.185	2,6
Benzophenone	metrafenone	disrupts the mitochondrial respiratory chain	1.880	2,3
Phenylpyrroles	fluopyrame	targets fungal cell wall biosynthesis	1.693	2,0
Phthalimides	folpet	disrupts fungal respiration and energy production	1.055	1,3
Carboxamides	fenpicoxamide	mitochondrial respiration	191	0,2
Quinazolinone	proquinazid	inhibits spore germination	127	0,2
Benzimidazole	thiophanate-methyle	inhibits microtubule formation	38	0,0
Anilinopyrimidines	cyflufenamid	inhibits fungal methionine biosynthesis	0	0,0
	cyprodinil		7	0,0
Carboxamides	krésoxym-méthyl	mitochondrial respiration	New molecule	
Phosphonates	Potassium phosphonates	disrupts fungal metabolism	New molecule	
Pyridione	pyriofenone	similar to strobilurins	New molecule	

Table is ordered accordingly to the total amount by group, then by molecule. Estimated quantities are extrapolated from sales data in Belgium in 2021, obtained from Corder asbl (2023)

3.3 The impact of chemical pesticides on epiphytic microbiomes

In 2021, 126.480 hectares were cultivated with winter wheat in Belgium. All active ingredients combined, more than 200 tons of chemicals were sprayed on these fields, representing a mean application of 1,83 $\pm$ 0,12 kg/ha. Among them, 83,3 tons were fungicides, representing 41% of the total, followed by growth regulators (39%) and herbicides (18%).

Insecticides represent less than 1% (Corder asbl, 2023). Considering the timing of their application, fungicides exert the most significant impact on the phyllospheric microbiome, as they are applied directly to the leaves. In contrast, herbicides are utilized prior to and during the initial growth stages of wheat. Growth regulators are typically sprayed in early spring, usually between the phenological development stages BBCH 30 (beginning of stem elongation) and 32 (node 2 at least 2 cm above node 1), when only the first few leaves of wheat have fully expanded. The last two leaves, which are crucial for yield, develop later and are thus the primary concern for farmers aiming to ensure high yields. Consequently, these leaves will be the main target of fungicide applications, which inevitably affects their associated microbiome. Interestingly, growth regulators and fungicides are applied in similar quantities, despite typically applying one to three fungicide treatments per season and only one growth regulator treatment. This discrepancy can be attributed to the concentration levels of the products, with growth regulators generally being about twice as concentrated as fungicides.

Agrochemical inputs have significant effects on microbial communities in various plant habitats, such as the soil, rhizosphere, phyllosphere, and endophytic tissues (Newman et al., 2016; Ali et al., 2019; Cernava et al., 2019; Chen et al., 2020c; Köberl et al., 2020). The impact of these chemicals has been well documented across multiple sources, revealing that they not only affect the microbial populations but also alter the dynamics between positive and negative responders within the plant microbiota (Chen et al., 2020b). For example, *Acinetobacter*, a genus within the Gamma-proteobacteria, is known to thrive upon fungicide exposure, while members of the Alpha-proteobacteria, intrinsically associated with plants, often decrease in numbers (Köberl et al., 2020; Chen et al., 2021), highlighting the selective pressures imposed by pesticide treatments.

These chemical interventions lead to shifts in the bacterial community composition of the phyllosphere. *Sphingomonas*, a typical dominant genus, is evenly distributed between chemically treated plots or not, whereas *Pseudomonas*, another dominant genus, faces a decline in abundance with increasing pesticide concentrations. The bacterial genera *Methylobacterium*, *Massilia*, *Aureimonas*, *Pantoea*, and *Burkholderia*, all important colonizers of plant surfaces, experience altered abundance profiles under pesticide stress (Chen et al., 2021), which suggests a reconfiguration of the microbial ecosystem in response to chemical applications (Perazzolli et al., 2014; Cernava et al., 2019). Pesticide treatment can also induce a negative effect on the abundant Alpha-proteobacteria and Gamma-proteobacteria, exhibited by the negative response of *Stenotrophomonas*, *Serratia*, and *Flavobacterium*, whereas *Paracoccus* is a positive responder (Chen et al., 2021). On the other hand, Gamma-proteobacteria, characterized by their fast growth, are

believed to recolonize the phyllosphere more quickly than other microbes after pesticide treatment, effectively filling the vacated niches during the recovery phase (Whipps et al., 2008a). This is particularly consequential for genera such as *Stenotrophomonas* and *Serratia*, which are closely connected to their plant hosts and are known to confer protection against various stresses (Hagemann et al., 2008; Pang et al., 2009). Their functions may be compromised when exposed to certain agrochemicals.

Moreover, Actinobacteria, a group that houses many promising biocontrol strains, are likely to be adversely affected by pesticide-impacted agroecosystems. The use of pesticides is also associated with an increase in particular Proteobacteria members and some Actinobacteria, with organisms like *Brevundimonas* exhibiting resistance to harsh conditions (Dartnell et al., 2010). However, certain treatments can shift the balance in favor of genera such as *Brevundimonas*, *Arsenophonus*, and *Candidatus Portiera*, while disadvantaging other bacterial groups known for plant commensalism and support in natural environments, such as *Massilia*, *Rhodanobacter*, and *Brachybacterium* (Ofek et al., 2012; Singh et al., 2016; Volpicella et al., 2014).

Triazole fungicides are widely used in Belgian wheat agriculture, with 42% of the total quantity of fungicide used. Among this family, prothioconazole is the most sprayed with about 20% of the total. Triazoles' effect on soil microbial diversity is profound, as highlighted by several studies (Onwona-kwakye et al., 2020; Storck et al., 2018; Sułowicz and Piotrowska-Seget, 2016). In general, it results, at first, in an increase in bacterial diversity and a decrease in fungal diversity, as shown with metaconazole (Nair et al., 2019) and prothioconazole (Lloyd et al., 2021). A major consequence is the observed increase in Actinobacteria and in specific bacterial genera such as *Bacillus* alongside a decrease in phyla Acidobacteria and Nitrospirae. The observed decreases in these two taxa suggest potential risks to the microbial ecology and associated biogeochemical processes as they are involved in carbon, iron, nitrogen, and hydrogen cycles (Daims et al., 2001; Ivanova et al., 2020). Moreover, the impact of pesticides is not just taxonomic but functional, as indicated by reductions in the bacterial network complexity and the positive response of some core operational taxonomic units (OTUs), classified as *Nocardioides*, *Sphingomonas*, *Ensifer*, *Rhodococcus*, and *Streptomyces*, that correlate with the degradation of chemicals like difenoconazole (Zhang et al., 2021a). On the same line, *Burkholderia*, *Dermacoccus*, *Hyphomicrobium* and *Methylobacterium* (Han et al., 2021) as well as *Alcaligenes faecalis* WZ-2 (Sun et al., 2020) have shown considerable capability to degrade tebuconazole, yet *Methylobacterium* bacteria were particularly susceptible to its impacts (Han et al., 2021). Tebuconazole can also disturb the Firmicutes/Bacteroidetes ratio (Ku et al., 2023). In another study, a proliferation of organotrophic bacteria

and fungi was observed after tebuconazole application, whereas Actinobacteria were found to be less tolerant to the molecule. The fungicide also promoted the proliferation of Proteobacteria, increased the relative OTU number of bacteria from *Arthrobacter*, *Kaistobacter*, *Rhodococcus* and *Streptomyces* genera and reduced the abundance of these from *Bacillus*, *Gemmata* and *Sphingomonas* genera, which may point to their sensitivity to this chemical (Baćmaga et al., 2022).

Among strobilurines molecules, another widely used fungicide family in wheat, pyraclostrobin has a multifaceted impact on microbial communities: it enhances bacterial diversity while diminishing fungal diversity, accompanied by an increase in specific bacterial genera such as *Bacillus* (Nair et al., 2019). However, it also significantly raises the proportion of the *Diaporthe* fungus, negatively affecting plant health. Conversely, it reduces foliar *Alternaria* isolates, suggesting potential adverse effects on seed quality when used without disease pressure (Batzer and Mueller, 2020). Interestingly, strains of *Bacillus* and *Paenibacillus* have shown the capability to degrade pyraclostrobin, indicating their role in mitigating some of its impacts (Birolli et al., 2020).

Authorized in organic agriculture, sulfur application generates higher microbial abundance and diversity. Interestingly, a negative response was observed for the plant-associated Sphingobacteria, alongside a positive response of Acidobacteria, Blastocatellia, Delta-proteobacteria and Verrucomicrobia (Damo et al., 2023).

Treatments with prochloraz, an imidazole often used in conventional agriculture, negatively affected non-target organisms, notably reducing the presence of potentially beneficial *Papiliotrema flavescens* on the avocado fruit surface (Bill et al., 2022). Furthermore, the application of prochloraz leads to alterations in the presence of *Bacillus subtilis*, alongside a decrease in both fungal and bacterial diversity (Bill et al., 2021).

The interactions between agrochemicals and microbiomes are multifaceted, affecting microbial abundance, diversity, and ecosystem functions (Muñoz-Leoz et al., 2013). Observed shifts depend greatly on considered active ingredient, but also on the applied concentration. Generally, elevated levels of pesticides can lead to more profound and lasting shifts in bacterial diversity, whereas lower concentrations tend to cause only temporary or minor effects (Chen and Edwards, 2001; Storck et al., 2018; Torabi et al., 2020; Wang et al., 2009). The observed shift is also widely context-dependent, as pyraclostrobin was observed to reduce bacterial diversity (Lloyd et al., 2023) or to increase it (Nair et al., 2019) and *Acidobacteria* are often susceptible to pesticides, as seen earlier, but sometimes thriving in pesticides-polluted soil (Regar et al., 2019). In all cases, these changes have implications for plant health, soil quality, and broader environmental sustainability, emphasizing the need for a

nuanced approach to agrochemical application that considers the ecological ramifications.

3.4 Biocontrol agents as an alternative

BCAs present a sustainable alternative to conventional agrochemicals, promising to enhance or maintain the health of the epiphytic microbiome crucial for plant vitality. Generally, the development process for BCAs candidates goes from laboratory screenings to field applications and is based on direct antagonism and/or various plant growth-promoting characteristics such as phosphate solubilization, nitrogen fixation, and the production of antibiotics and siderophores (Backer et al., 2018). Despite their success in controlled environments, translating these benefits to field conditions remains challenging, as evidenced by mixed results in increasing yields for crops like maize and wheat with strains such as *Azospirillum brasilense* (Fukami et al., 2016; Hungria et al., 2010) and *Kosakonia radicincitans* (Berger et al., 2018), or *Rhizobium leguminosarum* bv. *trifolii*'s impact on rice (Kecskés et al., 2016).

However, there are very few success stories with BCAs when applied at a large scale. Their inconsistent field performance is a significant hurdle for farmer adoption, primarily due to the often inadequate integration of BCAs into natural microbial communities, which can be attributed to several factors. Introduced microbes face competition from well-adapted resident microflora, with the competitive ability often overlooked during the selection process. The dosage, cell vitality, suitable formulations for delivery, and the specific relationship between the BCA, plant genotype, intrinsic characteristics of the pathogen, pathogen inoculum density, and environmental conditions significantly influence the outcomes (Samad et al., 2017; Bonaterra et al., 2022). Moreover, the timing and method of application can affect BCA performance, highlighting the need for strategies that consider the complex interactions within the plant holobiont (Chowdhury et al., 2015). A specific microbial inoculant's effectiveness can fluctuate, not guaranteeing consistent outcomes across all host cultivars or at all times (Finkel et al., 2017).

The interaction between BCAs and the plant microbiome is complex. On one hand, studies have demonstrated that BCAs can beneficially modify the foliar microbiome by suppressing harmful microbes while enhancing the presence of beneficial ones, thus potentially improving plant health and resilience against diseases (Cernava et al., 2019; Chen et al., 2018). For example, the introduction of *Bacillus amyloliquefaciens* QST713 into the banana rhizosphere has been shown to increase *Bacillus* and *Pseudomonas* populations, indicating a positive shift towards beneficial microbial communities (Tian et al., 2023). On the other

hand, the impact of BCAs on microbiomes is not universally positive or transformative. Some studies suggest that the introduction of certain biocontrol agents might have minimal effects on the foliar microbiome, underscoring the importance of the specific bacterial strains used as BCAs (Vacheron et al., 2013).

Pseudomonads, particularly in the phyllosphere of wheat, have shown potential in contributing to natural plant protection. However, there is a pronounced gap in our understanding of their impacts, especially concerning the foliar microbiomes of wheat, with limited studies addressing the effects of *Pseudomonas* or other BCAs on these critical microbial communities (Müller and Ruppel, 2014). The interaction between chemical fungicides and natural microbiomes has been explored to some extent, revealing both positive and negative impacts on microbial diversity and structure, which underscores the nuanced effects of such treatments on plant microbiomes (Cernava et al., 2019; Ray et al., 2020). Despite these insights, the specific influences of *Pseudomonas* as a BCA on wheat's foliar microbiome remain largely uncharted, necessitating further research to harness its full potential in sustainable agriculture practices.

This calls for enhanced efforts in tracking BCA efficacy and integration into existing microbial communities to better leverage their benefits while minimizing negative impacts. The future of sustainable agriculture may well depend on our ability to fine-tune these biological interventions, ensuring they complement the complex ecology of plant microbiomes. The focus on *Pseudomonas* as a BCA for wheat aligns with the urgent demand for sustainable farming practices, representing a pivotal area of research due to the contrast between its significant potential and the relatively few success stories. This duality underscores the necessity for deeper investigation into optimizing the application and integration of *Pseudomonas* in agricultural settings, ensuring that its benefits can be fully realized while addressing the challenges that have limited its widespread adoption in the field.

Chapter 4
Pseudomonas, a keystone genus associated with wheat

Pseudomonas is a large genus of Gram-negative bacteria regrouping more than 300 species (Girard et al., 2021) of motile rod-shaped bacteria that are unable to form spores. They are ubiquitous and are considered as fundamental components of bacterial communities (Mendes et al., 2013; Girard et al., 2021). A vast diversity of species carries a significant ecological importance and offers promising applications in the realms of biocontrol and bioremediation. Renowned for their adaptability, versatility, and metabolic capabilities, *Pseudomonas* species flourish across a multitude of environments, underscoring their role in environmental sustainability, agriculture, and microbial ecology studies.

These bacteria stand out as potential biocontrol agents, capable of suppressing plant pathogens and promoting plant growth. They achieve this through the production of antibiotics and siderophores, alongside inducing systemic resistance within plants. The use of *Pseudomonas* strains in agriculture has showcased their potential in safeguarding various crops, presenting an eco-friendly alternative to traditional chemical pesticides.

Since its initial description in 1894, the taxonomy of the *Pseudomonas* genus has undergone significant evolution, reflecting advances in bacterial taxonomy techniques. Initially identified as one of the oldest bacterial taxa, *Pseudomonas* has been reclassified multiple times, particularly with the introduction of molecular methods like DNA-DNA hybridization, 16S rRNA gene sequencing, and Multi-Locus Sequencing Analysis.

These techniques revealed that many species classified within the genus did not form a monophyletic group, prompting calls for taxonomic reorganization.

The advent of phylogenomic analysis has further refined our understanding of *Pseudomonas* taxonomy. This latest approach, utilizing whole-genome sequences, allows for the construction of a detailed phylogenetic tree, clearly delineating *Pseudomonas* species into distinct clusters based on shared genomic features and evolutionary relationships. In 2022, two parallel studies based on different phylogenomic approaches ended with similar classification trees (Lalucat et al., 2022; Passarelli-Araujo et al., 2022). The findings demonstrate that the genus, as traditionally defined, is not monophyletic, indicating a need for significant taxonomic revision (Fig. 4-1).

Phylogenomic analysis has led to the proposal of dividing the genus into several new genera, aiming to accurately represent the genetic diversity and evolutionary history of these bacteria. This methodological advancement marks a pivotal moment in bacterial taxonomy, especially for a genus of such broad ecological, industrial, and clinical relevance, providing a more precise and scientifically grounded classification system for *Pseudomonas* species.

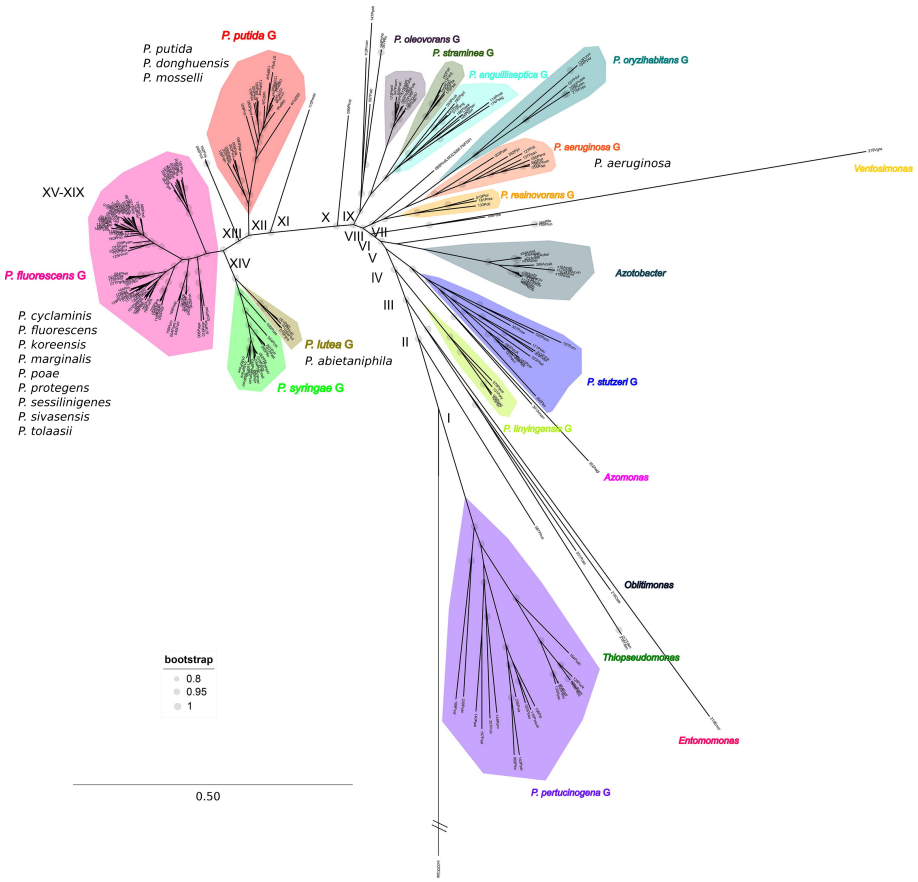

Figure 4-1: Phylogenomic classification of *Pseudomonas* genus.
The genus is not monophyletic and contains 13 groups, indicated by the capital G's. The species listed in each group are related to biocontrol and will be mentioned in the following sections. Adapted from Lalucat et al. (2022).

The *Pseudomonas fluorescens* group and its eight *Pseudomonas* subgroups (*P. chlororaphis*, *P. corrugata*, *P. fluorescens*, *P. fragi*, *P. gessardii*, *P. jessenii*, *P. koreensis*, *P. mandelii*) are well-known for the numerous strains associated with biocontrol activities (Hofte, 2021). *Pseudomonas* strains are known to produce a broad range of bio-active compounds, including lipopeptides, siderophores, polyketides, and volatile compounds with interesting bactericidal properties against plant pathogens (Caulier et al., 2018; Anderson et al., 2020).

On the other hand, all *Pseudomonas* are not beneficial to plants, some species being phytopathogens and cause diseases in important plants. A prominent example is *P. syringae*, which is responsible for various plant diseases, including bacterial speck, bacterial blight, and bacterial canker. Numerous pathovars have been described, these pathogens infecting a wide range of host plants, such as tomatoes (pv. tomato),

beans (pv. phaseolicola), kiwifruit (pv. actinidiae), soybean (pv. glycinea) and peas (pv. pisi) leading to significant agricultural losses. Among *P. syringae* pathovars, pv. syringae has the largest host range with about 180 different plant species, including major economic crops such as stone fruits, nut and fruits trees, vegetables and ornamentals (Morris et al., 2019).

P. syringae employs a type III secretion system to inject effector proteins into plant cells, disrupting normal cellular functions and promoting infection. Another example is *P. cichorii*, which causes bacterial rot in lettuce and celery, affecting the leaves and leading to tissue decay. *P. savastanoi* pv. savastanoi and pv. phaseolicola infect olive and beans, respectively, whereas *P. marginalis* can infect potato, carrot and lettuce, *P. corrugata* infects tomato and pepper, *P. fuscovaginae* causes rice rot and *P. viridiflava* can infect tomato, pepper and lettuce.

These phytopathogenic strains possess genes that code for toxins, enzymes, and other virulence factors, making them significant threats to crop production and plant health.

P. aeruginosa is the most well-known human pathogen within the *Pseudomonas* genus. It is a Gram-negative bacterium that can cause a wide range of infections, particularly in individuals with weakened immune systems. *P. aeruginosa* is notorious for its role in hospital-acquired infections, including pneumonia, urinary tract infections, and bacteremia. It possesses several virulence factors, such as biofilm formation, antibiotic resistance mechanisms, and the production of toxins, which make infections difficult to treat and control. This pathogen is a significant concern in healthcare settings due to its high resistance to many antibiotics and its ability to thrive in various environments.

The presence of potentially problematic genes in some of these *Pseudomonas* taxa can diminish their attractiveness as *Pseudomonas* used as BCAs. Horizontal gene transfer mechanisms, including conjugation, transformation, and transduction, can facilitate the spread of these pathogenicity genes among different *Pseudomonas* strains and other bacterial species in the environment. This genetic exchange can complicate the use of *Pseudomonas* as BCAs, as beneficial strains may acquire virulence factors, reducing their safety and effectiveness.

4.1 Biocontrol activities of Pseudomonads

The most important mechanisms of disease suppression by *Pseudomonas* biocontrol agents include competition for nutrients or space (Kamilova et al., 2005), antibiosis (Haas and Défago, 2005; Raaijmakers et al., 2002; Raaijmakers and Mazzola, 2012), secretion systems and effector delivery (Anantharajah et al., 2016; Galle et al., 2013; Green and Mecsas, 2016; Hernandez et al., 2020; Viana et al., 2021), and induction of systemic resistance (Bakker et al., 2007; Pieterse et al., 2014). Secondary metabolite production in *Pseudomonas* has been extensively reviewed (Hofte, 2021) and a comprehensive overview of the biosynthesis, chemistry, and biological significance of *Pseudomonas* secondary metabolites is provided by Gross and Loper (2009). The literature on secondary metabolites involved in biocontrol has been summarized in several studies (Haas and Défago, 2005; Mishra and Arora, 2018; Olorunleke et al., 2015; Raaijmakers and Mazzola, 2012). It has become evident that only a limited number of bioactive compounds play a clear role in biocontrol of plant diseases (Pieterse et al., 2014). Properties of these major secondary metabolites are synthesized in Table 4-1 and their chemical structures are illustrated in Appendix 1.

Table 4-1: Major Bio-active Compounds Involved in Biocontrol by *Pseudomonas*

Class	Metabolite	Precursor	Biosynthetic gene cluster	References
Alkyresorcinols	2-hexyl-5-propyl-alkylresorcinol (HPR)	Octanoid acid	darABC	(Nowak-Thompson et al., 2003; Calderón et al., 2013) (Biessy et al., 2019)
Biosurfactant	Rhamnolipids	alkanoic acids (HAAs)	RhlABCRI	(D'aes et al., 2010; Raaijmakers and Mazzola, 2012) (Bengtsson et al., 2015; Khattari et al., 2015) (Olorunleke et al., 2015; Caulier et al., 2018) (Wood et al., 2018; Geudens and Martins, 2018) (Crouzet et al., 2020; Götze and Stallforth, 2020) (Déziel et al., 2003)
Heterocyclic aromatic compounds: Phenazines	Phenazine-1-carboxylic acid (PCA)	chorismic acid	PhzABCDEFG	(Chin-A-Woeng et al., 2003; Price-Whelan et al., 2006) (Gross and Loper, 2009; Chincholkar and Thomashow, 2013) (Mavrodi et al., 2001; Huang et al., 2011) (Mavrodi et al., 2006; Briard et al., 2015) (Wang et al., 2016a; Biessy and Filion, 2018) (Morrison et al., 2017; Roquigny et al., 2018) (Mavrodi et al., 2013; Biessy et al., 2019) (Wan et al., 2021)
	1-hydroxyphenazine (1-OH-PHZ)	PCA	PhzS	
	2-hydroxy-phenazine (2-OH-PHZ)	PCA	PhzO	
	2-hydroxy-phenazine-1-carboxylic acid (2-OH-PCA)	PCA	PhzCDO	
	Phenazine-1-carboxamide (PCN)	PCA	phzH	
	Pyocianine (PCN-)	PCA	PhzM	
NRPS/PKS	Pyoluteorin	proline	pltABCDEFGLM	(Howell, 1980; Maurhofer et al., 1994) (Nowak-Thompson et al., 1999; Ramette et al., 2011) (Wu et al., 2011; Leclère et al., 2022)
Phenylpyrrole	Pyrrolnitrin	Tryptophan	prnABCD	(Hill et al., 1994; Kirner et al., 1998) (Ligon et al., 2000; Okada et al., 2005) (Biessy et al., 2019; Anderson et al., 2020) (Afonso et al., 2021)
Polyketide	2, 4-diacetylphloroglucinol (DAPG)	malonyl coA	phlACBD	(Weller et al., 2007; Almario et al., 2017) (Wang et al., 2023)
Siderophore	Pyochelin	salicylic acid and cysteine	pchDCBA, pchEFGHI, pchR	(Serino et al., 1997; Meyer, 2000) (Martínez-Viveros et al., 2010; Cornelis, 2010) (Wu et al., 2011; Schalk et al., 2020)
Siderophore	Pyoverdin	2,4-diaminobutyrate and tyrosine	Variable, includes PvdLIJDAF and pvcABCD	(Wu et al., 2011; Ringel and Brüser, 2018)
	HCN	glycine	hcnABC	(Laville et al., 1992; Blumer and Haas, 2000) (Frapolli et al., 2012; Wu et al., 2011) (Anderson et al., 2020; Anand et al., 2020)

These major bioactive compounds are detailed in the section below. For the sources of this information, please refer to Table 4-1.

- **2-hexyl-5-propyl-alkylresorcinol**: HPR is structurally related to DAPG but biosynthesized differently from octanoic acid. It exhibits antifungal and antibacterial activities, with its gene cluster identified in *P. chlororaphis*.
- **Biosurfactants**: Biosurfactants, including rhamnolipids and (cyclic) lipopeptides, are produced by *P. aeruginosa* and other *Pseudomonas* species, playing roles in biocontrol and plant interaction, with their structures and biosynthesis extensively studied. Their amphiphilic properties result from their combinations of polar peptide head with a lipophilic fatty acid tail.
- **Phenazines**: Phenazines, nitrogen-containing pigments produced by *Pseudomonas* and other Gram-negative Proteobacteria, include various structures. Phenazine-1-carboxylic acid (PCA) is derived from chorismic acid and is the precursor of the other related structures such as phenazine-1-carboxamide (PCN), 1-hydroxyphenazine (1OH-PHZ), 2-hydroxyphenazine (2OH-PHZ) and pyocyanin (PYO), the latter being related to *P. aeruginosa*. Biosynthesis genes for phenazines are conserved within a seven-gene locus, phzABCDEFG, with phenazine diversity arising from modifications by specific enzymes. These compounds exhibit anti-pathogenic properties and can induce systemic resistance in plants, also playing a role in biofilm formation and iron reduction. Phenazines are primarily found in specific *Pseudomonas* subgroups, including *P. aeruginosa*, *P. chlororaphis*, and *P. fluorescens*.
- **Pyoluteorin**: Pyoluteorin inhibits seedling diseases caused by *Pythium ultimum*, with its biosynthesis involving a 17-gene cluster.
- **Pyrrolnitrin**: Pyrrolnitrin, derived from tryptophan, displays broad antifungal activity and is synthesized by a four-gene cluster found in various bacterial genera, including *Pseudomonas*.
- **2,4-diacetylphloroglucinol (DAPG)**: DAPG, a polyketide antibiotic produced by specific *Pseudomonas* strains, suppresses a variety of soil pathogens such as *Gaeumannomyces graminis* var. *tritici* or *Macrophomina phaseolina* and is phytotoxic at high concentrations, with its biosynthesis initiated from malonyl CoA through a conserved nine-gene cluster.
- **Siderophores**: Siderophores contribute to biocontrol by competing for iron, directly antagonizing pathogens, or inducing systemic resistance. Most *Pseudomonas* strains produce pyoverdine, with around 100 variants identified and categorized into four groups. Besides pyoverdine, these strains often produce additional siderophores

like pyochelin, enantio-pyochelin, achromobactin, pseudomonine, corrugatin, and (thio)quinolobactin, which generally have a lower iron affinity than pyoverdine. These siderophores vary in chemical structure.

- **Hydrogen cyanide (HCN)**: HCN, inhibiting cytochrome c oxidase, is produced by several *Pseudomonas* strains, with production governed by the hcnABC gene cluster and regulated by various factors.

- **Others bioactive molecules**: Several *Pseudomonas* strains produce unique bio-active metabolites, likely acquired through horizontal gene transfer. These include rhizoxins, with fungicidal and oomyceticidal properties, found in some *P. protegens* strains and in *P. chlororaphis* MA342 (Ligon et al., 2000; Loper et al., 2008; Takeuchi et al., 2015). Sessilin, a cyclic lipopeptide with antifungal effects, is specific to the biocontrol strain *Pseudomonas* sp. CMR12a (D'aes et al., 2014). Syringotoxin, a peptide with antibiotic activity, is produced by *P. syringae* and *P. fuscovaginae* (Flamand et al., 1996). Promysalin, harboring salicylic acid, is an antibiotic from *P. putida* RW10S1 effective against both Gram-positive and Gram-negative bacteria (Kaduskar et al., 2017; Li et al., 2011). L-furanomycin, discovered in *P. fluorescens* SBW25, targets Gram-negative plant pathogens (Trippe et al., 2013). Several others bio active molecules, for which associated biosynthetic gene clusters has been identified, are listed in appendix Table S-1.

Among these numerous compounds, several have been linked to a specific effect against fungi on different plant species. Unsurprisingly, strains from aeruginosa, fluorescens and putida, along with lutea, groups are the most studied for biocontrol abilities (Table 4-2).

In the specific case of wheat and its pathogens, all the major compounds produced by *Pseudomonas* (Table 4-1) have been shown to be effective against soil-borne fungi (*Gaeumannomyces graminis* var. *tritici, Pythium ultimum*) as well as against above-ground diseases such as head blight (*Fusarium graminearum*), rust (*Puccinia recondita* f. sp. *tritici*) and STB (*Z. tritici*), with phenazines and 2,4DAPG as spearheads (Table 4-3).

Table 4-2: Metabolites production of *Pseudomonas* spp.

Pseudomonas group	Species	Strains	Target	Metabolites	References
aeruginosa	*P. aeruginosa*	M18, PNA1, BA5, sp.	*Fusarium oxysporum f. sp. cucumerinum*, *Pythium myriotylum*, *Phytophthora drechsleri*	pyoluteorin, phenazine-1-carboxylic acid, phenazine-1-carboxamide, HCN, siderophores, VOCs, non-volatile compounds	(Wu et al., 2011; Tambong and Höfte, 2001) (Islam et al., 2018; Maleki et al., 2011)
fluorescens	*P. fluorescens*	9, HC1-07, sp.	*Macrophomina phaseolina*, *P. drechsleri*, *Rhizoctonia solani*	phenazines, non-volatile compounds, viscosin	(Maleki et al., 2011; Castaldi et al., 2021) (Yang et al., 2014)
	P. korensis	S150	*P. nicotianae*, *R. solani*	lokisin	(Gu et al., 2020)
	P. poae	RE*1-1-14	*R. solani*	poaeamide	(Zachow et al., 2015)
	P. protegens	ML15, FD6, DSMZ13134	*Heterobasidion spp.*, *Botrytis cinerea*, *M. fructicola*	siderophores, hydrogen cyanide, 2,4-diacetylphloroglucinol, pyrrolnitrin and pyoluteorin	(Ajijah et al., 2023; Zhang et al., 2020) (Pellicciaro et al., 2022)
	P. sessilimigenes	CMR12a	*P. myriotylum*	sessilin, phenazine, orfamides, pseudodesmin	(Ferrarini et al., 2022)
	P. tolaasii	CH36	*P. myriotylum*	tolaasin, phenazine, orfamides, pseudodesmin	(Ferrarini et al., 2022)
lutea	*P. abietaniphila*	ODB36	*B. cinerea*	oxalate degrading enzyme	(Lee et al., 2020)
putida	*P. putida*	sp.	*Magnaporthe oryzae*, *P. drechsleri*	VOCs, non-volatile compounds	(Patel et al., 2021; Maleki et al., 2011)
	P. donghuensis	P482, SVBP6	*R. solani*, *Fusarium culmorum*, *Verticillium dahliae*, *P. ultimum*, *M. phaseolina*	pseudopyronine, fragin, pyoverdine, lankacidin, 7-hydroxytropolon	(Krzyzanowska et al., 2021; Muzio et al., 2020)
	P. mosselii	923	*Xanthomonas*, *Magnaporthe oryzae*	pseudoiodinine	(Yang et al., 2023)
Unknown	Unknown	CMR12a, COW3, PA14H7, spp.	*P. myriotylum*, *Pyricularia oryzae*, *Dickeya solani*, *Verticillium wilt*	phenazines, sessilins, orfamides, bananamides, 7-hydroxytropolone, viscosin, viscosinamide a, wlip and pseudodesmin a, pyoverdins, siderophores, HCN	(Oni et al., 2019) (Omoboye et al., 2019) (Munier-Lépinay et al., 2023) (Geudens et al., 2017) (Tao et al., 2020) (Mercado-Blanco et al., 2004) (Deketelaere et al., 2017)

Table 4-3: *Pseudomonas* spp. metabolites targeting wheat fungal pathogens

Target pathogen	Organism	Metabolites or effect	References
Zymoseptoria tritici	*P. aeruginosa* LEC1, *P. putida, P. fluorescens*	2,4-diacetylphloroglucinol (2,4-DAPG), 1-hydroxyphenazine (1-OH-PHZ), Siderophore, HCN, Pyoverdin, pyocianine, Dihydroaeruginoic Acid, Rhamnolipids	(Levy et al., 1989, 1992) (Flaishman et al., 1990, 1996) (Carmi et al., 1994) (Platel et al., 2022)
Puccinia recondita f. sp. tritici	*P. aeruginosa* LEC1, *P. putida*	2,4-DAPG, 1-OH-PHZ, Siderophore, HCN	(Levy et al., 1989, 1992; Flaishman et al., 1996)
Fusarium graminearum	*P. piscium,* Fluorescent *Pseudomonads, P. chlororaphis* Pcho10, *P. chlororaphis* BZR 245-F and *Pseudomonas* sp. BZR 523-2	Phenazine-1-carboxylic acid (PCA), pyoluteorin, pyrrolnitrin, Siderophore, HCN, Phenazine-1-carboxamide (PCN), pyocyanin, lipopeptides, viscosin, massetolides	(Alimi et al., 2012; Hu et al., 2014; Sidorova et al., 2023; Müller et al., 2018)
Gaeumannomyces graminis var. tritici	*Pseudomonas fluorescens* HC1-07, *P. chlororaphis* H18, *P. chlororaphis LX24*	viscosin-like cyclic lipopeptide, 1-OH-PHZ, 2-Hydroxyphenazine (2-OH-PHZ), 2,4-DAPG	(Shirzad et al., 2012; Yang et al., 2014; Liu et al., 2021; Wan et al., 2021)
Pythium ultimum	*P. chlororaphis* 30-84	PCA, 2-hydroxyphenazine-1-carboxylic acid (2-OH-PCA), 2-OH-PHZ	(Yu et al., 2018)

The development of resistance by fungal pathogens to control strategies is a significant concern in agriculture, where sustainable management practices are essential. Chemical fungicides have been widely used for decades; however, their efficacy is increasingly compromised by the emergence of resistant fungal strains. This resistance often arises due to the high selection pressure imposed by these chemicals, which typically target specific biochemical pathways. The repetitive and widespread use of these fungicides exerts a consistent selective force on fungal populations, leading to the survival and proliferation of resistant mutants.

In contrast, the risk of resistance development in fungal pathogens against biocontrol agents such as *Pseudomonas* species is notably lower. *Pseudomonas* spp. are versatile microorganisms that exert their antifungal effects through a multifaceted mechanism of action, including the production of secondary metabolites, competition for space and nutrients, induction of systemic resistance in plants, and direct antagonism of pathogens. This multifactorial mode of action reduces the likelihood of fungal pathogens developing resistance, as it is considerably more challenging for pathogens to simultaneously adapt to multiple stressors compared to a single-target chemical fungicide.

However, it is important to note that depending on the complexity of their mode of action, phytopathogens may display a wide range of sensitivities to biocontrol agents (BCAs), including extremely low sensitivity. In a few generations, some pathogens can adjust to the selection pressure imposed by BCAs (Bardin et al., 2015). The idea that biological control lasts longer than chemical control is a widespread misconception. According to research on pest management in fields, this assumption may not always be true. For exemple, numerous pests have developed tolerance to one or more *Bt* toxins, while the codling moth *Cydia pomonella* has established resistance to the *C. pomonella* granulovirus (Lahlali et al., 2022).

The durability of biological control of plant diseases, in contrast with pest management, has received little attention (Akutse et al., 2020). Similar to single-mode chemical fungicides, populations of pathogens could change into forms that are resistant to BCAs. This would make it less likely that BCAs will be able to reduce plant diseases for a long time. Therefore, a weak spot in the pathogen life cycle must be found as an opportunity window for successful biocontrol (Bardin et al., 2015).

Moreover, biocontrol *Pseudomonas* spp. exhibit a dynamic interaction with the environment and the plant microbiome, contributing to their effectiveness and adaptability. They can modulate their activity based on environmental conditions and the presence of pathogens, further complicating the ability of fungal pathogens to develop resistance. This ecological adaptability, combined with the diversity of antifungal mechanisms employed by *Pseudomonas*, creates a complex and less predictable environment for fungal pathogens, reducing the selective pressure that typically drives resistance development.

4.2 Regulation of secondary metabolites production

The production of secondary metabolites is tightly regulated through complex networks integrating environmental signals and metabolic feedback. These networks ensure the production occurs only under optimal conditions to confer ecological advantages while minimizing metabolic costs (Yan et al., 2018). Indeed, it has been shown that lineages of *P. aeruginosa* produce surfactants when they can mitigate the oxidative stress generated by primary metabolism and possess surplus resources beyond their basic requirements, enabling them to support secondary metabolism. This finding introduces an additional dimension to the regulation of a secondary metabolite that is not crucial for primary metabolism but plays a key role in altering the physical properties of the environments surrounding bacterial communities (Santamaria et al., 2022). Lipopeptide (LP) regulation in *Pseudomonas* has been recently reviewed by Zhou et al. (2024).

4.2.1 Global regulation of lipopeptide-associated genes

The most extensively studied global regulatory system involved in LP production is the Gac/Rsm signal transduction pathway (Haas and Défago, 2005). This cascade starts with the GacS/GacA two-component system, consisting of the GacS sensor kinase and the GacA response regulator. GacS autophosphorylates in response to an unidentified signal at high cell densities, transferring the phosphate to GacA, which then activates the expression of small RNA genes (Sonnleitner and Haas, 2011). These small RNAs (rsmX, rsmY, rsmZ) bind to repressor proteins (RsmA, RsmE), alleviating their inhibition on mRNA translation of genes involved in bioactive molecule biosynthesis, including LPs. Mutations in gacS or gacA generally lead to a complete loss of LP production (Koch et al., 2002; De Bruijn et al., 2007; Song et al., 2014; Olorunleke et al., 2017), and similarly, rsmXYZ mutants in *P. protegens* CHA0 and *P. lactis* SS101 exhibit loss of LP production (Sobrero et al., 2017; Song et al., 2015a).

Global regulators can also activate silent gene clusters. For example, in *P. fluorescens* Pf0-1, a silent gene cluster encoding a novel CLP was activated by complementing a defective gacA (Jahanshah et al., 2019).

4.2.2 Regulation of LP production in mono-producers

LP mono-producers, found within groups like *P. fluorescens* and *P. putida*, play roles in motility, biofilm formation, nutrient solubilization, and interactions with plants and insects (D'aes et al., 2010; Raaijmakers et al., 2010; Götze and Stallforth, 2020; Oni et al., 2022). These strains typically have well-conserved NRPS genes arranged in operons or split configurations (Cesa-Luna et al., 2023). CLPs are secreted via the PleABC efflux system, homologous to the MacAB-TolC system found in many Gram-negative bacteria (Fitzpatrick et al., 2017; Girard et al., 2022). Regulatory genes, often of the LuxR family, are positioned near these NRPS clusters and are crucial for LP production (De Bruijn and Raaijmakers, 2009; Washio et al., 2010; Dubern et al., 2008; Sobrero et al., 2017).

For example, mutation in luxR1 (viscAR) or luxR2 (viscBCR) in *P. fluorescens* SBW25 results in the loss of viscosin production (De Bruijn and Raaijmakers, 2009), while mutation of luxR1 in *Pseudomonas* sp. MIS38 leads to the loss of arthrofactin production (Washio et al., 2010). The role of LuxR2 regulators varies, with some LP biosynthetic gene clusters (BGCs) lacking these genes altogether (Girard et al., 2021).

Mono-producers of LLPs, such as syringafactin and cichofactin, are distributed across different *Pseudomonas* phylogroups (Bricout et al., 2022; Cesa-Luna et al., 2023). These LLPs are generally encoded by two NRPS genes arranged in operons and are regulated by upstream and downstream LuxR homologues (Berti et al., 2007). However, certain LLP producers, like *P. bijieensis* L22-9, lack these regulatory genes, and some strains, such as *P. syringae* DC3000, lack associated transporter genes (Berti et al., 2007; Cesa-Luna et al., 2023).

While quorum sensing (QS) regulation is uncommon among mono-producers, exceptions exist. For example, *P. fluorescens* 5064 regulates viscosin production via the 3-OH-C8-HSL quorum sensing signal (Cui et al., 2005). Similarly, the ppuI-rsaL-ppuR QS system regulates putisolvin production in *P. putida* PCL1445, with AHL signals inducing LP biosynthesis genes (Dubern et al., 2006). Additional regulators include heat shock proteins and the ClpAP complex, which are necessary for LP production under certain conditions (Dubern et al., 2005; Song et al., 2014; Washio et al., 2010; Song et al., 2015b). Environmental signals also play a role: for instance, protozoal exposure upregulates LP biosynthesis genes in *P. lactis* SS101 and *P. fluorescens* SBW25, enhancing predation protection (Mazzola et al., 2009).

4.2.3 Regulation of LP production in poly-producers

Poly-producers, such as those in the *P. syringae* group, often co-produce multiple CLPs and LLPs. Their regulation is more complex than in mono-producers and can be QS-dependent or independent.

Production of Mycins and Peptins in various plant-pathogenic *Pseudomonas* species is regulated by the GacS/GacA system and LuxR-type transcription factors, including SalA, SyrF, and SyrG, which respond to plant signals (Lu et al., 2002; Vaughn and Gross, 2016). These factors work hierarchically, with SalA regulating syrG and syrF, and SyrF activating syringomycin and syringopeptin biosynthesis (Lu et al., 2005; Kitten et al., 1998; Wang et al., 2006b,a). Environmental signals, such as arbutin and D-fructose, further regulate these BGCs via GacS, SalA, and SyrF (Burch et al., 2014; Hockett et al., 2013).

Other plant pathogens, such as *P. fuscovaginae*, produce multiple CLPs with distinct functions, including fuscopeptin, syringotoxin, and asplenin. These strains lack luxR-type regulatory genes in certain BGCs, suggesting a unique regulation mechanism (Ferrarini et al., 2022). Similarly, *P. cichorii* produces cichopeptins and cichofactins, regulated by multiple LuxR-type genes (Huang et al., 2015; Pauwelyn et al., 2013; Girard et al., 2020).

Some poly-producers, particularly those within the *P. asplenii, P. mandelii*, and *P. corrugata* subgroups, regulate LP production via QS systems. These systems involve the JesR1, JesI, RhtB, and JesR2 genes, which are crucial for the production and secretion of CLPs (Licciardello et al., 2009; Arp et al., 2018; Pflanze et al., 2023). In *P. nunensis* In5, for example, fungal signals activate a specific regulator, NunF, necessary for antifungal CLP production (Hennessy et al., 2017; Christiansen et al., 2020).

The QS systems in these strains often involve AHL signals like C6-AHL, which regulate CLP production in conjunction with LuxR-type proteins (Hennessy et al., 2017; Berry et al., 2014; Gu et al., 2023). The regulatory proteins JesR1 and JesR2 cluster phylogenetically with other LuxR-type regulators, indicating a conserved mechanism across various strains (Pflanze et al., 2023).

Tolaasin, a key virulence factor in mushroom pathogens like *P. tolaasii* and *P. costantinii*, is regulated by reversible genetic elements that alter the GacS sensor kinase, leading to phenotypic switching (Heeb and Haas, 2001; Han et al., 1997). These strains also produce a second CLP, such as pseudodesmin or viscosinamide, essential for mushroom colonization (Hermenau et al., 2020; Cesa-Luna et al., 2023).

In *P. sessilinigenes*, a well-studied biocontrol strain, sessilin and orfamide production is regulated by LuxR-type genes (ofaR1, ofaR2, sesR1), with regulation tied to the Gac/Rsm system (D'aes et al., 2014; Olorunleke et al., 2017). Gene loss and genomic rearrangements further complicate the regulatory mechanisms in these strains, highlighting the dynamic nature of LP regulation (Omoboye et al., 2019).

4.2.4 Environmental Influences

Factors like nutrient availability and osmotic stress heavily influence the stability and expression of genes involved in secondary metabolism. In large-scale fermentations, nutrient-rich conditions often lead to the accumulation of regulatory mutants, which outcompete the wild type, reducing the overall efficacy of the inoculant (Yan et al., 2018).

4.3 Market of *Pseudomonas*-based bioproducts

Adopting Biocontrol Agents (BCAs) in agriculture presents several risks and challenges that can hinder their widespread market entry. One significant challenge is the variable efficacy of BCAs under field conditions. For example, certain BCAs like *Trichoderma* species may perform well in controlled environments but show inconsistent results when applied in different climates or soil types. This inconsistency can deter farmers who rely on predictable outcomes for their crops.

Another risk is the slower action of BCAs compared to chemical pesticides. Farmers often need immediate pest control to protect their crops, and the slower response of BCAs can lead to economic losses if pests are not managed promptly. For instance, in cases where crops like tomatoes or strawberries face sudden pest outbreaks, relying solely on BCAs could result in yield losses before the BCAs take effect.

Moreover, the sophisticated management practices required for effective BCA use can be a barrier, especially for small-scale farmers. BCAs often need precise application techniques, specific environmental conditions, and continuous monitoring, which can be resource-intensive. For example, the combined use of multiple BCAs, as seen in trials with *P. fluorescens* and *Bacillus subtilis*, requires careful planning and understanding of microbial interactions, which may not be feasible for all farmers.

The market entry of BCA products also faces regulatory and eco-

nomic challenges. Obtaining approval from regulatory bodies like the EFSA or EPA can be a lengthy and expensive process, delaying the availability of new BCAs to farmers. Additionally, the cost of producing and formulating BCAs can be high, making them less competitive compared to chemical pesticides.

Addressing these risks and challenges is crucial for the successful adoption and market entry of BCAs. Practical solutions include conducting extensive field trials to demonstrate consistent efficacy, providing education and support to farmers on BCA application, and developing cost-effective production methods to make BCAs more accessible and affordable.

Despite these risks and challenges, *Pseudomonas*-based bioproducts are already commercialized in EU, in the USA, and other parts of the world to manage fungal diseases. Among them, several are authorized to control wheat diseases such as Cedomon, Cerall, Cedress, AtEze, Spot-Less, or Howler (Table 4-4).

Table 4-4: Examples of commercially available *Pseudomonas*-based bio-products worldwide

Species	BCA Strain	Disease/ Pathogen	Area where authorized	Commercial product name	Commercially produced by
Pseudomonas aureofaciens	Tx-1	*Rhizoctonia solani, Sclerotinia homeocarpa, Colletotrichum graminicola, Pythium aphanadermatum, Michrodochium nivale*	USA	Bio-Ject Spot-Less	EcoSoil Systems
P. chlororaphis	MA342	*Tilletia caries, Septoria nodorum* and *Fusarium* spp.	EU	Cerall Cedomon	BioAgri AB BioAgri AB
	63-28			Cedress AtEze	BioAgri AB EcoSoil Systems
	AFS009	*Pythium, Fusarium*	EU,Africa, Middle East and Central Asia	Howler	AgBiome and BASF
P. fluorescens	sp.	Leaf spot, *Pythium, Fusarium*	India	pSeudocon, Green Heal combat, BACTVIPE, Katyayani	Tari, Greenlife Biotech, bighaat, Katyayani Organics
	DSMZ 13134	*Rhizoctoniasolani, Helmintosporium solani*	EU	Pseudomonas Fluorescens Proradix	SP Sourcon Padena GmbH
	A506	*Erwinia amylovora*	USA	BlightBan	Nufarm Americas Inc.
P. syringae	ESC-10 and ESC-11	*Penicillium expansum, Botrytis cinerea, Mucor piriformis, Helminthosporium solani, Rhizopus stolonifer*	USA, EU	Bio-Save®10 LP and Bio-Save®11 LP	Lallemand Plant Care

The promising potential of *Pseudomonas* strains as biocontrol agents (BCAs) against fungal pathogens in wheat fields is gaining attention due to the urgent need for sustainable agricultural practices and reducing the environmental impact of chemical pesticides. Despite their *in vitro* success, the field application of BCAs, including *Pseudomonas*, is constrained by inconsistencies and varying degrees of effectiveness. These challenges stem from incomplete knowledge regarding the fate of BCAs in natural environments and their influence on existing microbiomes. Such gaps in understanding likely contribute to the observed variability in outcomes. However, the significance of *Pseudomonads* within the wheat microbiome cannot be overstated, particularly in their role as a keystone genus that influences both pathogen control, such as *Z. tritici*, and the health of the crop's microbiome. Recognizing *Pseudomonas* as a pivotal player paves the way for leveraging their capabilities more effectively, marking a step towards achieving more resilient and sustainable wheat production systems.

Chapter 5
Objectives

This thesis emphasizes the significant role of *Pseudomonas* within the wheat bacterial microbiome, focusing on the phyllosphere. Given *Pseudomonas* demonstrated capability to combat fungal pathogens *in vitro*, an increase in the use of these strains as biocontrol agents (BCAs) in wheat cultivation is anticipated. However, the fate of BCAs upon application to leaf surfaces and their effects on the natural microbiome remain largely unexplored, which may contribute to the observed variability in their efficacy. To shed light on this issue, our research explores the consequences of introducing a *Pseudomonas* biocontrol agent on the wheat phyllosphere microbiome. Specifically, wheat-associated *Pseudomonas* population will be investigated and the effects of the introduction of a strain of *Pseudomonas* on the composition and equilibrium of the foliar bacterial communities will be studied. This will include the potential shifts in the abundance of *Pseudomonas* and their impact on keystone taxa and microbial community structure. The research will address the following questions:

- What is the prevalence and distribution of *Pseudomonas* species and how do they interact within the wheat-associated microbiome?
- Is the *Pseudomonas* strain CF10PS3 biocontrol agent able to establish, survive and develop on wheat leaves ?
- What is the baseline wheat foliar microbiota and how does the introduction of the *Pseudomonas* biocontrol agent impact it?

To address these questions, we employed a multifaceted approach, integrating genomic, molecular, and ecological methodologies.

First, to identify and characterize major *Pseudomonas* species asso-

ciated with wheat, we conducted a systematic screening and selection of bacterial strains. Initial characterization was performed using *in vitro* methods, followed by DNA extraction and whole genome sequencing. The sequencing data was then assembled, annotated, and mined for insights into the potential role of *Pseudomonas* in the wheat microbiome. Phylogenomic analyses, including core and pan-genome analyses, were utilized to delineate species boundaries and to identify genes potentially involved in biocontrol.

Second, to specifically track the selected *Pseudomonas* strain during field experiments, we designed and tested qPCR probes. These probes were developed based on the unique genetic SNP identified in the strain's genome and its counterpart present in the other related strains' genomes. The qPCR assays underwent rigorous testing for validation, including specificity and limit of detection determination. We also evaluated the transferability and robustness of the assays across different conditions. These validated probes were then used to monitor the persistence of the introduced strain across the wheat growing season. The qPCR data provided precise quantification of the strain, enabling the assessment of its establishment and survival on wheat leaves under varying field conditions.

Furthermore, the impact of the introduced *Pseudomonas* strain on the natural wheat microbiome was assessed using an advanced and innovative metagenomic approach with the new bioinformatic tool PRONAME. This approach allowed for species-level discrimination, revealing shifts in species abundance and community structure. The sequencing data brought detailed insights into the impacts of the introduced strain on the native bacterial community. These findings shed light on the ecological role of *Pseudomonas* within the wheat phyllosphere and its potential to influence microbial dynamics at the phytobiome level.

Chapter 6
Pseudomonas arvensis sp. nov. and *Pseudomonas sivasensis* are associated with cereals

6.0 Forewords

The preceding chapters have laid a foundation on the importance of sustainable agricultural practices and the role of microbial communities in crop health. In line with this, Chapter 6 delves into the potential of *Pseudomonas* spp. as biocontrol agents. This chapter explores the presence of these bacteria in cereal fields, their antifungal properties, their mechanisms of action, and their potential to replace chemical pesticides in wheat cultivation. This chapter have been submitted for publication in the PeerJ journal, in the section Microbiology. Several collaborators were associated to the work presented in this chapter; Mathieu Delitte wrote the manuscript. Mathieu Delitte and Louis Morandini collected field samples. Mathieu Delitte and Benjamin Dubois carried out the experiments. Claude Bragard, Alain Bultreys, Jacques Mahillon and Frederic Debode acquired and managed funding. All authors designed the study, interpreted the results, reviewed and approved the final version of the manuscript.

Pseudomonas arvensis sp. nov. and *Pseudomonas sivasensis* are associated with cereals.

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Competing interests:
P. sivasensis and related strains are under patenting process and might be used as BCA by the UCLouvain spin-off company BIOCSOL.

Authors' contribution:
MD wrote the manuscript. MD and LM collected field samples. MD and BD carried out the experiments. CB, AB, JM and FD acquired and managed funding. All authors designed the study, interpreted the results, reviewed and approved the final version of the manuscript.

6.1 Abstract

The role of microbial communities in plant health and productivity has become increasingly evident. This study examines the presence of *Pseudomonas* spp. within the phytobiomes of Belgian cereal crops, particularly wheat, and evaluates their contributions to agricultural ecosystems. Specifically, we explored the antagonistic activities of these bacteria against major wheat pathogens, the colonization capabilities of *P.*

sivasensis on wheat, and its effectiveness in reducing symptoms of *Zymoseptoria tritici* infection. Additionally, we introduced *Pseudomonas arvensis* sp. nov., identified as a new inhabitant of the wheat microbiome, with DR1PS3 proposed as the type strain. These findings not only deepen our understanding of microbial interactions in cereal crops but also underscore the potential of native bacterial species in the development of sustainable disease management strategies.

- **Subjects:** Microbial Ecology, Agricultural Microbiology, Plant Pathology, Biocontrol Agents

- **Keywords:** *Pseudomonas sivasensis*, *Pseudomonas arvensis* sp. nov., Wheat, Phytobiome, Biocontrol, *Zymoseptoria tritici*, Whole Genome Sequencing

6.2 Introduction

Wheat is a major component of global agriculture, playing a vital role in ensuring food security and nutrition worldwide. Its widespread use and ability to thrive in various environments have established it as a dietary staple globally, contributing significantly to the economic and cultural landscape of societies. Despite its importance, wheat cultivation faces major threats from pathogens like *Zymoseptoria tritici*, the causative agent of Septoria Tritici Blotch (STB), which poses challenges due to its evolving resistance to fungicides (Fones and Gurr, 2015).

The microbial community within the wheat phytobiome plays a critical role in nutrient cycling, growth enhancement, and particularly in disease resistance. This complex system, consisting of the rhizosphere, phyllosphere, and endosphere, interacts dynamically with the plant, significantly influencing its health and productivity (Bulgarelli et al., 2012; Lundberg et al., 2012; Turner et al., 2013; Zhang et al., 2021b; Lengrand et al., 2024). Understanding this intricate microbiome is key to developing sustainable disease management strategies.

Among the microbial constituents, *Pseudomonads* have been highlighted as keystone species in the wheat microbiome, crucial for their biocontrol properties and overall plant health (Sivakumar et al., 2020; Kumar et al., 2021). However, the specific role and prevalence of *Pseudomonas sivasensis* in cereals have not been well-documented until now. The species has been linked to diverse environments ranging from the canola rhizosphere (Świątczak et al., 2023b) to the *Bletilla striata* (hyacinth orchid) root endosphere (Wu et al., 2022), and even in aquatic or industrial environments (Duman et al., 2020), as well as in a high plateau

wetland ecosystem (Xiong et al., 2023), suggesting a notable adaptability. This study marks the first reported association of *P. sivasensis* with cereal crops, significantly broadening our understanding of its ecological range and biocontrol potential.

Moreover, our investigation led to the discovery of a new *Pseudomonas* species, *Pseudomonas arvensis* sp. nov., enhancing our taxonomic and ecological knowledge of the genus. This discovery underscores the importance of advanced taxonomic tools in identifying and characterizing microbial species within the cereal microbiome.

This study seeks to investigate the biocontrol potential of *P. sivasensis* and *P. arvensis* sp. nov. within cereal crops. We aimed to understand how these bacteria colonize wheat, their prevalence, and their antagonistic effects against major wheat pathogens, contributing to sustainable agricultural practices through the use of native biocontrol agents.

6.3 Methods

6.3.1 Bacterial strains selection

Four cereal fields in Belgium (Table 6-1) were sampled in 2019 at four periods (ends of March, April, May, and June) to collect *Pseudomonas* isolates from wheat heads, leaves, roots, and soil. These were obtained by isolation on CFC agar (cephaloridine, fucidin, and cetrimid), a medium designed for *Pseudomonas* growth.

Table 6-1: Characteristics of the sampled fields.

Field Letter	Field Location	GPS Coordinates	Host	Agricultural Practices	Tillage
A	Jemeppe-sur-Sambre	50°29'47.0"N 4°37'32.6"E	Winter wheat	Organic	Yes
B	Jodoigne	50°43'13.4"N 4°51'22.4"E	Winter wheat	Conventional	Yes
C	Walhain	50°39'22.2"N 4°38'52.3"E	Spelt	Organic	No
D	Mont-Saint-Guibert	50°39'08.2"N 4°38'20.4"E	Winter wheat	Conventional	No

To select strains with biocontrol potential, a rapid prescreening was performed by qualitative observation of direct *in vitro* antagonism against *Fusarium graminearum*, adapting antibiosis tests described for toxins (Bultreys and Gheysen, 1999), but using lysogeny broth (LB) agar. In a second step, their ability to antagonize four wheat fungal pathogens was assessed through *in vitro* confrontation with a quantitative approach, focusing on the mycelial growth reduction in *F. graminearum* (causing head blight), *Gaeumannomyces graminis* var. *tritici* (causing take-all),

Oculimacula yallundae (causing eyespot), and *Z. tritici* (causing STB). For the first three fungi, a gelose piece from a one-week-old fungus culture grown on potato dextrose agar was placed at the center of a LBA plate. Subsequently, three 10 µl drops of a 24-h LB liquid culture (30°C, 180 rpm) of the bacteria were introduced at 2.5 cm from the center in three opposite directions. The plates were then incubated at 22°C for 7 days (*Fusarium graminearum*) or 12 days (*Gaeumannomyces graminis* var. *tritici* and *O. yallundae*) and the inhibition zone was measured at the end of the incubation period.

Considering the slow mycelial growth of *Z. tritici*, an alternative top-agar method was used for evaluating the strain antagonistic ability against this pathogen. Briefly, conidia from a 10-day old *Z. tritici* LB liquid culture (22°C, 150 rpm) were harvested, adjusted to 10^6 spores/mL. One milliliter of the spore suspension was mixed with freshly autoclaved LBA at 45°C and immediately spread over a previously constituted thin PDA plate. When solidified, a 10-µl drop of a bacterial suspension, prepared as described earlier, was placed at the center of the plate. The inhibition area was measured 10 days later. All antagonism tests were conducted in three biological repetitions and in two temporal repetitions. Statistical analyses were performed in RStudio (v. 4.3.3) using t.test of the stats package.

6.3.2 Bacterial strain initial identification

Following a 24-h incubation in LB medium (yeast extract, 5 g/L, NaCl, 5 g/L, bacto tryptone, 5 g/L) at 28°C and 150 rpm, the bacterial suspensions underwent centrifugation, and the resulting pellets were used for DNA extraction using the Invitrogen PureLink Genomic DNA Kit (Fisher Scientific, USA) in accordance with the manufacturer's recommendations. Molecular identification involved a Multi-Locus Sequence Analysis (MLSA) targeting the 16S rRNA, *gltA, rpoB, glnS, gapA, gyrB, ileS, nuoD, recA* and *rpoD* genes. PCR amplification conditions were as follows: initial denaturation at 94°C for 5 min, 30 cycles of denaturation at 94°C for 30 sec, annealing for 30 sec, elongation at 72°C for 60 sec, and a final extension step at 72°C for 5 min. The characteristics of primers and their annealing temperatures are detailed in Table 6-2. Amplicon length was checked on agarose gel. Sequencing was conducted by Eurogentec (Belgium) using Sanger sequencing. Subsequently, sequences were concatenated and queried against the RefSeq Representative Genomes database at the National Center for Biotechnology Information (NCBI).

Table 6-2: Characteristics of primers and annealing temperature

Target gene	Primer name	Sequence 5' ¿ 3'	Amplicon size (bp)	Annealing temperature (°C)	Reference
16S rRNA	27F 1492R	AGAGTTTGATCCTGGCTCAG GGTTACCTTGTTACGACTT	1400	51	Richert et al. (2005)
Citrate synthase (*gltA*)	gltA_F gltA_R	AGTTGATCATCGAGGGCGCWGCC TGATCGGTTTGATCTCGCACGG	529	60	Nikolić et al. (2018)
DNA-directed RNA polymerase subunit beta (*rpoB*)	LAPS5 LAPS27	TGGCCGAGAACCAGTTCCGCGT CGGCTTCGTCCAGCTTGTTCAG	1230	50	Nikolić et al. (2018)
Glutaminyl-tRNA synthetase (*glnS*)	glnS_F glnS_R	ACCAACCCGGCCAAGAAGACCAGG TGCTTGAGCTTGCGCTTG	710	60	Andreani et al. (2014)
Glyceraldehyde-3-phosphate dehydrogenase A (*gapA*)	GapA_F GapA_R	CGCCATYCGCAACCCG CCCAYTCGTTGTCGTACCA	476	60	Andreani et al. (2014)
Gyrase subunit B (*gyrB*)	GyrB-2F GyrB-4R	ACCGTCGAGTTCGACTACGA CCTCGGTGTTGCCSARCTT	1461	57	Andreani et al. (2014)
Isoleucyl-tRNA synthetase (*ileS*)	ileS_F ileS_R	TTCCCAATGAARGCCGGCCTGCC GGGGTGGTGGTCCAGATCACG	633	60	Andreani et al. (2014)
NADH dehydrogenase subunit D (*nuoD*)	nuoD_F nuoD_R	GAAGTCCTGACCTTCCTGC GAAGAACTCGGCCATCATG	771	60	Ait Tayeb et al. (2005)
Recombinase A (*recA*)	recA_F recA_R	TGGCTGCGGCCCTGGGTCAGATC ACCAGGCAGTTGGCGTTCTTGAT	537	60	Mulet et al. (2009)
RNA polymerase sigma factor (*rpoD*)	PsEG30F PsEG790R	ATYGAAATCGCCAARCG CGGTTGATKTCCTTGA	743	55	Mulet et al. (2010)

6.3.3 *In vitro* characterization

To evaluate the cellulolytic activity of the bacterial strains, the method described by Thomloudi et al. (2021) was followed. Briefly, 10 μL of bacterial liquid culture was placed in the center of a Petri dish containing the CMC medium. After 7 days of incubation at 37°C, plates were flooded with 2 mL of Lugol's solution. Cellulolytic activity was measured by the size of the translucent halo around the bacterial colonies. Proteolytic activity was assessed using the method outlined by Durham et al. (1987). Briefly, bacterial suspensions were inoculated on plates containing the skimmed milk medium. After overnight incubation at 30°C, activity was measured by the size of the translucent halo surrounding the bacterial colonies. Siderophore production was evaluated as explained by Bultreys and Gheysen (2000), but using King B agar. The orange halo around the bacteria after 24 hours of incubation at 30°C indicated siderophore production. Swimming and swarming motility assays were conducted following Thomloudi et al. (2021). Briefly, 10 μL of overnight bacterial culture was inoculated on NA medium amended with 0.3% and 0.5% agar, respectively. Petri dishes were incubated at 28°C for one day. Biofilm formation was evaluated using the 96-well PVC plate assay as described by O'Toole (2010) and Thomloudi et al. (2021). Briefly, diluted (1:100) overnight bacterial cultures (100 μL) were inoculated in each well and incubated at 28°C for 2 days. Wells were washed three times with distilled water before adding 200 μL of crystal violet. After incubating at room temperature for 30 minutes, the biofilm was solubilized with a solution of 20% ethanol and 80% acetone and measured by the intensity of the purple color. Indole production was assessed as described by Baldan et al. (2015) and Thomloudi et al. (2021). Briefly, the supernatant of a 48-hour bacterial culture amended with 1% L-Tryptophan was mixed with Salkowski reagent. A positive result was indicated by a pink-orange-red coloration in the dark. All

tests were conducted in three biological repetitions and in three temporal repetitions.

6.3.4 DNA extraction, genome sequencing, assembly, annotation and mining

DNA was extracted from pelleted 24h bacterial cultures using the NucleoSpin Soil kit (Macherey-Nagel, UK), with SL1 and SX as lysis solutions. After purification with AMPure XP beads, the quality of the DNA was checked on a 0.8% agarose gel and with a Nanodrop spectrometer (ThermoFisher Scientific, USA). The DNA concentration was assessed using a Qubit 4 fluorometer (ThermoFisher Scientific,USA). Sequencing libraries were constructed with the Ligation Sequencing Kit V14 (SQK-LSK114, Oxford Nanopore Technologies, UK) following the manufacturer's recommendations. Each library was loaded on an R10.4.1 Flongle flowcell, and a MinION device from ONT (Oxford Nanopore Technologies) was used to carry out the sequencing for 24h. For the CF10PS3 strain, an additional WGS assay was conducted using Illumina technology to allow a (near-)perfect hybrid genome assembly using both ONT and Illumina sequencing data. For this trial, the extracted DNA was sent to Eurofins Genomics (Constance, Germany) to be sequenced on an Illumina NovaSeq device using the NovaSeq 6000 S4 PE150 XP sequencing mode.

The raw ONT sequencing data (POD5) was basecalled using Dorado (v0.3.4) with the basecalling model dna_r10.4.1_e8.2_400bps_sup@v4.2.0. Adapters were removed using Guppy (v6.5.7) and genomes were then assembled using Trycycler (v0.5.4) (Wick et al., 2021). For the CF10PS3 strain, an ONT + Illumina hybrid assembly was performed following the approach described in Wick et al. (2023). Briefly, Trycycler was used to build a long-read assembly, which was then polished using Medaka (v1.8.0) (ONT, 2023). This assembly without structural errors was then polished with Illumina data using Polypolish (v0.5.0) (Wick and Holt, 2022) and Polca (Zimin and Salzberg, 2020) to remove remaining small-scale errors.

The assembled genomes were then annotated using the Prokaryotic Genome Annotation Pipeline (PGAP) (Li et al., 2021). Functional pathways were retrieved using the Kyoto Encyclopedia of Genes and Genomes (KEGG) (Ogata et al., 1999) and the KEGG Automatic Annotation Server (Moriya et al., 2007). To evaluate the strain potential for secondary metabolite production, AntiSMASH (Blin et al., 2023) and the virulence factor database (VFDB) with VFanalyser (Liu et al., 2022) were used. VFanalyzer constructs orthologous groups within the query genome and pre-analyzed reference genomes from VFDB to avoid

potential false positives due to paralogs, then conducts iterative and exhaustive sequence similarity searches among the hierarchical pre-build datasets of VFDB.

To confirm MLSA identification of the strains with a whole genome-based taxonomic analysis, genome sequence data were uploaded to the Type (Strain) Genome Server (TYGS), a free bioinformatics platform available at `https://tygs.dsmz.de`, accessed on 27 March 2024 (Meier-Kolthoff et al., 2022). Information on nomenclature, synonymy, and associated taxonomic literature was provided by TYGS's sister database, the List of Prokaryotic names with Standing in Nomenclature (LPSN, available at `https://lpsn.dsmz.de`) (Meier-Kolthoff and Göker, 2019). TYGS analysis involved using the MASH algorithm (Ondov et al., 2016) and 16S rDNA gene sequences extracted using RNAmmer (Lagesen et al., 2007) to determine the closest type strain genomes. Digital DNA-DNA Hybridization (dDDH) values were computed using the GGDC 4.0 method (Meier-Kolthoff et al., 2013). A balanced minimum evolution tree with branch support was inferred using FASTME 2.1.6.1 (Lefort et al., 2015) and visualized with PhyD3 (Kreft et al., 2017). Type-based species and subspecies clustering was performed using a 70% dDDH threshold (Meier-Kolthoff and Göker, 2019). In parallel, Average Nucleotide Identity (ANI) was calculated with orthoANIu method, considered as the most accurate method (Yoon et al., 2017), with a threshold for species delineation set at 96% (Madhaiyan et al., 2017).

6.3.5 Plant colonization

Transformation to express GFP was performed following the recommendations of Choi and Schweizer (2006). Briefly, plasmids were obtained from an overnight liquid culture (37°C, 220 rpm) of *Escherichia coli* strain DH5α containing either *Tn7::mNeonGreen* plasmid or helper plasmid *pTNS3*. Culture was conducted in LB supplemented with 10 µg/mL gentamycin and 0,2 mM diaminopimelate (DAP). Plasmids were extracted with QIAprep Spin Miniprep kit (Qiagen) following manufacturer recommendations. To prepare competent cells, strains in the exponential growing stage were washed three times in a sucrose solution (102.7 g/l) and transformed by electroporation. Briefly, 100 µl of competent cells were mixed with 500 ng of *Tn7::mNeonGreen* and 500ng of helper plasmid *pTNS3* and electroporated at 2,5kV. Transformed cells were spread on LBA plates containing gentamycin antibiotic.

Bacteria expressing GFP were then cultivated in LB for 24 h (30°C, 180 rpm) before being washed twice and resuspended in sterile $MgCl_2$ + 1% Tween20. Seeds from the Chevignon wheat cultivar were soaked in this suspension for 1 h before sowing in an autoclaved 70/30 mix of loam/perlite. Plants were maintained in a growth chamber with a

day/night cycle of 8 h light and a temperature of 20°C. Hydathode morning dew and total leaves were harvested 9, 15 and 30 days after sowing. Drops exiting hydathodes were immediately spread on LBA plates supplemented with gentamycin and CFC plates. Apex of the leaves were dipped in 100 µl of sterile $MgCl_2$ + 1% Tween20 for 10 min (Suspension A). A 3-cm section of the mid leaf (for suspension M), situated at 8 cm of the apex, and a 3-cm section situated 1 cm away from the leaf base (for suspension B) were cut and dipped in 1 ml of sterile $MgCl_2$ + 1% Tween20 for 10 min. After removing the leaf parts, suspensions M and B were centrifuged at 13,000 g for 2 min and 900 µl of supernatant were discarded. Pellet was resuspended in the remaining 100 µl before spreading on CFC plates and LBA plates supplemented with gentamycin. All plates were incubated at 28°C for 24 h.

The following detached leaves protocol was applied to follow the epiphytic spatial distribution of the strain expressing GFP. Wheat seeds of the Chevignon cultivar, showing intermediate resistance to *Z. tritici*, were grown in an autoclaved 70/30 mix of loam and perlite in plastic trays in a glass-house with 16 h of light and a constant temperature of 22°C until the second leaf was fully expanded. Water agar (15 g/L) containing 100 mg/L benzimidazole (Sigma), used to retard senescence, was dispensed into sterile clear polystyrene boxes (10 x 10 cm). Two rectangular sections (2,5 x 10 cm) were cut from the center of the agar. The plantlet second leaf sections were laid, top surface uppermost, across the gap so that the cut ends remained on the agar. The gap below the leaves helped to prevent water soaking and contamination by other microorganisms. In this gap, a sterile paper piece soaked with 1 mL sterile water was introduced to maintain high humidity in the box. Eight leaf sections were fitted into each box. Strips of agar were then laid over the cut edges of the leaf sections so that they were not exposed, thereby delaying senescence. Bacterial suspension was constituted with a 24 h LB culture washed and resuspended in $MgCl_2$ + Tween20 at a 10^8 CFU/mL concentration. In each box, 2 ml of this suspension was applied. Leaf surfaces were observed under a laser confocal microscope after inoculation and every 5 days for up to 40 days.

6.3.6 Plant protection

To evaluate the ability of the strains to protect the plant by reducing STB infection and its pycnidia production, a detached leaves protocol was designed. Boxes with detached leaves and their inoculation with bacterial suspensions were performed as explained in the last paragraph. All boxes containing detached leaves were constituted on the first day, whereas bacterial and mock treatments (negative control, treated with the $MgCl_2$ + Tween20 buffer only) took place that same day, the day after, and three days later. After bacterial inoculation on the last day,

boxes were left for 30 min under a laminar flow to allow leaves to dry before pathogen application.

Fungal inoculum of *Z. tritici* was produced from an active culture grown on LBA. An isolated mycelium mound derived from a single spore was placed in 10 mL of LB in a 50 mL Falcon tube. After 10 days of incubation at 22°C under shaking, spores were harvested by centrifugation and washed twice in sterile $MgCl_2$ + 1% Tween 20. The suspension concentration was then adjusted to 10^6 spores/mL. In each box where bacteria had been applied two or three days before, or 30 minutes earlier, 2 mL of this suspension was applied, and boxes were closed and incubated at 20°C with a light/dark period of 8/16 h. Symptomatic area and number of pycnidia were scored 28 days after inoculation. All assessments were carried out using a dissecting microscope at 40X magnification. Statistical analysis was performed in Rstudio (v. 4.3.3) using t test of the *stats* v4.3.3 package. This experiment was conducted twice at different time intervals.

6.4 Results

6.4.1 Isolates characterization

From the 444 *Pseudomonas* isolates obtained at the end of the samplings, 114 exhibited direct antagonism against *F. graminearum*. Among them, 33 showed antagonist activities against at least three of the four tested pathogens in quantitative tests, where mycelium growth inhibition was measured (Table 6-3). They were all active against the two soilborne pathogens *G. graminis* var. *tritici* and *O. yallundae*, and showed various abilities to antagonize *F. graminearum* and *Z. tritici*. For isolates obtained from heads, 11/12 were able to control *F. graminearum*. Similarly, 5/6 foliar isolates are able to antagonize *Z. tritici*.

All isolates were able to produce siderophores, protease, and cellulase, except for isolates BF6PS3 and CR3PS2 (*Pseudomonas germanica* and *Pseudomonas granadensis*, respectively), which did not exhibit cellulase production.

MLSA identification based on 10 taxonomically relevant genes compared to the NCBI representative genome database allowed retrieving 11 *Pseudomonas* species in this wheat-associated community (Table 6-4). *Pseudomonas fluorescens*, *Pseudomonas lurida*, *Pseudomonas poae*, *Pseudomonas salmasensis*, *Pseudomonas orientalis*, and *Pseudomonas simiae* are known to colonize wheat, some strains of these species being known as potential biocontrol agents. On the other hand, *Pseudomonas*

tensigenes, *Pseudomonas granadensis*, *Pseudomonas germanica*, *Pseudomonas lactis*, and *Pseudomonas sivasensis* had never been associated with wheat or cereal, to the best of our knowledge. This is particularly interesting for *P. sivasensis* and closely related species since they represent more than 25% of the 33 isolates (Table 6-4). These isolates originate from all plant compartments and were retrieved from the first sampling which occurred at the end of March until the last sampling which took place at the end of June. They were obtained from the four fields no matter the tillage use and agricultural practices (whether organic or conventional) nor the plant host (spelt or winter wheat).

Table 6-3: *In vitro* antagonist activities of the *Pseudomonas* isolates and their bioactive molecules production

	Antagonisitc activity								Bioactive molecules		
Isolate	*F. graminearum*		*Z. tritici*		*G. graminis* var. tritici		*O. yallundae*		Protease	Cellulase	Siderophore
AE10PS1	5.8	**	0.0	ns	7.2	**	17.7	****	+	+	+
BE11PS1	5.8	***	0.0	ns	4.7	***	13.7	****	+	+	+
BF6PS1	4.7	*	5.3	*	14.2	***	14.0	***	+	-	+
CR3PS2	3.0	**	0.0	ns	13.8	***	18.0	****	+	-	+
AE11PS2	5.7	****	5.2	***	5.2	**	13.3	****	+	+	+
AE11PS3	5.8	***	5.8	****	6.8	**	16.3	****	+	+	+
AE11PS4	6.0	***	6.3	****	3.0	***	15.8	****	+	+	+
BF10PS1	3.5	*	4.8	***	14.0	***	15.7	****	+	+	+
BF8PS1	7.2	****	4.3	****	10.7	****	15.3	****	+	+	+
BR8PS4	3.3	*	4.7	***	13.0	***	16.3	****	+	+	+
BR8PS5	3.8	*	3.8	****	10.0	****	11.8	***	+	+	+
CF11PS1	2.3	ns	0.0	ns	6.0	**	6.5	ns	+	+	+
CF10PS4	1.7	ns	3.5	***	12.0	****	6.5	ns	+	+	+
AE12PS1	7.0	***	4.0	***	3.8	**	13.4	***	+	+	+
AE12PS4	4.8	***	4.5	****	2.8	**	15.0	****	+	+	+
AE12PS5	3.8	**	3.7	****	3.3	*	14.8	****	+	+	+
BS3PS2	3.8	**	3.5	***	12.7	****	16.0	***	+	+	+
BS3PS3	3.2	**	4.0	**	12.7	****	14.3	****	+	+	+
CE10PS1	3.3	*	0.0	ns	3.7	**	14.0	***	+	+	+
CR6PS3	5.5	**	7.2	***	11.0	****	15.0	***	+	+	+
CR6PS4	6.0	***	5.8	**	9.5	****	14.0	***	+	+	+
CR6PS5	6.0	***	5.2	**	10.5	****	13.0	***	+	+	+
DR10PS5	5.0	*	0.0	ns	6.5	**	14.7	****	+	+	+
AR12PS3	2.8	ns	0.0	ns	17.8	***	23.7	****	+	+	+
BE10PS1	3.2	***	0.0	ns	3.2	****	13.3	****	+	+	+
BE10PS3	3.1	***	0.0	ns	3.2	****	13.1	****	+	+	+
CF10PS3	1.7	ns	2.7	***	5.7	**	7.3	ns	+	+	+
CR7PS1	5.8	**	0.0	ns	7.0	**	17.3	****	+	+	+
DR1PS3	3.5	*	0.0	ns	9.4	**	7.2	ns	+	+	+
DR4PS3	5.3	**	5.8	***	9.0	****	16.6	****	+	+	+
DS3PS4	2.7	**	0.0	ns	8.5	***	19.3	****	+	+	+
DS3PS5	4.3	*	0.0	ns	8.0	****	18.0	****	+	+	+
AR11PS3	2.5	*	0.0	ns	15.8	****	18.7	****	+	+	+

The numbers represent the mean inhibition measures in millimeters (n=6), and stars denote the significance of the measured inhibition. Significance was evaluated by comparing measures with the negative control in the Student t-test (ns : not significant; *: p-value <0,5 ; ** <0,01; *** <0,001; **** <0,0001)

Table 6-4: Identity percentages between 10 genes used in the MLSA approach and their best-match in the NCBI representative genome database, with the information about isolate origin

Identification based on MLSA against refseq representative genomes database	% ID with type-strain	Isolate	Plant organ	Field location	Host	Tillage	Agric. practices	Sampling date
P. fluorescens	96.95	AE10PS1	Head	J-s-S	WW	Yes	O	19/06/19
P. fluorescens	97,57	BE11PS1	Head	Jodoigne	WW	Yes	C	18/06/19
P. germanica	99,04	BF6PS1	Leaf	Jodoigne	WW	Yes	C	24/04/19
P. granadensis	99,42	CR3PS2	Root	Walhain	S	No	O	27/03/19
P. lactis	99,78	AE11PS2	Head	J-s-S	WW	Yes	O	19/06/19
P. lactis	98,54	AE11PS3	Head	J-s-S	WW	Yes	O	19/06/19
P. lactis	99,5	AE11PS4	Head	J-s-S	WW	Yes	O	19/06/19
P. lurida	98,39	BF10PS1	Leaf	Jodoigne	WW	Yes	C	18/06/19
P. lurida	99,04	BF8PS1	Leaf	Jodoigne	WW	Yes	C	21/05/19
P. lurida	99,34	BR8PS4	Root	Jodoigne	WW	Yes	C	21/05/19
P. lurida	98,71	BR8PS5	Root	Jodoigne	WW	Yes	C	21/05/19
P. orientalis	97,7	CF11PS1	Leaf	Walhain	S	No	O	20/06/19
P. poae	98,99	CF10PS4	Leaf	Walhain	S	No	O	20/06/19
P. salmasensis	98,79	AE12PS1	Head	J-s-S	WW	Yes	O	19/06/19
P. salmasensis	99,32	AE12PS4	Head	J-s-S	WW	Yes	O	19/06/19
P. salmasensis	99,56	AE12PS5	Head	J-s-S	WW	Yes	O	19/06/19
P. simiae	97,21	BS3PS2	Soil	Jodoigne	WW	Yes	C	25/03/19
P. simiae	99,32	BS3PS3	Soil	Jodoigne	WW	Yes	C	25/03/19
P. simiae	99,2	CE10PS1	Head	Walhain	S	No	O	20/06/19
P. simiae	99,79	CR6PS3	Root	Walhain	S	No	O	29/04/19
P. simiae	99,71	CR6PS4	Root	Walhain	S	No	O	29/04/19
P. simiae	99,57	CR6PS5	Root	Walhain	S	No	O	29/04/19
P. simiae	99,53	DR10PS5	Root	MSG	WW	No	C	14/06/19
*P. sivasensis** (tmp)	93,82	AR12PS3	Root	J-s-S	WW	Yes	O	19/06/19
P. sivasensis	98,33	BE10PS1	Head	Jodoigne	WW	Yes	C	18/06/19
P. sivasensis	99,29	BE10PS3	Head	Jodoigne	WW	Yes	C	18/06/19
P. sivasensis	98,9	CF10PS3	Leaf	Walhain	S	No	O	20/06/19
*P. sivasensis** (tmp)	97,39	CR7PS1	Root	Walhain	S	No	O	23/05/19
*P. sivasensis** (tmp)	99,22	DR1PS3	Root	MSG	WW	No	C	01/04/19
*P. sivasensis** (tmp)	99,79	DR4PS3	Root	MSG	WW	No	C	25/04/19
*P. sivasensis** (tmp)	98,67	DS3PS4	Soil	MSG	WW	No	C	01/04/19
*P. sivasensis** (tmp)	99,11	DS3PS5	Soil	MSG	WW	No	C	01/04/19
P. tensigenes	96,58	AR11PS3	Root	J-s-S	WW	Yes	O	19/06/19

MSG: Mont-Saint-Guibert, J-s-S: Jemeppes-sur-Sambre, WW: winter wheat, S: spelt, O: organic, C: conventionnel
* Strain initially identified as *P. sivasensis* via MLSA, subject to reidentification as *P. arvensis* sp. nov. - see below.

6.4.2 Genome sequencing

Considering the unexpected prevalence of *P. sivasensis* isolates in this community, whole genomes of the nine strains were sequenced using a MinION device and the V14 sequencing chemistry. The genome sequences are deposited on NCBI under BioProject reference number PRJNA1141912 and the strains are deposited in the LMG bacterial collection in Gent, Belgium, with accession numbers provided in Table 6-5.

All 11 genome assemblies, including reference strains BsEB-1 and W6, have one contig with a similar length of 6.30 ± 0.14 Mbp, a GC content of $60.02 \pm 0.32\%$, and 5639 ± 118 proteins (Table 6-5). No plasmid was retrieved in the genomic contigs.

Table 6-5: Genomic characteristics of 11 *P. sivasensis* strains

Strain	BsEB-1	W6	CF10PS3	BE10PS3	BE10PS1	CR7PS1	DR1PS3	DS3PS5	DS3PS4	AR12PS3	DR4PS3
LMG accession	/	/	S-33705	33707	33706	33710	33711	33712	S-33704	33708	33709
NCBI accession	SAMN23530418	SAMN15393973	SAMN42904002	SAMN42493717	SAMN42493716	SAMN42493718	SAMN42493719	SAMN42493721		SAMN42493722	SAMN42493720
Size (Mbp)	6.101	6.109	6.351	6.279	6.279	6.305	6.408	6.246	6.246	6.448	6.576
Percent G+C	59.8	59.8	59.6	59.7	59.7	60.2	60.1	60.2	60.2	60.4	60.6
Protein number	5463	5506	5687	5609	5611	5659	5729	5568	5573	5744	5880

In-depth genome alignments performed via TYGS delineated phylogenetic relationships across 27 distinct species clusters, as illustrated in Fig. 6-1. Four clusters were assigned to the query strains, indicating notable genetic delineations. Notably, in TYGS tool strain P7 is the reference genome for *P. sivasensis*, which closely grouped with two additional queried reference genomes, BsEB-1 and W6, alongside with strains BE10PS1, BE10PS3, and CF10PS3, as evidenced by short branch lengths indicating minor genetic distances (red numbers). Conversely, strains CR7PS1, DR1PS3, DS3PS4, and DS3PS5 formed a distinct cluster, characterized by longer branch lengths from known species, suggesting a potential new species. This hypothesis was further supported by robust bootstrap values above 70% (blue numbers), confirming the reliability of these phylogenetic relationships. Additionally, AR12PS3 and DR4PS3 showed close affiliations with *P. cyclaminis* and *P. marginalis*, respectively.

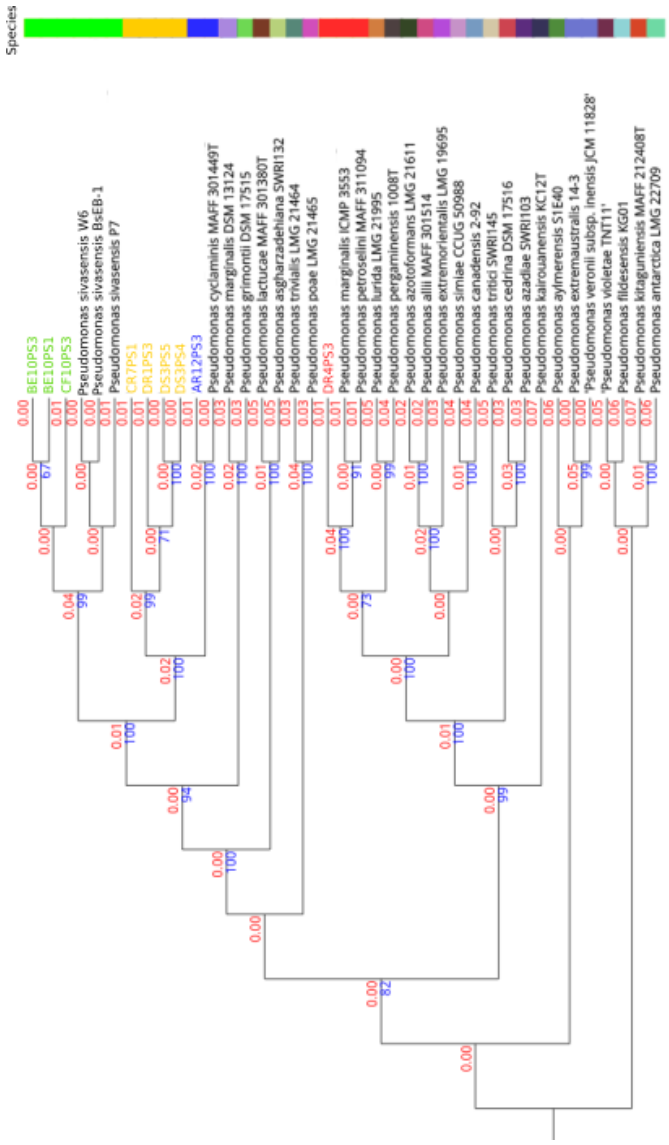

Figure 6-1: Phylogenetic tree based on complete genome alignments.
Tree was build using FastME 2.1.6.1 in TYGS. All query genomes were aligned with the 50 most closely related genomes among the 20886 genomes in the database. Closest alignments were selected to build the tree. Red numbers are the branch length value, scaled in terms of GBDP distance. The blue numbers above branches are GBDP pseudo-bootstrap support values > 60% from 100 replications, with an average branch support of 79.9%. The tree was rooted at the midpoint. Species delineation is represented by the color bar on the right.

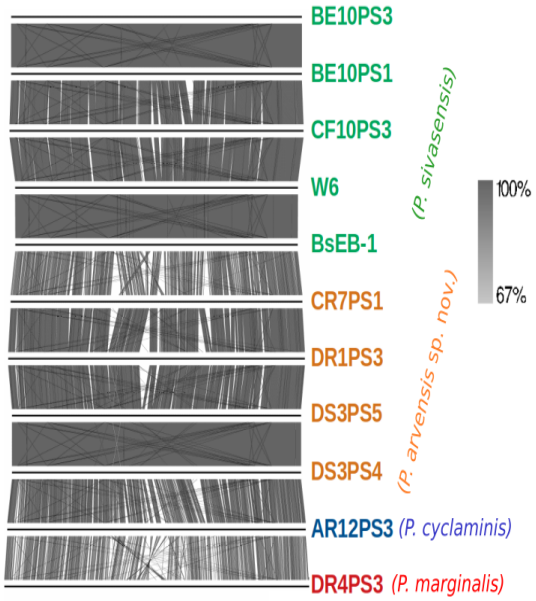

Figure 6-2: Genome organization of sequenced strains

Plain horizontal lines represent the complete aligned genomes. Orthologous clusters between genomes are connected by shades of gray indicating the percentage of similarity, as depicted in the right part.

Detailed comparison of complete genome organization across strains, as visualized in Fig. 6-2, aligned consistently with phylogenetic relationships established by TYGS. Each genome is marked distinctly, with *P. sivasensis* genomes shown in green, illustrating homogeneous genomic structures indicative of close evolutionary relationships. In contrast, genomes attributed to a *P. arvensis* sp. nov. demonstrated a unique organizational pattern, emphasizing potential genomic divergence. Notably, the comparison between the BsEB-1 and CR7PS1 genomes highlighted significant organizational variations. Additionally, the genome of *P. cyclaminis* AR12PS3 was more similar to that of the new species than that of *P. sivasensis* in its organization, suggesting nuanced evolutionary relationships. In contrast, *P. marginalis* DR4PS3 exhibits distinct genomic features, underscoring its phylogenetic distance.

6.4.3 Genome mining

In the results presented in Table 6-6, the genomic comparisons between various strains of *P. sivasensis* and related species were quantified using orthoANIu and dDDH metrics. The orthoANIu values, typically ranging above 99% among closely related *P. sivasensis* strains (BsEB-1, W6, CF10PS3, BE10PS1 and BE10PS3), underscore a strong genetic

relatedness. Conversely, dDDH values, crucial for delineating species boundaries, highlighted more pronounced differences, especially when comparing *P. sivasensis* with distinct species such as *P. cyclaminis* and *P. marginalis*. The lower dDDH values between these species, often below the 70% species delineation threshold, support their classification as separate species. The genomic analysis reinforces that strain AR12PS3 is close to *P. cyclaminis* strain MAFF 301449T and the strain DR4PS3 to *P. marginalis* ICMP 3553, given their ANI and dDDH values (AR12PS3: 98.96%, 91.4%; DR4PS3: 98.87%, 90.0%). The proposed new *Pseudomonas* species comprising strains CR7PS1, DR1PS3, DS3PS4, and DS3PS5 showed high intra-group ANI values exceeding 98.82%, signifying close genetic relationship. Although their ANI values with *P. cyclaminis* stood at approximately 95.80%, just under the threshold (Rosselló-Móra and Amann, 2015), their dDDH values with a species representative genome or with the isolate AR12PS3 ranged from 65 to 66%, below the 70% species delimitation threshold (Meier-Kolthoff et al., 2013).

Table 6-6: OrthoANIu and dDDH values calculated between genomes.

			orthoANIu												
			P. sivasensis						*P. arvensis* sp. nov.			*P. cyclaminis*	*P. marginalis*	*P. cyclaminis*	*P. marginalis*
			BsEB-1	W6	CF10PS3	BE10PS3	BE10PS1	CR7PS1	DR1PS3	DS3PS5	DS3PS4	AR12PS3	DR4PS3	MAFF 301449T	ICMP 3553
dDDH	*P. sivasensis*	BsEB-1	/	99.98	98.93	99.05	99.05	91.63	91.65	91.69	91.69	91.69	88.14	91.36	87.79
		W6	100	/	99.06	99.04	99.07	91.42	91.37	91.29	91.28	91.52	87.92	91.6	87.85
		CF10PS3	91.8	91.7	/	99.06	99.05	91.43	91.33	91.28	91.28	91.52	87.78	91.52	89.55
		BE10PS3	91.9	91.8	92	/	99.99	91.25	91.38	91.56	91.56	91.46	88.04	91.48	89.56
		BE10PS1	91.9	91.8	92	100	/	91.27	91.44	91.52	91.52	91.46	87.99	91.53	89.56
	P. arvensis sp. nov.	CR7PS1	44.4	44.4	44.3	44.6	44.6	/	98.82	98.88	98.89	95.78	88.1	95.76	89.54
		DR1PS3	44.3	44.3	44.2	44.6	44.6	91	/	99.05	99.05	95.78	88.09	95.78	89.55
		DS3PS5	44.3	44.3	44.3	44.5	44.5	91.6	92	/	99.99	95.86	88.14	95.81	89.52
		DS3PS4	44.3	44.3	44.3	44.5	44.5	91.6	92	100	/	95.86	88.15	95.8	89.52
	P. cyclaminis	AR12PS3	44.4	44.4	44.5	44.5	44.5	66.1	65.6	65.9	65,9	/	88.17	98.96	89.58
	P. marginalis	DR4PS3	34.5	34.5	34.7	34.8	34.8	35.1	35	35	35	35.4	/	88.26	98.87
	P. cyclaminis	MAFF 301449T	44.7	44.7	44.7	44.8	44.8	66.1	65.2	65.5	65.5	91.4	35.5	/	88.12
	P. marginalis	ICMP 3553	34.5	34.5	34.6	34.8	34.8	35.1	35	35	35	35.3	90	/	/

OrthoANIu values are in the upper right part and dDDH values in the lower left part.

Fig. 6-3 illustrates the core and pan-genome of *P. sivasensis* and related strains, using a Venn diagram to depict orthologous gene clusters. For the sake of clarity, one isolate of each species and the reference genome BsEB-1 were used. This analysis provides insights into the genetic conservation and variability within these strains, highlighting the core genes essential for basic cellular functions and shared across all examined strains. Additionally, it also delineates genes unique to specific strains, reflecting their evolutionary adaptations and potential specialized functions. The pan-genome size indicates the total genetic diversity found across all strains, essential for understanding the species'

adaptability and ecological fitness.

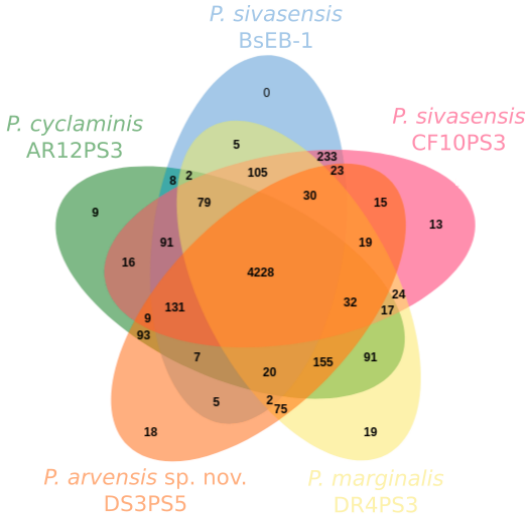

Figure 6-3: Core and pan-genome analysis of one strain of each species compared with *P. sivasensis* reference strain (BsEB-1).
Venn diagram of orthologous clusters generated by OrthoVenn3

In this analysis, 4,950 ± 76 clusters were detected within each genome. Of these, 4,228 were shared across all five isolates. Additionally, 233 clusters were exclusively shared by the two representatives of *P. sivasensis*, BsEB-1 and CF10PS3. The later strain harbored 13 unique clusters, 12 of which being of unknown function, while the remaining one contained the *GspE* gene, involved in the type II secretion system. Strains DR4PS3, DS3PS5, and AR12PS3 possessed 19, 18, and 9 unique clusters, respectively. These clusters were predominantly of unknown function, except in the case of AR12PS3, where processes related to flavonoid and macrolide biosynthesis were identified, along with a fimbrial protein associated with cell adhesion.

Fig. 6-4 illustrates the genetic profiles of each strain concerning their biocontrol potential. Here, we present a subset of biocontrol-related genes from the VFdb and ANTISMASH databases, focusing on genes for which some strains showed a match. There were no discernible trends in the presence or absence of genes among different species. However, one notable exception is the presence of the efflux pump encoded by *AcrAB* genes, which was exclusively found in *P. sivasensis* strains and *P. cyclaminis* AR12PS3. Notably, *P. sivasensis*, along with *P. arvensis* sp. nov. DR1PS3, possessed the complete operon for koreenceine production. All genomes analyzed in this study contained operons for viscosin, APE-Vf, fragin and pseudopyronine production. Conversely, the *pvdD* gene, a key component of the pyoverdine biosynthetic gene cluster, was not identified in any genomes, although all strains pos-

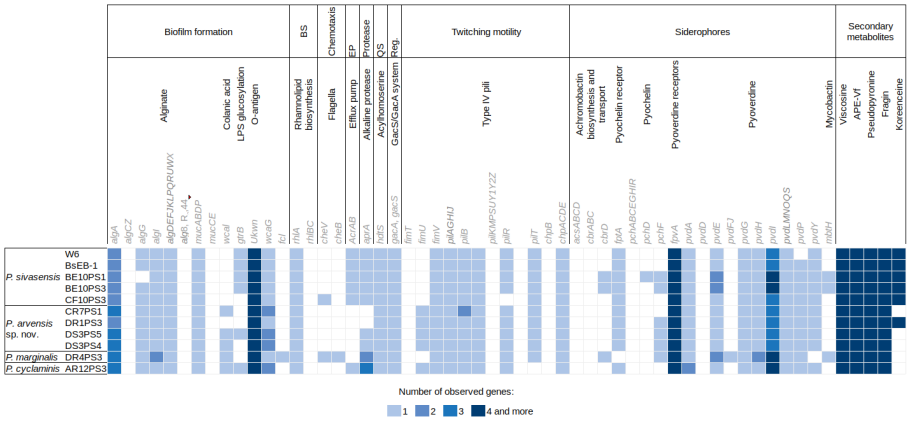

Figure 6-4: Distribution of genes potentially involved in biocontrol in the nine sequenced genomes and two *P. sivasensis* reference genomes.

sessed the *fpvA* gene which encodes the pyoverdine receptor. Similarly, none of the strains possessed a complete operon for pyochelin production, but all except DR4PS3 had the *fptA* gene encoding the pyochelin receptor. Notably, all strains exhibited potential quorum sensing capability through Acyl-homoserine lactone and possessed the *GacA/GacS* regulatory system.

6.4.4 Plant colonization

Soaking of wheat seeds in GFP bacterial suspensions followed by immediate sowing allowed bacteria to enter the seedling and to reach the upper parts, as exhibited by the spreading on LBA supplemented with gentamycin of morning dew in the following days after emergence (Fig. 6-5a). Among *P. sivasensis* strains, CF10PS3 was isolated from leaf and was able to antagonize *Z. tritici* during *in vitro* tests. It was used in subsequent tests to evaluate its plant colonization abilities. Following seed inoculation, CF10PS3 was transported through xylem and exited leaves through hydathodes. From there, it was able to colonize the leaf surface. As shown with confocal imagery after leaf spraying, CF10PS3 can thrive on leaf surface. Immediately following application, single bacteria were found homogeneously distributed on the surface (Fig. 6-5b). Two days later, it was found in large aggregates that colonized trichomes and their base (Fig. 6-5c), veins (Fig. 6-5d) and more importantly, they occupied the niche around stomata (Fig. 6-5e).

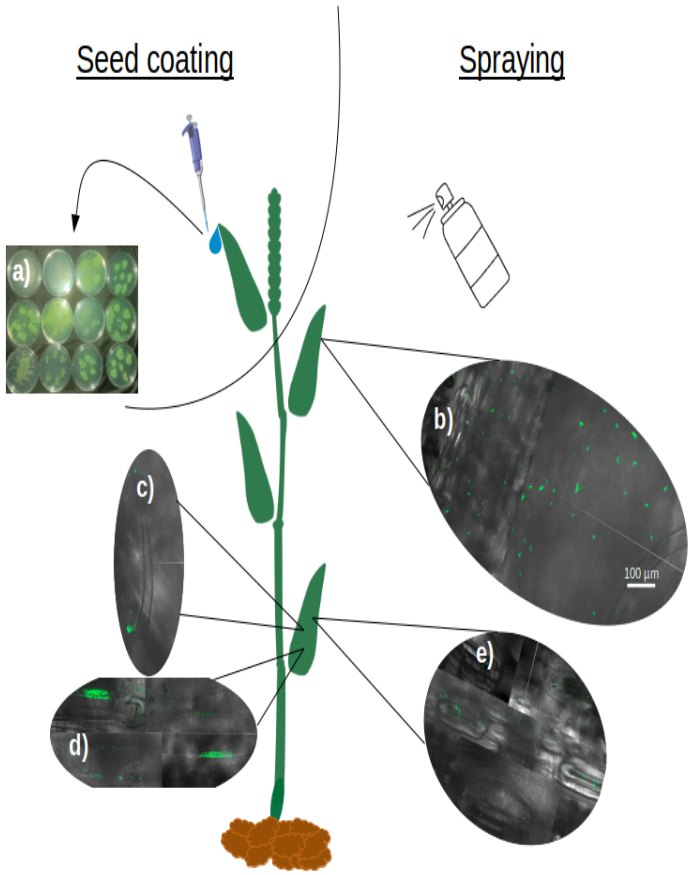

Figure 6-5: Ability of all strains to exit leaves through hydathodes (a), and ability of CF10PS3 to colonize trichomes (c), veins (d) and stomata (e) from initial homogenous distribution of single cells (b). *Images b, c, d and e have been taken with a confocal microscope and its associated laser exiting green fluorescent protein of transformed CF10PS3. All these images has the same scale, represented by the bar on image b and where taken under 630X magnification.*

6.4.5 Plant protection

CF10PS3 also demonstrated the capability to reduce the symptomatic area of *Z. tritici*. In the positive control boxes, where *Z. tritici* was applied alone to evaluate the infection level without protection, leaf area covered by lesion was about 50% (Fig. 6-6a) and mean pycnidia number produced by these lesions on each leaf was 87,3 (Fig. 6-6b). When CF10PS3 was applied just before the pathogen, symptomatic area decreased to about 20% and mean pycnidia number dropped to 31.

This effect was even more noticeable when applied two and three days before infection (dbi), with symptomatic area of 5 and 2%, respectively. Considering these low infection levels, no pycnidia were produced on the few symptomatic tissues.

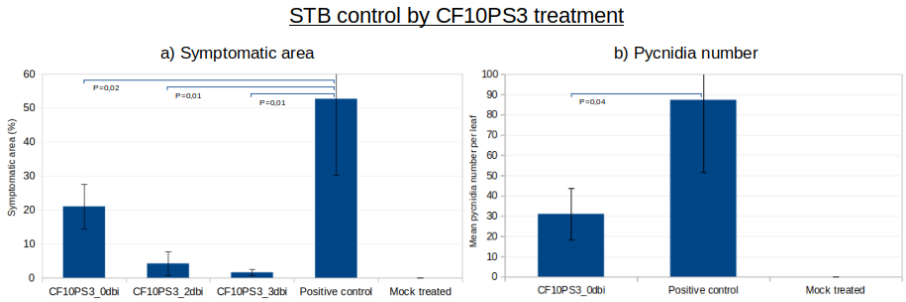

Figure 6-6: *Zymoseptoria tritici* control by CF10PS3 treatment.
a) symptomatic area on leaves in boxes where bacteria was applied 3 days before pathogen inoculation (3dbi), 2 dbi or the same day than Z. tritici conidia (0dbi). b) Mean pycnidia number per leaf produced by the lesions with or without CF10PS3 application. Student test P values are indicated (n=8). Positive control were inoculated with Z. tritici conidia only, whereas mock treatment (negative control) were sprayed with the buffer only.

6.5 Discussion

The *Pseudomonas* genus, known for its significance in association with a variety of plants (Preston, 2004; Poncheewin et al., 2022), and particularly cereals (Mehmood et al., 2023), has once again demonstrated its potential in biocontrol. In this study, from 444 isolates obtained in several cereal fields in Belgium, 33 showed biological activity against at least three of the four wheat pathogens tested (*F. graminearum*, *G. graminis* var. tritici, *O. yallundae*, and *Z. tritici*), underscoring the biocontrol potential of this genus. Initially, Sanger sequencing of 10 taxonomically relevant genes (16S rRNA, *gapA, glnS, gltA, gyrB, ileS, nuoD, recA, rpoB,* and *rpoD*) through a Multi-Locus Sequence Analysis (MLSA) approach allowed for the identification of 11 species. Among these, *P. fluorescens*, *P. lurida*, *P. poae*, *P. salmasensis*, *P. orientalis*, and *P. simiae* are known to colonize wheat, some strains of these species being known as potential biocontrol agents (Kron et al., 2020; Gómez-Lama Cabanás et al., 2022; Ibrahim et al., 2023). In addition, isolates have also been identified as *P. tensinigenes*, known to colonize wheat rhizosphere and produce tensin (Girard et al., 2021), *P. granadensis* already isolated from soil (Pascual et al., 2015), *P. germanica* obtained from *Iris germanica* rhizomes (Atanasov et al., 2022), known soil inhabitant *P. lactis* (Havryliuk et al., 2020), and *P. sivasensis*. The

MLSA reported nine isolates, more than one quarter, as belonging to *P. sivasensis* with high percentages of identity, greater than 97%, except for isolate AR12PS3 for which the best match was still *P. sivasensis*, but with a lower 93% identity. *P. sivasensis* was known to promote the growth of canola (Świątczak et al., 2023b) and was spotted in different environments but was never associated with cereals, to the best of our knowledge. Its high prevalence in the four fields and all plant compartments observed with the samplings encouraged us to investigate further. Whole genome sequencing of these nine strains confirmed only three of the isolates as true *P. sivasensis*, despite initial MLSA findings. This highlights the superior resolution of WGS over MLSA in species identification (Mulet et al., 2023). The remaining isolates were reclassified; one as *P. cyclaminis* (AR12PS3), a species phylogenetically close to *P. marginalis* and *P. grimontii* (Sawada et al., 2021), and another as *P. marginalis* (DR4PS3). The other four could constitute a new species, with the proposed name *Pseudomonas arvensis* sp. nov., with DR1PS3 as the type-strain and strains CR7PS1, DS3PS4, and DS3PS5 as other representatives. This new species, phylogenetically closer to *P. cyclaminis* and close to *P. sivasenis*, *P. marginalis*, and *P. grimontii*, presents an interesting case with Average Nucleotide Identity values calculated with orthoANIu method, – considered as the most accurate method (Yoon et al., 2017) - around 95.80% and digital DNA-DNA Hybridization values between 65 to 66%, just below the ANI and dDDH thresholds commonly used for species delineation (96% and 70%, respectively) (Madhaiyan et al., 2017). The close evolutionary relationships among these species are reflected in their genomic structure and gene composition, with 4,228 of 4,950 gene clusters being common across the genomes studied. Significantly, the conserved regions comprise operons responsible for the production of viscosin, APE-Vf, pseudopyronine, and acyl-homoserine lactone production. Viscosin is a lipopeptidic biosurfactant involved in motility and biofilms, and harboring PGP traits and antifungal properties (Alsohim et al., 2014). APE-Vf is an arylpolyene with antifungal and biocontrol activities in *P. fluorescens NBC275* (Dutta et al., 2020). Pseudopyronine is a pyrone with significant activity against agriculturally important fungi (Nishanth Kumar et al., 2016). The diazeniumdiolate secondary metabolite fragin is a metallophore with reported antifungal activity (Jenul et al., 2018). Acyl-homoserine lactone (AHL) is a quorum-sensing (QS) signaling molecule with a potent effect on pathogenic microbes on leaves (Chaudhry et al., 2021). All *P. sivasensis* strains and *P. arvensis* sp. nov. strain DR1PS3 shared the complete operon for koreenceine production, a polyketide with antifungal effects (Riera et al., 2023). Among the strains, *P. sivasensis* CF10PS3 was isolated from leaf and demonstrated notable *in vitro* antagonistic activity against *Z. tritici*. It was further explored for its plant colonization capabilities. Following seed inoculation, CF10PS3 was demonstrated to move via the xylem, exited leaves through hydathodes, and demonstrated effective colonization of

leaf surfaces. It was shown that the strain will preferentially establish itself around stomata, along the veins, and at the bases of trichomes, considered as preferential sites for microbial colonization as reviewed by Vacher et al. (2016). Translocation and proliferation of *Pseudomonas* in plants have already been highlighted, notably with *P. chlororaphis* (Hernandez-Jerez et al., 2020). These bacteria applied to seeds could indeed migrate from the seed to various plant tissues, including roots and foliage, albeit often at lower concentrations in the foliage. Other studies have also demonstrated that *P. chlororaphis* can internally colonize plant tissues, indicating that seedborne applications can effectively distribute the biocontrol agent within the plant (Mercado-Blanco and Bakker, 2007; Finkel et al., 2017). This internalization suggests that the bacteria not only adhere to external plant surfaces but can also penetrate and establish within, utilizing pathways like root hairs, cracks, and wounds. This movement is crucial for the bacteria's role in biocontrol, enhancing their effectiveness by inhabiting spaces that are critical for pathogen interaction and plant health support. Following spray application on leaves, confocal microscopy revealed initial homogeneous distribution of bacteria, which then aggregated significantly around trichomes, veins, and stomata within two days. Most epiphytes survive on the leaf surface by forming large aggregates which help them to cope with the surrounding milieu and maintain a hydrated surface by the production of extracellular polymeric substances (Morris and Kinkel, 2002; Lindow and Brandl, 2003; Baldotto and Olivares, 2008; Vorholt, 2012). CF10PS3's ability to produce hormones and surfactants, as well as its motility and biofilm formation are likely to be key factors in the colonization success (Nadakuduti et al., 2012; Braga et al., 2016; Ueda et al., 2018; Leveau, 2019; Oso et al., 2019; Streletskii et al., 2019; Flores-Núñez et al., 2020). This strategic colonization presumably facilitates the observed reduction in symptomatic areas of *Z. tritici* infection and the reduction of pycnidia produced by lesions. Preinfection applications were the most effective. *Z. tritici* conidia germinate on the leaf surface, undergo an epiphytic growth phase (Fones et al., 2017), and then invade the plant through the stomata (Battache et al., 2022). Near the stomata, the fungus can encounters CF10PS3, which can exert its antagonistic effects to reduce the number of hyphae that penetrate the stomata and start an infection.

6.6 Conclusions

This study highlighted the significant prevalence of *P. sivasensis* and the newly discovered *P. arvensis* sp. nov. in cereal crops, underscoring their potential roles in agricultural ecosystems. The work confirmed the natural occurrence of *Pseudomonas* species in association with cereals and demonstrated the extensive colonization capabilities of these bac-

teria, particularly *P. sivasensis*. The discovery of *P. arvensis* sp. nov. enriches our understanding of microbial diversity in the cereal phytobiome and its implications for agricultural practices. While the study sheds light on species-specific interactions within the wheat microbiome, it also raises considerations about how environmental factors influence the efficacy and stability of these bacteria. These findings prompt a reevaluation of how we might better leverage microbial interactions for crop health and yield, potentially leading to more sustainable and ecologically friendly agricultural practices. Further research into the genetic and environmental interactions of these *Pseudomonas* strains could provide deeper insights into their roles and management within agricultural systems.

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Chapter 7
Monitoring *Pseudomonas sivasensis* strain CF10PS3 in cereal fields

7.0 Forewords

Building upon the insights gained previously, Chapter 7 transitions to a focused case study on *Pseudomonas sivasensis* strain CF10PS3. This chapter, which will be submitted for publication in MicrobiologyOpen, examines the behaviour of this specific strain in a wheat field. By employing advanced qPCR techniques, the study monitors the dynamics of CF10PS3 in the wheat phyllosphere. The findings provide valuable insights into the strain's environmental behavior and its potential benefits for wheat disease management, thus paving the way for more targeted and effective biocontrol strategies in sustainable agriculture. Several collaborators were associated to the work presented in this chapter; Mathieu Delitte led the conceptualization, formal analysis, and wrote the original draft of the manuscript. Benjamin Dubois contributed to the conceptualization, equally participated in the formal analysis, and supported the writing of the original draft. Jacques Mahillon contributed to the conceptualization, supported the review and editing, and shared responsibility for acquiring funding. Frédéric Debode also contributed to the conceptualization, supported the review and editing, and shared responsibility for acquiring funding. Claude Bragard contributed to the conceptualization, led the review and editing, and shared responsibility for acquiring funding. All authors reviewed and approved the final version of the manuscript.

Monitoring *Pseudomonas sivasensis* strain CF10PS3 in cereal fields.

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Competing interests:
P. sivasensis and related strains are under patenting process and might be used as BCA by the UCLouvain spin-off company BIOCSOL.

7.1 Abstract

The persistence and efficacy of biocontrol agents (BCAs) in agricultural fields are crucial for sustainable crop production. In this study, we investigated the persistence of the introduced bacterial strain *Pseudomonas sivasensis* CF10PS3 in the wheat phyllosphere using a novel qPCR probe protocol. The CF10PS3 strain, known for its *in vitro* biocontrol properties against wheat pathogens, was applied through foliar spray, and its persistence was monitored over seven weeks. Our qPCR assays, designed to specifically detect CF10PS3, distinguished it from naturally occurring *P. sivasensis* strains, providing precise insights into its dynamics in the field.

The experimental results indicated that CF10PS3 was already present on the wheat leaves prior to its application, suggesting its natural adaptation to the foliar environment. Following initial application, a significant increase in CF10PS3 was observed, though subsequent environmental factors such as rain and wind might have caused notable fluctuations in its population. Despite these variations, the introduced strain showed considerable persistence, with population levels significantly higher than those in untreated plots by the end of the study period.

This research underscores the importance of understanding bacte-

ria dynamics in the field, highlighting the influence of environmental conditions on their efficacy. The use of specific qPCR probes proved effective in monitoring introduced strains, offering valuable insights for optimizing BCA application strategies. Our findings contribute to the development of robust biocontrol methods, promoting sustainable agricultural practices and enhancing crop protection.

Keywords: *Pseudomonas sivasensis*, Biocontrol agents (BCAs), Wheat microbiome, Phyllosphere, qPCR monitoring, Plant-microbe interactions, Environmental impact, Sustainable agriculture, Microbial persistence

7.2 Introduction

The use of beneficial microorganisms in agriculture has recently gained significant attention as an eco-friendly and sustainable approach. They allow for enhancing crop productivity and reduce the environmental impact of chemical treatments. *Pseudomonas* spp. strains have emerged as pivotal beneficial microorganisms due to their diverse array of plant growth-promoting and biocontrol capabilities (Mehmood et al., 2023). They have exhibited remarkable potential for increasing crop yields, improving soil health, and protecting plants against phytopathogens (Martínez et al., 2019; Ghadamgahi et al., 2022; Ridene et al., 2023). Despite such promising abilities, to date, no biocontrol agent has consistently demonstrated effective control of wheat foliar diseases, particularly *Zymoseptoria tritici*, the causative agent of Septoria Tritici Blotch.

In the phyllosphere, encompassing the above-ground parts of plants, such as leaves and stems, the survival and colonization of *Pseudomonas* spp. strains are subject to a complex interplay of environmental factors (Chaudhry et al., 2021). High humidity levels often create a more favorable environment by reducing desiccation stress on the leaf surfaces, thus promoting microbial growth (Aung et al., 2018). However, the relationship between humidity and microbial colonization is also influenced by additional factors. Temperature fluctuations, for example, modulate the metabolic activity and growth rates of *Pseudomonas* spp. (Kahl et al., 2022; Bisht, 2023). Rainfall events introduce another dimension, potentially washing away or diluting microbial populations, but also providing moisture essential for survival (Pietrarelli et al., 2006). Moreover, the presence of other microorganisms on leaf surfaces, forming the phytobiome, and the specific characteristics of the plant host further influence the dynamics of microbial communities (Leach et al., 2017; Sivakumar et al., 2020; Zhang et al., 2021b; Bashir et al., 2022). Consequently, the interaction between *Pseudomonas* spp. and the phyl-

losphere is a multifaceted process, where humidity, temperature, rain, and a myriad of ecological factors collectively shape the outcomes of microbial colonization and its potential biocontrol activities against plant pathogens. Understanding this intricate balance is pivotal for harnessing the full potential of *Pseudomonas*-based strategies in sustainable agriculture.

The successful use of *Pseudomonas* spp. in agricultural field trials necessitates the development of robust tools for their detection, quantification and monitoring. Understanding the presence and persistence of these strains in the field is essential for optimizing their application strategies, but also for assessing their long-term impacts on both crop health and the broader ecosystem. Traditional culture-based methods and generic molecular techniques have been valuable in elucidating the presence of *Pseudomonas* populations in soil and plant-associated niches (Elad and Kirshner, 1993; Paul et al., 2017; Pujol et al., 2006). However, they often lack the specificity required to discriminate between closely related *Pseudomonas* strains, limiting their use in strain-specific monitoring.

To address this limitation, we present in this study the design and validation of a strain-specific probe tailored for the precise detection of a *Pseudomonas* strain used in a recent agricultural field trial. The development of such a probe represents a critical step towards elucidating the fate and dynamics of this specific strain in the complex environment of cereal fields.

Here, we describe the rational design of the strain-specific probe, its molecular validation, and its application up to the field trial stage. We also discuss broader implications of our findings for the use of Pseudomonads in sustainable agriculture and the advantages of strain specific monitoring approaches.

The ability to selectively detect and monitor specific *Pseudomonas* sp. in agricultural field trials holds promise for enhancing our understanding of their behavior in natural ecosystems and optimizing their application for sustainable crop production. Furthermore, it opens up new avenues for tailoring biocontrol and plant growth-promoting strategies based on an in-depth understanding of strain-specific interactions with the crop.

7.3 Material and methods

7.3.1 Bacterial strain and formulation

Pseudomonas isolate CF10PS3 was obtained from a flag leaf in a spelt field (*Triticum spelta*) located in Walhain, Belgium. The field was managed under organic agriculture, without tillage. The isolate was identified as *Pseudomonas sivasensis* strain CF10PS3 (Delitte et al., submitted). Briefly, identification was performed through Multi-Locus Sequence Analysis targeting 16S rRNA, *ileS, gapA, glnS, gltA, gyrB, nuoD, recA, rpoB* and *rpoD* genes and later confirmed via whole genome sequencing performed with both Illumina and MinION technologies. *In vitro* assessments revealed the strain's ability to produce siderophores, proteases, and cellulase, as well as its ability to solubilize phosphate, and exhibit swimming and swarming behaviors. It has also shown great ability to colonize wheat leaves both through direct spray applications and through translocation from seed coating. Both *in vitro* and *in planta* studies demonstrated CF10PS3's efficacy against *Z. tritici*, the causal agent of Septoria Tritici Blotch in wheat. According to Delitte et al. (submitted), strain CF10PS3 possesses the genetic determinants for the production of several biocontrol-related metabolites. These include viscosin, fragin, pseudopyronine, the aryl polyene APE-Vf, koreenceine, and siderophore. Additionally, strain CF10PS3 also possesses flagella and type IV pili (Delitte et al., submitted). The strain is available via the LMG collection in Belgium, under collection number LMG S-33705. It can also be obtained through a Material Transfer Agreement (MTA) from the Plant Health Laboratory at the Earth and Life Institute – Applied Microbiology, UCLouvain.

Prior to each field application, CF10PS3 was grown on lysogeny broth (LB) agar plates at 28°C for 24 h (10g/l tryptone, 5g/l yeast extract, 5g/l NaCl). A single colony was selected and inoculated into 20 mL of LB. Following 24 h of growth at 30°C, 180 rpm, the bacterial suspension was used to inoculate 2 L of LB, incubated at 30°C, 180 rpm for 24 h. Following incubation, the bacterial suspension was centrifuged and the bacterial pellets were resuspended in buffer to reach a final concentration of 10^7 CFU/ml. The buffer consisted in 10 mM $MgCl_2$ and 0.1% Tween20. All liquids were sterilized by autoclaving at 121°C, 3 bars for 1 h. Sterile buffer was used as negative control in mock treatment.

7.3.2 Plant treatment and sampling

Winter wheat cultivar Chevignon was sown in an agricultural field in Perwez, Belgium, on 15/10/2022. The sowing followed a potato crop rotation. Trial plots, each measuring 10 by 2 m, were randomly assigned to four replicates per treatment modality. Bacteria suspended in buffer, as well as sterile buffer alone (which will be referred as the mock-treatment), were applied to the corresponding plots on 27/04/23 (BBCH32) to protect the third leaf, on 11/05/23 (BBCH37) to protect the second leaf and on 17/05/23 (BBCH39) to protect the flag leaf. BBCH scale provide a standardized method for describing phenological development (Meier et al., 2009). Leaf sampling was conducted by cutting the base of seven leaves within each plot. The harvested leaves were immediately placed in sterile Falcon tubes and stored on ice until they could be transferred to a laboratory for weighing and freezing at -20°C. Leaf samplings were performed following this schedule: for the second leaf, sampling occurred before and 1 h after treatment, as well as at 1, 4, and 6 days post-treatment. On the 6th day after the second leaf treatment, additional bacterial application was made to protect the first leaf. Consequently, both the first (F1) and second (F2) leaves were sampled at each designated sampling time: before and 1 h after F1 treatment, 2 days after F1 treatment, 5, 7, 14, 22, 29, 36, 43 and 50 days after the F1 treatment. This comprehensive sampling approach allowed a detailed monitoring of *P. sivasensis* strain CF10PS3 dynamics in the wheat field.

7.3.3 Harvest of epiphytic microbial communities and DNA extraction

To recover the diverse epiphytic microbial communities present on the leaves, we adopted the leaf harvest protocol as follow: leaves were placed in sterile 250 mL wide-neck Erlenmeyer flasks, each containing 50 mL of a sterile peptone-phosphate buffer (peptone 10 g/l, NaCl 5 g/L, KH_2PO_4 7,5 g/L and $KH_2PO_4.3H_2O$ 9,5 g/L). Following an incubation period of 30 min at room temperature, the Erlenmeyer flasks were subjected to agitation at 150 rpm for 75 min. This was followed by a 45 min resting period at room temperature. The liquid from each flask was then transferred back into the initial Falcon tubes and centrifuged for 20 min at 6,000 rpm. Subsequently, the supernatant was discarded, while the pelleted microorganisms were stored at -20°C for subsequent analysis.

DNA extraction was performed with DNeasy PowerSoil Pro Kit (Qiagen), following the manufacturer's protocol with one modification: the initial lysis buffer was used to resuspend the pellet. The resuspended

pellet was then placed into the Power-Beads tube, and the remainder of the protocol proceeded without further changes. Extracted DNA was quantified using Qubit4 system (Thermo Fisher Scientific).

7.3.4 Probes and primer design

A single-nucleotide-polymorphism (SNP) was identified in the alignment of the 319 *rrn* sequences (16S-ITS-23S operon) obtained from NCBI complete genomes of wheat-associated *Pseudomonas*, as previously described by Delitte et al. (submitted), and the complete sequenced genome of isolate CF10PS3 (Table S-2). *P. chlororaphis* was also considered, as it is one of the two commercialized *Pseudomonas* strains in Belgium. The other one being of undetermined species (strain DSMZ 13134), it was not considered in this study. To specifically detect the presence of *P. sivasensis* strain CF10PS3 among bacterial communities in leaf washing samples, a set of two primers was designed to amplify the region encompassing the targeted SNP (either a "T" in isolate CF10PS3 or a "G" in all the other sequences) in the *rrn* operon. Complete alignment of the 319 sequences can be found in supplementary Table S-2.

The TaqMan probe sequence was custom-designed by adjusting the size and number/position of Locked Nucleic Acid (LNA) bases to enhance its affinity for the specific target sequence (https://eu.idtdna.com/calc/analyzer) while minimizing stability towards non-target DNA sequences, allowing for effective mismatch discrimination (You et al., 2006). Primers were selected using the Primer3 webtool (http://bioinfo.ut.ee/primer3-0.4.0/) to amplify a region of 100-200 bp surrounding the probe sequence, ensuring optimal compatibility with the designed probes (Table 7-1).

The compatibility of all designed primers to work in a single reaction was evaluated using the multiple primer analyzer webtool (www.thermofisher.com). The specificity of the probe and primer pair was verified on GenBank using primer-Blast and Blastn algorithms (Ye et al., 2012). The combination of T-probe and primers only hits the targeted *P. sivasensis* CF10PS3. The G-probe and primers combination allowed for the detection of all targeted *P. sivasensis* strains (reference strains 2RO45, BsEB1, P7, CCUG57209, W6, and strains BE10PS1, BE10PS3).

Table 7-1: Primers and probes characteristics

Primers and probes characteristics	Sequence (5' > 3')	Tm °C	Amplicon length (bp)
Ps_sivasensis_F	CGTTTGGCTCCACCACTACT	60	145
Ps_sivasensis_R	ACGTTCAGTCTATCTTTCTATCACA	58	
Ps_sivasensis_T	FAM-CATTGT**T**A**T**GATGGTGAA-BHQ1	61.1	
Ps_sivasensis_G	HEX-CATTGT**G**GATGATGGTGAA-BHQ1	61.1	

Underscored bases are Locked Nucleic Acid (LNA) and bases in bold are the SNPs specific for each probe. Melting temperatures (Tm) are indicated in Celsius degrees. FAM and HEX are the fluorophores used with the BHQ-1 quencher.

7.3.5 Cloning of the target DNA and determination of plasmid copy number

To produce amplicons representative of the target sequences, the primer pair was used in a standard PCR reaction with Q5 High Fidelity DNA Polymerase (NEB) and DNA extracted from *P. sivasensis* strain CF10PS3 (for T-SNP) and strain DS3PS5 (for G-SNP). The resulting PCR products were visualized on an agarose gel to ensure specificity, and subsequently purified using a SmartPure PCR kit (Eurogentec). DNA purity and concentration were assessed with a NanoDrop spectrophotometer (Thermofisher Scientific) and a Quantus fluorometer (Promega). To standardize PCR reactions, DNA fragments with either T or G SNP were ligated into *pK18mobsacB* plasmid following the Gibson Assembly Cloning Kit instructions (NEB) and introduced in competent *E. coli* using the heat shock transformation protocol. Briefly, 10 μl of Gibson Assembly products were added to 200 μl of competent cells. The mixture was kept on ice for 15 min before going to a 42°C bath for 42 sec and getting back on ice for 2 min. The mixture was supplemented with 950 μl of LB medium and incubated at 37°C, 150 rpm, for 1h. Bacteria were concentrated by centrifugation (5,000 g, 10 min) and spread on LBA plates containing kanamycin, X-gal, and IPTG. Plasmid DNA isolated from bacterial cultures was extracted with Qiaprep Spin Miniprep Kit (Qiagen) and adjusted to 10^8 copies with Thermofisher DNA Copy Number and Dilution Calculator with a molar mass per base pair set at 650 Da. The limit of detection, robustness, and efficiency were determined on diluted plasmid DNA.

7.3.6 Limit of detection

The absolute Limit of Detection (LOD) for our PCR assay, which includes primers, both probes, and amplification program, was determined using serial dilutions of plasmid DNA. For these dilutions, we aimed target copy numbers of 50, 20, 10, 5, 2, 1, and 0.1. We performed six PCR reactions for every dilution level. The LOD_6, defined as the lowest number of copies at which all six PCR tests yield positive results, was identified under the condition that the highest dilution, supposedly containing 0.1 copy per reaction, resulted in no more than one positive PCR out of six attempts (Association Française de Normalisation (AFNOR), 2003). We subjected the copy number at the LOD_6 to 60 additional tests within the same run to determine the LOD_{95}. The LOD_{95} criterion is met when at least 95% of these 60 tests produce positive results. The maximum acceptable copy number for both LOD_6 and LOD_{95} were set at 20 copies.

7.3.7 Dynamic linear range and efficiency

The dynamic range of our PCR assay was evaluated over eight orders of magnitude ranging from 10^8 to 10^1 copies. For each dilution, we conducted the assay in six replicates. We analyzed linearity by plotting the cycle threshold (Ct) values against the logarithmic scale of target concentrations (Stolovitzky and Cecchi, 1996). Dilutions from 10^8 to 10^1 copies in six replicates were also used in two plates, as well as in duplicates in eight other plates, to calculate PCR efficiency. To be considered as acceptable, efficiency has to be between 90 and 110% (Broeders et al., 2014).

7.3.8 Transferability and robustness

To assess the transferability of the method, LOD_6, LOD_{95}, linear range and efficiency were tested following the same protocols in another independent laboratory at the Walloon Agricultural Research Centre (CRA-W), located in Gembloux, Belgium. The robustness of our method was evaluated by implementing variations to the standard experimental procedures (Commission, 2010). The parameters we adjusted included the primer concentrations (either standard or reduced by 30%), probe concentrations (standard or reduced by 30%), and the volume of the real-time PCR Master Mix (standard or altered by $\pm$ 1 μL), resulting in a total reaction volume of 20 $\pm$ 1 μL. We conducted six replicates containing 20 copies of each plasmid targets, under the modified conditions detailed in Table 7-2. The key criterion for acceptance was that all al-

Table 7-2: Experimental conditions tested to evaluate robustness of the method.

PCR machine	CFX96 thermocycler (Bio-Rad)				
PCR reagent kit	Takyon No Rox Probe 2X MasterMix dTTP (Eurogentec)				
Primers concentration	Standard	Minus 30%	Standard	Standard	Standard
Probes concentration	Standard	Standard	Minus 30%	Standard	Standard
PCR volume	Standard (18μl mix + 2μl DNA)	Standard (18μl mix + 2μl DNA)	Standard (18μl mix + 2μl DNA)	Standard + 1μl (19μl mix + 2μl DNA)	Standard - 1μl (17μl mix + 2μl DNA)

Variations to the standard protocol tested in the robustness evaluation. Standard protocol is in the second column. Primers (-30%) and probes (-30%) concentration limitations were tested, as well as the reaction volume (plus or minus 1 μl of Master Mix).

terations to the standard protocol should consistently yield a positive result at a target level of 20 copies per reaction, as per the guidelines defined by Broeders et al. (2014).

7.3.9 Specificity

Specificity of the method was checked on 34 organisms from different taxonomic bacterial genera including 20 *Pseudomonas* spp. isolates. The possibility of cross-reaction with human DNA was considered and host wheat DNA was also checked. Briefly, human DNA was extracted from saliva and wheat DNA from a young leaf obtained from disinfected seed grown in sterile condition and surface-disinfected before extraction. Both DNA were extracted with DNeasy PowerSoil Pro Kit (Qiagen), following the manufacturer's recommendations and each DNA extract was tested at least in duplicates. Two nanograms of DNA were used in the PCRs. The tested organisms are outlined in Table 7-3. With the exception of *P. aeruginosa* and *P. paraeruginosa* which were obtained from humans, all other organisms were isolated from agricultural

environments, primarily associated with wheat, and all DNA was also extracted with PowerSoil DNA Extraction Kit (MO BIO Laboratories, USA), following the manufacturer's recommendations.

Table 7-3: List of organisms used to evaluate qPCR specificity.

Genus	Species	Strain	Origin	Name*
Bacillus	*subtilis*	BF12B1	Wheat leaf	UPB 1356
Brevundimonas	*alba*	18360	Soil	LMG 18360
Burkholderia	*anthina*	22950	Maize rhizosphere	LMG 22950
Enterobacter	*kobei*	BC_B1	Field soil	UPB 1352
Flavobacterium	*johnsoniae*	LMG1341	Soil	LMG 1341
Glutamicibacter	*creatinolyticus*	BC_F7	Field soil	UPB 1350
Homo	*sapiens*	/	/	/
Methylobacterium	*bullatum*	24788	Moss phyllosphere	LMG 24788
Microbacterium	*oxydans*	E109	Field soil	UPB 460
Pantoea	*agglomerans*	Pa19	Wheat head	UPB 1357
Pedobacter	*foliorum*	31463	Field soil	LMG 31463
Pseudomonas	*aeruginosa*	Pa01	Wound	LMG 1242
Pseudomonas	*cichorii*	P14	Field soil	UPB 461
Pseudomonas	*cyclaminis*	AR12PS3	Wheat root	UPB 1358
Pseudomonas	*fuscovaginae*	CB51	Wheat	UPB 526
Pseudomonas	*lurida*	BF10PS1	Wheat leaf	UPB 1359
Pseudomonas	*lurida*	BF8PS1	Wheat leaf	UPB 1355
Pseudomonas	*marginalis*	DR4PS3	Wheat root	UPB 1360
Pseudomonas	*arvensis* sp. nov.	CR7PS1	Wheat root	UPB 1361
Pseudomonas	*arvensis* sp. nov.	DR1PS3	Wheat root	UPB 1362
Pseudomonas	*arvensis* sp. nov.	DS3PS4	Field soil	UPB 1363
Pseudomonas	*arvensis* sp. nov.	DS3PS5	Field soil	UPB 1364
Pseudomonas	*orientalis*	CF11PS1	Wheat leaf	UPB 1365
Pseudomonas	*paraeruginosa*	8029	Infected ear	LMG 8029
Pseudomonas	*protegens*	44RP8	Field soil	UPB 1366
Pseudomonas	*sivasensis*	BE10PS1	Wheat head	UPB 1367
Pseudomonas	*sivasensis*	BE10PS3	Wheat head	UPB 1368
Pseudomonas	*sivasensis*	CF10PS3	Wheat leaf	UPB 1354
Pseudomonas	*syringae* pv. *aptata*	5143	*Beta vulgaris*	LMG 5143
Pseudomonas	*syringae* pv. *atrofaciens*	P64	Wheat	UPB 463
Pseudomonas	*viridiflora*	2352	*Phaseolus* sp.	LMG 2352
Sphingobacterium	*thalpophilum*	BC_B3	Field soil	UPB 1353
Sphingomonas	*albertensis*	32139	Wheat leaf	LMG 32139
Staphylococcus	*equorum*	BC_G2	Field soil	UPB 1351
Triticum	*aestivum*	/	/	/
Xanthomonas	*translucens* pv. *undulosa*	CB4	Wheat	UPB 513

**** The strains were obtained from either the BBCM/LMG collection in Ghent, Belgium, or from the UPB collection at the Plant Health lab of UCLouvain, Belgium.***

7.3.10 qPCR assay for bacteria quantification

The mixture for qPCR assay was prepared with Takyon No ROX Probe 2X MasterMix dTTP (Eurogentec), 300 and 200 nM of each primer and probe, respectively, and 2 μl of DNA template in a total volume of 20 μl. The amplification reaction was performed on a CFX96 thermocycler (Bio-Rad). The qPCR cycling protocol included an activation step at 95°C for 3 min, followed by 40 cycles of denaturation at 95°C for 10 sec and annealing/extension at 60°C for 45 sec, with fluorescence intensity measured at the end of each cycle. Each sample was run in two technical replicates. Eight tenfold serial dilutions of each plasmid (ranging from 10^8 to 10^1 DNA copies) were prepared and used as templates for each qPCR run. These dilutions were utilized to generate calibration curves, correlating known starting quantities with their corresponding Ct (Cycle threshold) values. These calibration curves were instrumental in achieving absolute quantification of the copies from both the T and G probes in the samples by plotting the Ct values against the established calibration curve.

7.3.11 qPCR assay for wheat DNA quantification

To assess the presence of plant DNA contaminants in DNA extracts, we conducted qPCR assays targeting the wheat ARF and DUF52 housekeeping genes. These genes were chosen due to their demonstrated stability in terms of expression within wheat genomes, as previously established by Dubois et al. (2017). Briefly, the qPCR reactions employing TaqMan probes were prepared using 10 μl of Takyon No ROX Probe 2x Mastermix dTTP Blue (Eurogentec), with a concentration of 300 nM for each primer and 100 nM for the TaqMan probe labeled with the FAM fluorophore and TAMRA quencher (Eurofins Genomics). Additionally, 2 μl of the extracted DNA and nuclease-free water (Thermo Scientific) were added to reach a total reaction volume of 20 μl. The prepared samples were loaded into a Hard-Shell 96-Well PCR skirted white plate and securely sealed using a Microseal 'B' PCR Plate Sealing Film (Bio-Rad). Subsequent PCR amplifications were conducted on the C1000 TouchTM thermal cycler, coupled with the CFX96TM Real-Time detection system (Bio-Rad). The thermal cycling program involved an initial denaturation step at 95 °C for 3 min, followed by 40 cycles of denaturation at 95 °C for 10 sec and annealing/extension at 69 °C for 1 min. Each sample was subjected to two technical replicates to ensure precision and reliability. For the quantification of relative wheat DNA content in each sample, we used the $2^{\Delta Ct}$ method, where ΔCt represents the difference between the Ct values for the wheat (Ct_{wheat}) and the sample (Ct_{sample}). DNA extracted from thoroughly disinfected leaves served as a reference for "pure" wheat DNA content. This method

allowed us to accurately estimate the proportion of wheat DNA in the samples.

7.3.12 Data analysis

All data analyses were performed using R Studio (version 2022.07.1 Build 554). The initial steps were to set the limit of detection to the one calculated in previous steps and normalizing DNA concentrations. To achieve this, the qPCR data for each wheat gene (ARF and DUF52) were processed separately. The mean Ct values of technical replicates were computed for each sample, as well as for the "pure" wheat DNA extract. ΔCt values were calculated by subtracting the mean Ct value of wheat from each Ct value of the samples. The relative abundance of wheat DNA in the samples was then calculated using the $2^{\Delta Ct}$ method. The final relative abundance (Relab) of wheat was determined by averaging the relative abundance values obtained from each gene for each sample. DNA concentration (DNA_cc) in each sample was subsequently normalized (nDNA) as follows: nDNA = (1 - Relab) * DNA_cc. Data obtained from qPCR assays targeting the FAM and HEX probes, which detect copies from CF10PS3 strain or the natural population, respectively, were utilized as "Starting Quantities", divided by the mass of the sample to express data by gram of tissue. All data beyond the LOD (see 2.6.) were discarded. The means of both values for technical replicates were calculated for each fluorophore and each sample, and normalization was carried out, taking into account the nDNA values calculated earlier. Additionally, the fluorophore copies for each treatment were averaged across the four field replicates, and their standard error values were calculated. Logarithms of these data were calculated to facilitate their representation in graphical form. Normality of the data was assessed by the Shapiro-Wilk normality test with p-values $< 0{,}05$. For statistical analysis, a mixed linear model was built for each foliar stage to take into account the dynamic of samplings. Hourly meteorological data used in the analysis were sourced from the CRA-W Gembloux meteorological station, 10 km away from the field.

7.4 Results

7.4.1 Strain characterization

P. sivasensis is found as a common bacterial species associated with small grain cereals (spelt, wheat) and plays a role as a key species within the small grain cereals phytobiome at field level (Delitte et al., submit-

ted). *P. sivasensis* strain CF10PS3 was isolated from a flag leaf in a spelt field in Walhain, Belgium, managed under organic agriculture. This strain was selected for its potential biocontrol-related metabolites production, its colonization abilities on wheat leaves and its potential control of Septoria Tritici Blotch. Interestingly, based on an alignment of 319 sequences *Pseudomonas* spp. strains (Table S-2), CF10PS3 can be distinguished from the other ones usually found in wheat fields, as it has a "T" SNP in the 16S-ITS-23S operon that allowed us to design a qPCR experiment to specifically detect it. Others sequences from *P. sivasensis* and closely related species (*P. arvensis* sp. nov. and *P. cyclaminis*) have a "G" SNP and are detected with the primers and G-probe combination. All others sequences were theoretically verified *in silico* and were neither amplified by the primers nor detected by the probes, excepted for 11 sequences among the 319, belonging to *P. fluorescens* and *P. poae* species and representing a low-level percentage of off-target detection (<3,5%) (Table S-2).

7.4.2 Method performances

We assessed the performance of the designed experiment across various parameters to ensure accuracy and reliability. This includes evaluating the specificity, limit of detection, linearity, efficiency and robustness, ensuring trustworthy results for the intended application.

The specificity of the method was checked on 34 organisms related to our case study. Human DNA was checked for cross contamination. Wheat DNA was verified for its lack of influence on the experiment. Probe labeled with FAM fluorophore only gave positive results for *P. sivasensis* CF10PS3, confirming the "T" SNP is only present in that strain (Table 7-4, FAM column). The other probe labeled with the HEX fluorophore and designed to detect "G" SNP effectively gave positive response for all other *P. sivasensis sensu lato*, confirming its relevance to evaluate the natural population of that group (Table 7-4, HEX column).

Table 7-4: Specificity of the method.

Target	Results FAM	Results HEX
P. sivasensis CF10PS3	+ (m=15,74+/_0,17)	-
P. sivasensis BE10PS1	-	+ (m=17,92+/_0,16)
P. sivasensis BE10PS3	-	+ (m=18,78+/_0,48)
P. cyclaminis AR12PS3	-	+ (m=17,84+/_0,15)
P. arvensis sp. nov. CR7PS1	-	+ (m=18,58+/_0,03)
P. arvensis sp. nov. DR1PS3	-	+ (m=19,02+/_0,11)
P. arvensis sp. nov. DS3PS4	-	+ (m=19,64+/_1,28)
P. arvensis sp. nov. DS3PS5	-	+ (m=19,31+/_0,05)
Bacillus subtilis BF12B1	-	-
Brevundimonas alba LMG 18360	-	-
Burkholderia anthina LMG 22950	-	-
Enterobacter tabaci BC_B1	-	-
Flavobacterium johnsoniae LMG1341	-	-
Glutamicibacter creatinolyticus BC_F7	-	-
Methylobacterium bullatum 24788	-	-
Microbacterium oxydans E109	-	-
P. aeruginosae Pa01	-	-
P. cichorii P14	-	-
P. fuscovaginae CB51	-	-
P. lurida BF10PS1	-	-
P. lurida BF8PS1	-	-
P. marginalis DR4PS3	-	-
P. orientalis CF11PS1	-	-
P. paraeruginosae 8029	-	-
P. protegens 44RP8	-	-
P. syringae pv. *aptata* 5143	-	-
P. syringae pv. *atrofaciens* P64	-	-
P. viridiflora 2352	-	-
Pantoea agglomerans 19	-	-
Pedobacter foliorum 31463	-	-
Sphingobacterium thalpophilum BC_B3	-	-
Sphingomonas albertensis 32139	-	-
Staphylococcus equorum BC_G2	-	-
Xanthomonas translucens pv. *undulosa* CB4	-	-
Human DNA	-	-
Wheat DNA	-	-

+ = Positive signal, - = No signal

For positive samples, mean Cq values with their standard deviations are given in brackets

This experimental setup confirmed *in silico* results where the combination of primers and probes allowed specific detection of either CF10PS3 or the other *P. sivasensis s.l.*.

The limit of detection was evaluated following two complementary approaches. The LOD_6 of the method was estimated at 20 copies for each target since this quantity was detected in all six replicates and the highest dilution, supposedly containing 0.1 copy per reaction, resulted in no more than one positive PCR out of six attempts, as required in Broeders et al. (2014). For LOD_{95}, at least 57 out of 60 attempts yielded positive results with both probes on targets plasmid DNA.

Linearity was observed over a range from 10^2 to 10^8 copies per reaction (Fig. 7-1). The coefficient of determination (R^2) was calculated as >0.99 for both probes. At 10 copies, only three of the six replicates were positive for each probe, since this number is under the LOD, and therefore was not considered for linearity evaluation.

For each plate, efficiency ranged between 90% and 110%, meeting the the criteria proposed by Broeders et al. (2014). The PCR method robustness was successfully evaluated on plasmid DNA. All tests with deviations to the standard protocol delivered positive results with 20 template copies with only slight deviations to the expected Cq value (Table 7-5), aligning with requirements described in Broeders et al. (2014).

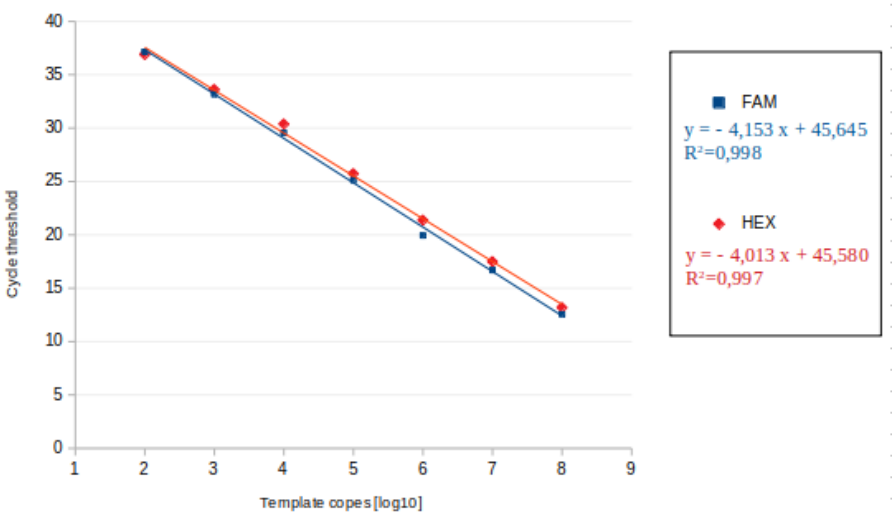

Figure 7-1: Linearity of the designed qPCR.

This was analyzed with serial dilutions from 10^8 to 10^2 copies of both probes (labelled with FAM fluorophore in blue or HEX fluorophore in red). Points represent the mean values of six replicates. Lines represent linear fits of the data with their associated equations of calibration function and correlation coefficients.

Table 7-5: Robustness of the qPCR method.

Deviations from standard protocol	FAM fluorophore			HEX fluorophore		
	Expected Cq	Observed Cq	Coefficient of variation (%)	Expected Cq	Observed Cq	Coefficient of variation (%)
-30% primers	35.19	34.5	2.05	36.47	35.1	3.68
-30% probes	35.19	35.9	-2.11	36.47	36.5	-0.19
-1% MasterMix	35.19	35.4	-0.48	36.47	37.1	-1.68
+1% MasterMix	35.19	35.3	-0.29	36.47	37.3	-2.17

7.4.3 Persistence of introduced *P. sivasensis* in wheat phyllosphere

The newly developed qPCR protocol was then used to investigate the persistence of *P. sivasensis* CF10PS3 in the wheat phyllosphere over key wheat growth stages following its application via foliar spray. A mock treatment consisting of the sterile buffer alone was also evaluated for comparison.

Initial situation

In this field experiment, natural populations of *P. sivasensis s.l.* were comparable across all plots and on F1 and F2, with no statistical difference (Fig. 7-2). The strain of interest was already present on the leaves prior to the initial treatment targeting F2 leaves on May 11 (foliar stage BBCH37). Indeed, a slight level of detection (4 copies per gram of leaf) of CF10PS3 was observed in plots where the bacterium has not been applied yet (Fig. 7-3b, green triangle). This could be expected, considering that the strain was isolated from a flag leaf, indicating its natural presence and adaptation to the foliar cereal leaf environment. Higher levels of detection, with about 10 copies per gram (Fig. 7-3b, blue triangles), were observed in plots where a previous treatment targeting F3 had been applied at BBCH32, 15 days before the first sampling of second leaves. At that time, the developing F2 leaves were probably impacted, with bacteria in the spray application likely landing on emerging F2. With very few exceptions, CF10PS3 can be detected around 100 copies in plots where it was not applied.

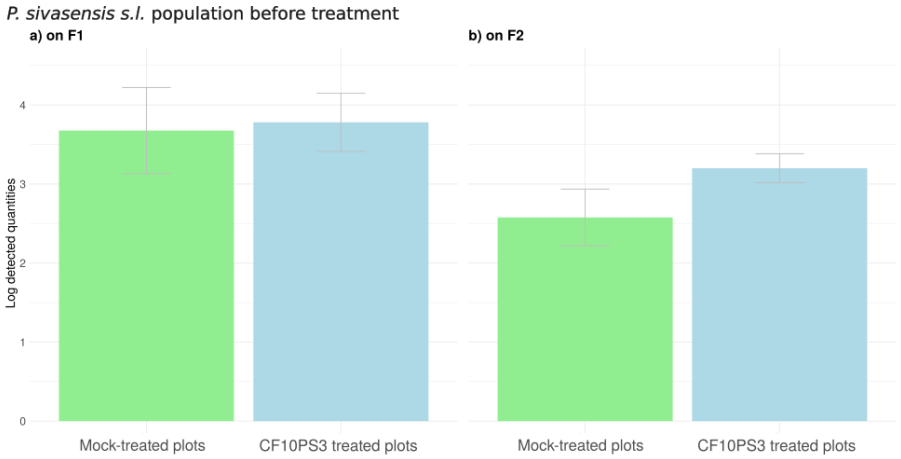

Figure 7-2: Natural population levels before treatments on F1 (a) and F2 (b).

Second leaf bacterial population dynamic

Following the first treatment targeting F2 on May 11 (BBCH37), a significant increase in CF10PS3 presence on treated leaves was noted, with a 100-fold increase (Fig. 7-3b, blue triangles). A slight 10-fold increase was also observed in untreated plots (green triangles), likely due to the wind drift during windy conditions at that moment of the day (Supplementary Fig. S-3).

Subsequent sampling conducted the day after treatment revealed a substantial decrease in bacterial detection across all plots. A 13-mm rain event, occurring 3 hours after treatment (Supplementary Fig. S-3), likely contributed to this decrease by washing off both applied and natural CF10PS3 populations. Additional rain episodes in the following days may further explained the continuous decrease in all plots, indicating potential wash off and dilution of bacterial populations. Bacterial levels were maintained until a new application on May 17 (BBCH39), aiming to protect F1. This treatment resulted in a 10-fold increase of CF10PS3 in plots treated with bacteria (Fig. 7-3b, blue triangle), since underlying F2 leaves were also impacted by the treatment targeting F1 leaves. Mock-treated plots were unaffected, and surprisingly, natural populations decreased by 10 times (blue crosses). In the following two days (May 18 and 19), CF10PS3 populations dropped by a tenfold factor, while natural populations remained stable. Windy conditions with lower humidity might have increased desiccation for the bacteria (Supplementary Fig. S-3). Over the next three days (until May 22), wind persisted, but with milder temperatures and higher humidity, potentially allowing bacteria to maintain their levels. Throughout the sub-

sequent five weeks, all populations followed similar increasing trends, with natural populations consistently and statistically close in number. At the end of the sampling campaign, detected amounts of CF10PS3 in plots treated with bacteria were 20 times higher than in mock-treated plots, reaching from 50 copies to nearly 1,000.

First leaf bacterial population dynamics

On F1 leaves (Fig. 7-3a), initial amounts of CF10PS3 were notably high, around 1,000 copies per gram on May 17 (BBCH39). Unsurprisingly, there was a tenfold difference between treated and untreated plots. Natural populations remained significantly consistent across all plots after bacterial application. A significant drop in all bacterial populations was observed in all plots until May 22. In the following five weeks, all curves followed a similar pattern, also observed on F2 leaves. At the end of the sampling campaign, natural *P. sivasensis s.l.* populations reached approximately 5,000 copies, whereas plots where CF10PS3 was applied or the untreated plots showed 1,000 and 100 copies per gram, respectively. Copies of CF10PS3 were consistently higher throughout the season when applied in the field compared with mock-treated plots.

Seasonal dynamics

The natural population levels of *P. sivasensis* and closely related species fluctuated between 500 and 5,000 copies per gram during the sampling campaign, conducted from the foliar stage BBCH37 to the senescence of F1 and F2 leaves (BBCH85 and 81, respectively). When CF10PS3 was applied, there was a notable increase in copies throughout the season, with fluctuations ranging between 10 (F2) or 50 (F1) and 5,000. Ultimately, the number of CF10PS3 copies was significantly higher in plots where the bacteria were applied compared to mock-treated plots. It is evident that foliar application can influence the bacterial population of a single species throughout the entire season.

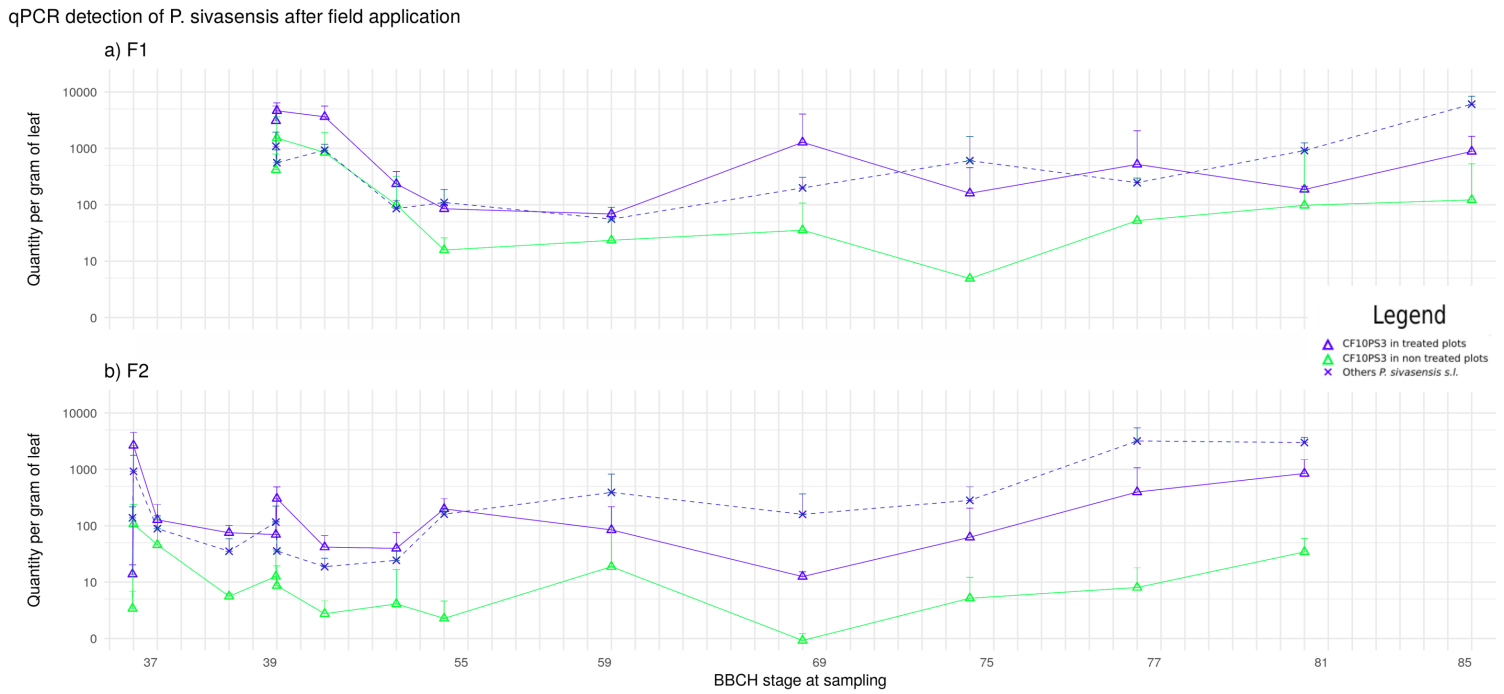

Figure 7-3: qPCR detection of *P. sivasensis* natural population and strain CF10PS3 on F1 (a) and F2 (b). *Points represent the mean qPCR copies from P. sivasensis CF10PS3 (triangles) or the natural population of P. sivasensis sensu lato (crosses), encompassing closely related species. Data are shown for plots (n=4) that were either mock-treated with buffer (green) or treated with CF10PS3 (blue). Error bars indicate standard deviation (SD). For the natural population analysis, data from bacteria-treated and mock-treated plots showed no significant statistical difference and were therefore combined. Each square of the grid represents one day, with the corresponding BBCH stage at sampling indicated on the X-axis.*

7.5 Discussion

P. sivasensis CF10PS3, a common bacterial species in small grain cereals, was isolated from a spelt field in Walhain, Belgium, under organic management. It produces siderophores, proteases, cellulases and biocontrol-related metabolites, and can antagonize wheat pathogens, particularly *Z. tritici* (Delitte et al., submitted). CF10PS3 is distinct from other *P. sivasensis* found in wheat fields, as it has a SNP in the 16S-ITS-23S operon that allowed us to design a qPCR experiment to specifically detect it.

The durability of a plant protection control method is crucial for its long-term efficacy, defined as its persistence in space and time (Bardin et al., 2015). However, various biocontrol agents (BCAs) have shown inconsistent efficacy under commercial field conditions, proving less effective or even ineffective compared to controlled conditions (Bonaterra et al., 2022). Traditionally, the persistence of introduced bacteria was assessed using series dilution and plating on selective agar media, which did not allow discrimination between introduced strains and related natural populations (Elad and Kirshner, 1993; Paul et al., 2017; Pujol et al., 2006). In this study, we developed qPCR probes to distinguish the introduced *P. sivasensis* strain CF10PS3 from naturally present *P. sivasensis* s.l. in the wheat phyllosphere. This tool was useful to investigate the persistence of the introduced strain over seven weeks post-application. The results also indicated the absence of noticeable effect of the CF10PS3 introduction on wild *P. sivasensis* naturally present on leaves. Similar experiments had already been conducted on strawberry, following the colonization of *Methylobacterium extorquens* and its impact on fruit flavor (Verginer et al., 2010), and on potato and black pepper to examine the endophytic colonization of *P. putida* strain P9 (Andreote et al., 2009) and BP25 (Agisha et al., 2017), respectively. Other works have developed strain-specific qPCR for soil and root-associated microorganisms (Mendis et al., 2018).

Our field study suggests that environmental factors, particularly rainfall and humidity, may play a significant role in shaping the dynamics of bacterial populations, including *P. sivasensis* strain CF10PS3. While the strain has demonstrated an ability to persist in the wheat phyllosphere, fluctuations observed post-treatment point to the possibility that environmental conditions could impact both introduced and naturally occurring bacterial populations. This raises the importance of carefully considering the timing of bacterial treatments to enhance colonization and efficacy. The decreases in CF10PS3 populations observed after treatment could plausibly be associated with weather events, such as rainfall, which may have contributed to bacterial wash-off. Such patterns hint at the sensitivity of bacterial persistence to external factors,

although further studies would be required to definitively link these changes to specific meteorological conditions. The potential for population recovery following these environmental challenges suggests a certain resilience in CF10PS3, a characteristic that may be beneficial for its continued development as a biocontrol agent. Additionally, our use of strain-specific probes allowed precise tracking of the introduced strain, distinguishing it from other *P. sivasensis* strains naturally present in the field. This level of specificity is vital for understanding the persistence and behavior of the strain in a complex field environment. Despite the variability in population levels, plots treated with CF10PS3 generally maintained higher bacterial counts compared to control plots. This observation indicates that targeted bacterial applications can positively influence foliar bacterial populations, even under fluctuating environmental conditions. Investigating methods to enhance the strain's environmental stability, perhaps through formulation or application timing adjustments, could further improve its potential in sustainable crop management.

The primary drawback for qPCR detection lies in the presence of non-degraded DNA in samples, with the rate of DNA degradation post-cell death being highly dependent on environmental conditions (Nielsen et al., 2007). While research has explored DNA persistence in soil, varying results have been reported, ranging from rapid degradation in some studies (Nielsen et al., 2000) to a prolonged persistence in others (England et al., 1997). Assessing DNA persistence in the phyllosphere requires further investigation. Nevertheless, our field trial results suggest that long-term DNA stability is unlikely in the phyllosphere. Two and five days after F1 field inoculation, all treated plots exhibited a 1- and 2-log decrease, respectively, in CF10PS3 population levels, indicating rapid population decline. In this case, the rain wash-off hypothesis can be excluded since no precipitation was recorded during these days.

Monitoring the presence and persistence of BCAs allows for the evaluation of their effectiveness in controlling plant pathogens under field conditions and their impact on native epiphytic communities. Understanding how BCAs react to environmental conditions enables researchers to optimize application strategies, including timing, reapplication, dosages, and formulations for more effective pest control. Assessing the interaction between different co-formulants and BCAs will help determine their influence on efficacy, stability, and persistence. This information is crucial for optimizing formulation combinations and identifying synergistic effects that enhance overall performance. Understanding the impact of co-formulants will contribute to the development of commercially viable biocontrol products.

The inconsistent efficacy observed in field settings, despite promising results in controlled conditions, underscores the need for a com-

prehensive understanding of BCA persistence. Traditionally, assessing BCA persistence relied on techniques unsuitable for discriminating between introduced strains and natural populations. Our innovative use of qPCR probes allowed us to overcome this limitation, specifically distinguishing the introduced *P. sivasensis* strain CF10PS3 from its naturally occurring counterpart in the wheat phyllosphere. This technological advancement enabled a detailed investigation of the persistence of the introduced BCA candidate for seven weeks. Comparing our detected levels with those reported in the literature is challenging due to the limited available data. However, Pujol et al. (2006) also observed a decline in populations of *Pseudomonas* spp. after application, with stabilization at approximately 5000 CFU per gram, which aligns with our findings of population stabilization at around 1000 copies per gram. Similarly, Paul et al. (2017) reported a reduction in *Bacillus thuringiensis* populations from 10^{12} to 10^4 within six days post-application, indicating that a decline post-inoculation is typical for bacterial biocontrol agents, yet sufficient numbers can remain to confer beneficial effects. As for the comparison between F1 and F2 inoculation, we observed that the bacterial population level on F1 leaves before application was already higher than on F2 leaves. This is in line with the origin of the strain, which was isolated from a flag leaf (F1), suggesting its natural adaptation to this part of the plant. On F2 leaves, after the initial introduction of the bacteria, the strain quickly established itself, occupying approximately 50% of the *P. sivasensis* population, as both signals were at the same levels. At growth stage 59, the strain still accounted for around 10% of the population, and this proportion was maintained until the last sampling. On the flag leaves (F1), the introduced strain was the dominant population for the first three weeks post-application, after which it stabilized at around 10% of the total population. These levels are promising, as they suggest that the strain is well-adapted to the leaf environment and can persist at levels that are hopefully sufficient to control diseases. However, the bacterial load required for effective disease control is a crucial factor that must be thoroughly investigated. Future studies should focus on determining the optimal bacterial concentrations needed for disease suppression in similar environments. This could involve testing varying application concentrations to better understand the relationship between bacterial load and disease control efficacy. Such studies are essential to substantiate any strain's potential as a biocontrol agent and to optimize its practical application in the field. Given the progression dynamics of Septoria tritici blotch (STB), which spreads from the lower leaves to the upper leaves via rain splash, it is crucial that the introduced strain is able to colonize all foliar levels. This is particularly important for protecting the flag leaves (F1), which contribute the most to wheat yield, as well as the second leaves (F2) to a lesser extent. Ensuring effective colonization of both leaves could provide critical protection against STB, maintaining healthy foliage and preserving crop yield potential.

The phyllosphere's dynamic nature, influenced by factors such as moisture, temperature fluctuations, UV radiation, and nutrient availability, presents a challenging environment for microorganisms. Despite these hurdles, our field experiment, targeting F1 and F2 leaves with a strain applied in a buffer, demonstrated a significant increase in *P. sivasensis* presence throughout the season. Transitory decreases, attributed to rain events and environmental factors, underscored the dynamic nature of bacterial populations. Despite fluctuations, the introduced bacteria exhibited persistence, hinting for its potential as a protective agent. The CF10PS3 strain was shown to be able to colonize foliar parts of the plant following seed coating (Delitte et al., submitted). Here, it was demonstrated that it can also directly colonize leaves after foliar application, suggesting a potential for biocontrol application.

Our research highlights the importance of tracking BCAs on leaves. This capability will help optimizing application strategies and allows for the study of co-formulants, improving biocontrol methods. Overall, this research increases our understanding of BCA behavior in complex field environments, leading to more effective and adaptable plant protection strategies.

7.6 Conclusion

Our study underscores the potential of the *P. sivasensis* CF10PS3 strain as a biocontrol agent in wheat fields, particularly against the pathogen *Z. tritici*. The development of qPCR probes enabled precise monitoring of CF10PS3, distinguishing it from naturally occurring *P. sivasensis*, and provided insights into its persistence and dynamics in the field. The results demonstrated that despite environmental challenges such as rainfall and temperature fluctuations, the CF10PS3 strain maintained a higher presence in treated plots compared to controls, suggesting its potential for effective foliar application.

The ability of CF10PS3 to persist and adapt to the phyllosphere environment, both as a seed coating and a foliar application, highlights its versatility as a biocontrol agent. This study also emphasizes the need for optimizing application strategies, including timing and formulations, to enhance the efficacy and stability of BCAs under variable field conditions. Understanding the interactions between BCAs and environmental factors, as well as co-formulants, is crucial for developing commercially viable and effective biocontrol products.

Overall, our research contributes to a deeper understanding of BCA behavior in field environments, advocating for the implementation of targeted bacterial applications in plant protection. This knowledge will

aid in improving biocontrol methods, ultimately leading to more sustainable and resilient agricultural practices.

7.7 Acknowledgements

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Chapter 8
Impact of *Pseudomonas sivasensis* CF10PS3 introduction on wheat phyllosphere microbiome

8.0 Forewords

Given that *P. sivasensis* is widely associated with wheat, that strain CF10PS3 can colonize the plant and thrive on leaves after foliar application, we wanted to understand its impact on the natural foliar microbiome. This chapter leverages the methodology developed with PRONAME (appendix 2), a new bioinformatic pipeline allowing species-level discrimination in metabarcoding studies focusing on 16S-ITS-23S (*rrn*) operon, and the field assay described in Chapter 7 to examine population dynamics in the weeks following the flag leaf treatment. These dynamics were compared with those resulting from a conventional chemical treatment program consisting of an application of metconazole and folpet at stage BBCH32, followed by a mixture of fluxapyroxad and mefentrifluconazole at stage BBCH59. This chapter has been drafted as a paper for publication in Science of the total environment journal. Several collaborators were associated to the work presented in this chapter; Mathieu Delitte wrote the manuscript and collected field samples. Mathieu Delitte and Benjamin Dubois carried out the experiments. Claude Bragard, Jacques Mahillon and Frédéric Debode acquired and managed funding. All authors designed the study, interpreted the results, reviewed and approved the final version of the manuscript

Impact of *Pseudomonas sivasensis* CF10PS3 introduction on wheat phyllosphere microbiome

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Keywords: *Pseudomonas sivasensis* CF10PS3, Wheat phyllosphere microbiome, Biocontrol agents, Metagenomic, Microbial community dynamics, Phytobiome, keystone species, *Zymoseptoria tritici*

Competing interests

P. sivasensis and related strains are under patenting process and might be used as BCA by the UCLouvain spin-off company BIOCSOL.

Authors' contributions

MD wrote the manuscript and collected field samples. BD and MD carried out the experiments. CB, JM and FD acquired and managed funding. All authors designed the study, interpreted the results, reviewed and approved the final version of the manuscript.

8.1 Abstract

In the quest for sustainable agriculture, biological control agents (BCAs) offer promising alternatives to chemical pesticides. While the impacts of chemical treatments on the microbiome are well-documented, studies on BCA impacts remain scarce. This work investigates the impact of introducing *Pseudomonas sivasensis* CF10PS3, a candidate bacterial biocontrol agent, on the wheat phyllosphere microbiome, compared to a conventional chemical treatment. Utilizing an innovative metagenomic technique with PRONAME, we assigned taxonomy at the species level

to microbial communities associated with wheat plants treated with CF10PS3, a chemical fungicide, and a buffer as a control. Our findings demonstrate that CF10PS3 has a dynamic impact on the phyllospheric microbial landscape, initially exerting a detrimental effect on numerous species, followed by a mixed effect with a similar number of depleted and promoted species. Ultimately, almost only promoted species were observed. The chemical treatment had more transient effects, with an equal number of depleted and promoted species by the end. These interactions underline the potential of using BCAs as microbiome modifiers, offering fine resolution since no broad effect was observed on the overall community, as revealed by alpha and beta diversity indices. This study contributes to the growing understanding of microbial interactions in the phyllosphere and highlights the importance of integrating microbial management strategies in crop cultivation to bolster agricultural sustainability.

8.2 Introduction

The microbiome is a fundamental component of plant health and productivity, contributing to nutrient cycling, growth enhancement, and disease resistance (Lyu et al., 2021). Recent researches have underscored the critical role of the plant microbiome, a dynamic and complex community of microorganisms that resides in and around plant tissues and is referred to as the phytobiome (Leach et al., 2017). Understanding these microbial communities, particularly within the context of wheat, is essential for advancing agricultural practices and ensuring sustainable crop production.

The term "microbiome" was initially defined by Whipps et al. in 1988 and later revisited by Berg et al. in 2020. The microbiome encompasses a specific microbial community within a well-defined habitat, including the microorganisms themselves, their interactions, and the resulting ecological niches (Whipps et al., 1988; Berg et al., 2020a). This concept highlights the interconnectedness and dynamic nature of these microbial communities, which are intimately linked to the health and functioning of their host plants.

The wheat phytobiome, an intricate ecosystem comprising bacteria, archaea, fungi, oomycetes, viruses, and other microorganisms, plays a pivotal role in plant health. This microbial community varies across different plant compartments, including the rhizosphere (soil zone around roots), phyllosphere (above-ground parts like leaves, stems, and flowers), and endosphere (interior plant tissues)(Bulgarelli et al., 2012; Lundberg et al., 2012; Turner et al., 2013; Zhang et al., 2021a; Lengrand et al., 2024). Each compartment hosts a unique microbial assembly that in-

teracts with the plant and its environment, influencing plant health and productivity (Mitter et al., 2016).

Focusing on the phyllosphere, the above-ground parts of wheat plants harbor a diverse microbial community. The wheat leaf microbiome includes a variety of bacteria along which *Actinobacteria, Proteobacteria, Bacilli, Clostridia*, and *Sphingobacteria* are key players (Kavamura et al., 2021; Chen et al., 2022; Barroso-Bergadà et al., 2023; Li et al., 2023). These microorganisms are crucial for nutrient cycling, disease control, and overall plant health (Hartman et al., 2018). However, the composition and dynamics of these microbial communities are influenced by numerous factors, including environmental conditions, plant genotype, developmental stage, and agricultural practices (Berg et al., 2015; Delitte et al., 2021).

Chemical treatments, including fungicides, herbicides, and insecticides, are commonly used in wheat cultivation to manage pests and diseases. These treatments significantly impact the phyllospheric microbiome, often altering microbial diversity and community structure (Muñoz-Leoz et al., 2013; Ku et al., 2023; Lloyd et al., 2023). For instance, fungicides applied to leaves can affect the abundance and composition of microbial populations, sometimes promoting the growth of specific genera like *Acinetobacter* while reducing others such as Alphaproteobacteria (Köberl et al., 2020; Chen et al., 2021). These shifts can disrupt the balance of beneficial and harmful microorganisms, potentially leading to unintended consequences for plant health.

Bacterial biocontrol agents (BCAs) offer a promising alternative to chemical treatments, controlling plant pathogens and promoting plant growth, thereby potentially reducing chemical inputs. *Pseudomonas* species have shown significant potential as BCAs in wheat cultivation due to their production of antimicrobial compounds and induction of systemic resistance in plants (Hofte, 2021). However, the introduction of BCAs into the wheat phyllosphere and their impact on the natural microbiome are not well understood.

The importance of plant microbiota in biocontrol research has been underestimated, yet it is crucial for the integration of BCAs and the ecological shifts they induce within the microbial community (Massart et al., 2015). The interactions between introduced beneficial microorganisms and native microbial populations vary with environmental conditions, emphasizing the need for microbial ecology research to enhance the efficacy of BCAs (Mendes et al., 2013). Inconsistent results with BCA applications may arise from complex interactions between introduced bacteria and the existing microbial community. Factors such as microbial competition, environmental conditions, and plant-specific responses can influence BCA effectiveness (Samad et al., 2017; Bonaterra

et al., 2022). Understanding these interactions is crucial for optimizing BCAs and achieving consistent, beneficial outcomes in wheat cultivation (Bonaterra et al., 2022).

The wheat microbiome, particularly the phyllosphere, is vital for plant health and productivity, hosting beneficial microbial communities. While chemical treatments can disrupt these communities, BCAs offer a sustainable alternative. This study aims to understand the impact of BCAs on the wheat microbiome to develop strategies for their successful integration and consistent performance, which is critical for advancing sustainable agricultural practices and enhancing wheat production in the face of increasing environmental challenges.

8.3 Materials and Methods

8.3.1 Bacterial strain characterization

Pseudomonas sivasensis strain CF10PS3 was obtained from a flag leaf in a spelt field (*Triticum spelta*) in Walhain, Belgium (Delitte et al., submitted). Whole genome sequencing was performed with Illumina and MinION technologies. The strain revealed *in vitro* capabilities to produce siderophores, proteases, and cellulase, solubilize phosphate, and exhibit swimming and swarming behaviors. It effectively colonized wheat leaves through direct spray and also following seed coating, and its role against *Zymoseptoria tritici*, the causal agent of septoria tritici blotch, was demonstrated. The strain possesses genes for producing biocontrol-related metabolites such as viscosin, fragin, pseudopyronine, aryl polyene APE-Vf, koreenceine, and siderophores, along with flagella and type IV pili (Delitte et al., in preparation).

8.3.2 Plant treatments

Winter wheat variety Chevignon was sown in an agricultural field in Perwez, Belgium, on October 15, 2022, following a potato crop rotation. Plots measuring 10 by 2 m were assigned to four replicates per treatment modality. CF10PS3 was grown on lysogeny broth (LB) agar plates at 28°C for 24 h, inoculated into LB, and incubated. The bacterial suspension was centrifuged, and pellets were resuspended in sterile buffer to a final concentration of 10^7 CFU/mL. Buffer components included 10 mM $MgCl_2$ and 0.1% Tween20. Buffer as control and bacterial applications occurred on April 27, May 11, and May 17, 2023, targeting specific leaf stages (BBCH32, BBCH37, BBCH39). BBCH scale pro-

vides a standardized method for describing phenological development (Meier et al., 2009). Chemical treatments involved metconazole and folpet at stage BBCH32, followed by a mixture of fluxapyroxad and mefentrifluconazole at stage BBCH59, on May 31.

8.3.3 Samplings

Leaf sampling involved cutting the base of seven leaves per plot, with two replicates. Leaves were stored on ice and frozen at -20°C. Flag leaf samplings followed a schedule: field plots were sampled before BBCH39 treatment and 1 h after for CF10PS3 and buffer controls, as well as 2, 7, 14 and 42 days after treatment. In chemically treated plots, leaves were harvested 1 h and 28 days after the treatment. This approach allowed a detailed assessment of the impact of *P. sivasensis* strain CF10PS3 and chemical treatments on the foliar microbiome.

8.3.4 Harvest of epiphytic microbial communities and DNA extraction

To recover the diverse epiphytic microbial communities present on the leaves, we adopted the leaf harvest protocol as follow: leaves were placed in sterile 250 mL wide-neck Erlenmeyer flasks, each containing 50 mL of a sterile peptone-phosphate buffer (peptone 10 g/l, NaCl 5 g/L, KH_2PO_4 7,5 g/L and $KH_2PO_4.3H_2O$ 9,5 g/L). Following an incubation period of 30 min at room temperature, the Erlenmeyer flasks were subjected to agitation at 150 rpm for 75 min. This was followed by a 45 min resting period at room temperature. The liquid from each flask was then transferred back into the initial Falcon tubes and centrifuged for 20 min at 6,000 rpm. Subsequently, the supernatant was discarded, while the pelleted microorganisms were stored at -20°C for subsequent analysis.

DNA extraction was performed with DNeasy PowerSoil Pro Kit (Qiagen), following the manufacturer's protocol with one minor modification: the initial lysis buffer was used to resuspend the pellet. The resuspended pellet was then placed into the Power-Beads tube, and the remainder of the protocol proceeded without further changes. Extracted DNA was quantified using Qubit4 system (Thermo Fisher Scientific).

8.3.5 PCR Amplification and sequencing libraries preparation

Extracted DNA was amplified using primers 16S-27F (5'-AGRGTTYGATYHTGGCTCAG-3') and 23S-U2428R (5'-CCRAMCTGTCTCACGACG-3') targeting the 16S-ITS-23S region of the ribosomal operon (*rrn*) (4500 bp) of bacteria (Martijn et al., 2019). The PCR mixture (20 µL total volume) included 2×GoTaq Long PCR Master Mix, 0.5 µM of each primer, 2 µM of mPNA (to block mitochondrial amplification), 2 µM of pPNA (to block plastid amplification), and 20 ng of DNA (Lundberg et al., 2012). The thermal profile consisted of an initial denaturation at 95°C for 2 min, followed by 30 cycles of 20 s at 95°C, 60 s at 67°C, 30 s at 55°C, and 4 min at 65°C, with a final extension at 72°C for 10 min. PCR was performed in triplicate and pooled before purification with AMPure XP beads (Beckman Coulter) using a 0.5X ratio. The quantity and quality of the PCR products were assessed using a Qubit4 Fluorometer (Invitrogen) and Nanodrop spectrophotometer, respectively, and visualized on agarose gel. Libraries were prepared using the Ligation Sequencing Kit V14 (SQK-LSK114) (Oxford Nanopore Technologies) according to the manufacturer's instructions and sequenced with Flongle Flow Cells (LSK114) during 24-hour runs.

8.3.6 Sequencing data processing and analysis of diversity

Raw data were analyzed with PRONAME (Dubois et al., in preparation). Raw data were imported into PRONAME, and adapters and primers were trimmed. A graph of simplex and duplex read distribution was constructed, and only duplex reads with lengths between 3500 and 5000 bp and a minimum quality score of 15 were kept. PRONAME refined clustered reads with a 90% identity percentage and polished the centroid sequence using the medaka model based on 300 subsampled reads. Representative sequences were blasted against the OEBG_fulloperon/lu16S8F_lu23S2490R_seqs_from_bacterial_genomes_complemented.fasta database included in PRONAME (Dubois et al., 2022).

8.3.7 Analysis on QIIME2

Identification of taxonomic groups with statistically significant differences in abundance between treatments was performed using ANCOMBC in QIIME2 (Lin and Peddada, 2020). We used parameters as –p-p-adj-method 'BH' –p-conserve True and –p-alpha 0.005. This method accounts for variations in microbiome datasets, such as unequal sampling

fractions and differences in sequencing efficiency, to limit the effects of group unevenness due to microbiome variability among individuals in the study. OTUs were summarized at the species level.

8.4 Results

8.4.1 Quantitative shifts in species abundance

In our study, we used ANCOMBC (Lin and Peddada, 2020) to assess the differential abundance of taxa at the species level in field plots subjected to treatments with CF10PS3 and chemical agents, compared to plots treated with buffer only (negative controls). The results are illustrated in Fig. 8-1 and reveal both enhancement and depletion effects on several bacterial species due to the treatments.

Depleted species: In the immediate following of treatment, 145 species were negatively impacted in CF10PS3-treated plots, and 72 species in chemically treated plots, indicating a significant decrease in these species' populations. The number of negatively impacted species rapidely decreased to 77 seven days post-treatment with CF10PS3. Unfortunately, we lack corresponding data for the chemically treated plots at an equivalent time point. Over the course of four weeks, this negative impact continued to vanish, with only a few species still exhibiting significant reduced abundance compared with negative control— three species (*Microlunatus phosphovorus, Pantoea agglomerans* and *Pseudomonas coleopterorum*) - in the CF10PS3-treated plots and seven (*Corynebacterium marinum, Dietzia kunjamensis, Hymenobacter* sp. BRD128, *Massilia* sp. H6, *Planococcus halotolerans*, *Pseudomonas coleopterorum* and *Sphingomonadaceae* bacterium OTU29MARTA1) in the chemically treated plots.

Enhanced species: Conversely, an enhanced abundance of several species was also observed following both types of treatments, more pronounced with CF10PS3. After the bacterial treatment, the abundance of 17 species increased immediately and 48 were promoted after 2 days, suggesting a thriving condition for these taxa. Remarkably, the benefit of bacterial treatment in terms of number of positively impacted species continued to show a sustained increase throughout the season, with 33 species still showing enhanced abundance at the end of the observation period. In contrast, the chemical treatments initially positively affected 27 species; however, this effect was more transient, with only 8 (*Exiguobacterium sibiricum, Kineococcus radiotolerans, Rhodococcus fascians, Rhodococcus* sp. RCBS9, *Clostridium chauvoei, Hymenobacter* sp. BRD128, *Erwinia persicina, Roseomonas haemaphysalidis*) species

continuing to exhibit an increased abundance by the end of the sampling period.

This detailed assessment underscores the intricate interactions among microbial communities when exposed to different treatments. The application of CF10PS3 has consistently shown more enduring benefits on several bacterial species than chemical treatments. Our results illuminate the dynamic nature of microbial ecosystems, revealing that some species not only withstand but adapt to new environmental conditions over time. These findings demonstrate the potential of targeted microbial interventions to favorably shift community dynamics, enhancing the resilience and adaptive capacities of beneficial species while reducing the prevalence of others.

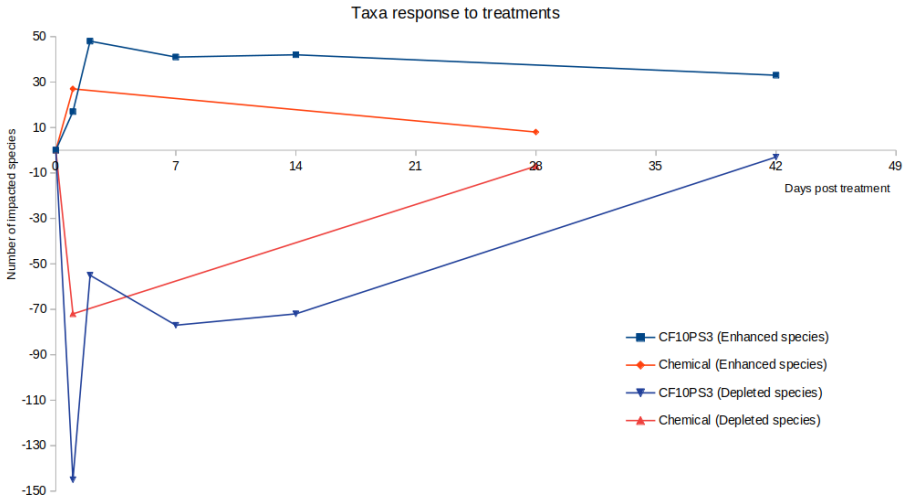

Figure 8-1: Taxa response to treatments.
Following treatments, the number of depleted (red) or enhanced (blue) species are represented for each sampling time point. To be noted, chemical treatment took place 14 days after bacterial treatment, so the last sampling (after 42 days for bacteria and 28 days for chemical) was the same day.

8.4.2 Species-specific responses to CF10PS3 and chemical treatments

Fig. 8-2 details the dynamic shifts in microbial species following the application of CF10PS3. This large-scale introduction of CF10PS3 into the environment had immediate and significant effects on the natural microbial populations. Within just one hour following the foliar application, a substantial depletion of numerous bacterial species was observed, while fewer species showed increased numbers. Notably, the taxa most

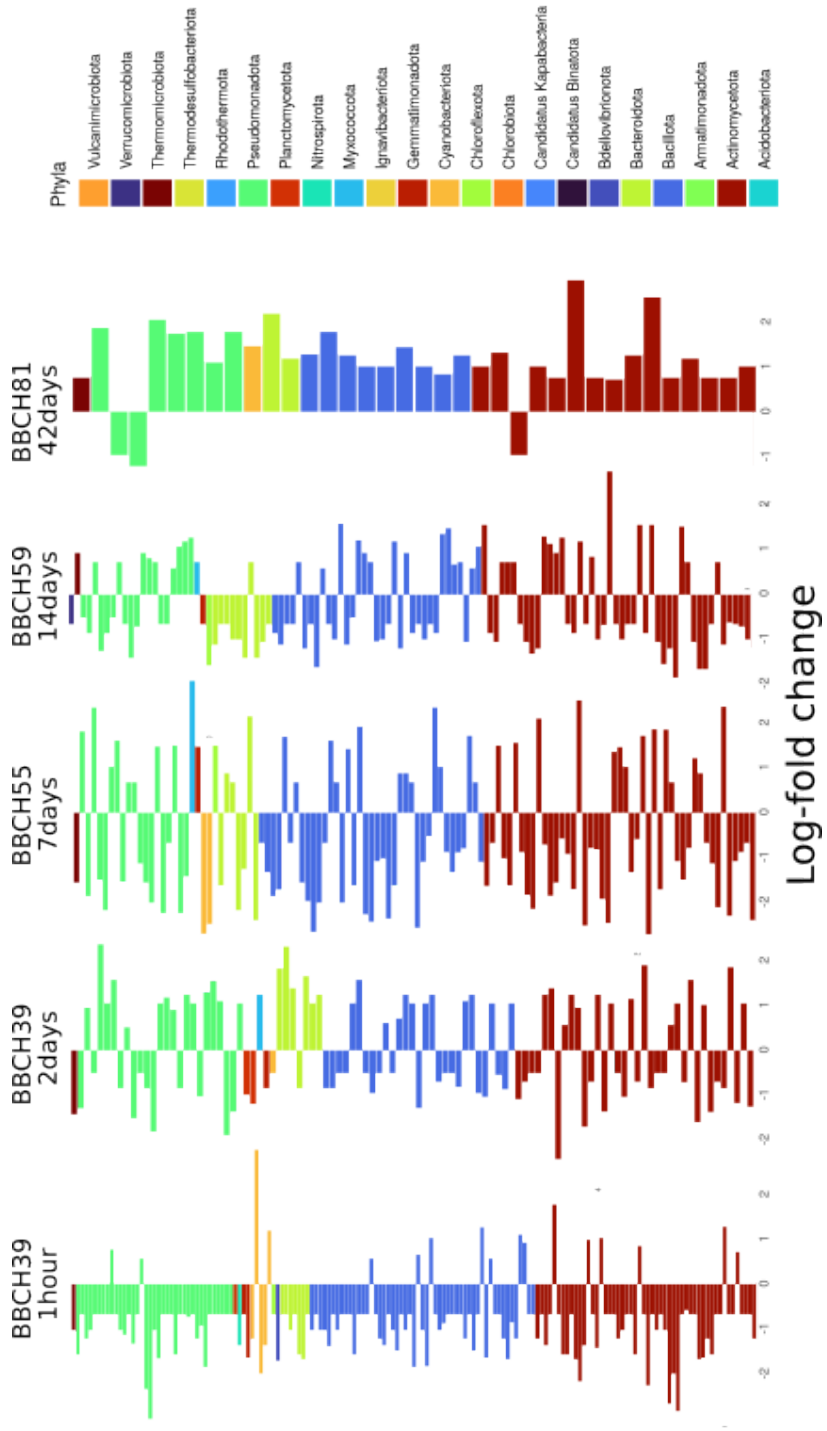

Figure 8-2: Positive and negative influence of CF10PS3 treatment on species.

Each bar in the graph represents a species, color-coded to indicate its phylum affiliation. This visualization highlights the positive and negative influences of CF10PS3 treatment on species diversity across different phyla. Bars length represent the log-fold change value between the species abundance in the treated sample compared to buffer control (n=2).

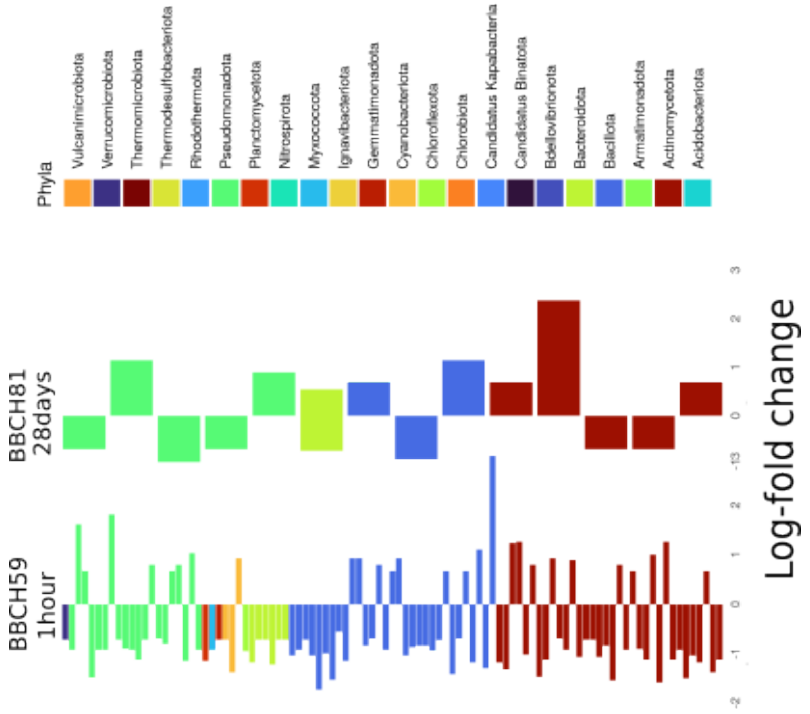

Figure 8-3: Positive and negative influence of chemical treatment on species.

Each bar in the graph represents a species, color-coded to indicate its phylum affiliation. This visualization highlights the positive and negative influences of chemical treatment on species diversity across different phyla. Bars length represent the log-fold change value between the species abundance in the treated sample compared to buffer control (n=2).

affected in terms of species number were *Acidobacteria*, *Bacilli*, and *Pseudomonata*. As the environment began to adjust to the introduction of CF10PS3, a balance was observed between depleted and promoted taxa over the next two days, which continued into the following two weeks. This suggests a period of adaptation and re-establishment of microbial equilibrium within the ecosystem. By the end of the observation period, 42 days after treatment, the overall impact of the CF10PS3 treatment on the microbiome diversity appeared to be positive: only 3 species remained depleted, while 33 species were promoted. Similarly, the immediate effects of the chemical treatment, as shown in Fig. 8-3, were comparable to those observed with CF10PS3, with numerous species being depleted and a few showing improvement. However, the long-term effects of the chemical treatment were more mixed. After 4 weeks, the data indicated a nearly balanced outcome, with 8 species enhanced and 7 species remaining depleted. These species names are

listed in the previous point. These observations highlight the transient nature of microbial disruptions caused by both CF10PS3 and chemical treatments. While both treatments initially disrupt the microbial communities, the CF10PS3 treatment seems to foster a more robust and beneficial adaptation over time, in contrast to the chemical treatment, which results in a more balanced and less clearly positive outcome in terms of promoted and depleted species number.

8.4.3 Species-level impact: Promoted and depleted responders

Taking advantage of the methodology used here, we were able to categorize species that were either promoted or depleted by the treatments, at the species level.

All the species impacted at any time points are listed in a supplementary table freely accessible on a Google Drive via the QR code or the link:
https://docs.google.com/spreadsheets/d/1dqiM7lBa9kQo2jVV_f2ZZKa4eHDvbK2U/edit?usp=sharing&ouid=112697845186401374116&rtpof=true&sd=true

In this section, we will concentrate on the major trends observed. Depletion: Immediate decline following chemical treatment was observed in numerous species, including *Arthrobacter* sp. EM1, *Bacillus smithii*, *Bradyrhizobium* sp. BWC-3-1, *Clostridium* (4 species), *Flavobacterium pallidum, Methylobacterium bullatum*, and *Sphingomonas* sp. NIC1. None of these species remained depleted until the end of the sampling. Populations of species belonging to *Hymenobacter* and *Massilia* genera, as well as *Pseudomonas coleopterorum* and *Sphingomonadaceae* bacterium (OTU29MARTA1) were diminished at 28 days post-treatment.

While many perturbations were observed following bacterial application, few were lasting. *Nakamurella* sp. was reduced from two days after treatment and lasting for 14 days and *Limnochorda pilosa* and *Austwickia chelonae* were lessened from immediately after treatment through to 14 days. At the conclusion of the experiment, only *Pantoea agglomerans* and *Pseudomonas coleopterorum* remained negatively impacted.

At the end of samplings, species from *Pseudomonas* genus were depleted in both treatments, as well as *Dietza* and *Massilia* members (chemical treatement) and *Pantoea* (bacterial treatment).

Enhancement: *Exiguobacterium sibiricum* population was consistently promoted from immediately after the chemical treatment through to the end of the experiment. Following CF10PS3 application, no species

showed an increased and sustained abundance response immediately until the end. *Sporosarcina thermotolerans* and *Clostridium chauvoei* were enhanced starting two days after CF10PS3 treatment and remained so until the end. *Rubrivivax gelatinosus* showed enhancement effects beginning two days after treatment and lasting for 14 days. At the end of sampling, *Erwinia, Exigobacterium, Rhodococcus* and *Kineococcus* genera, known to contain biocontrol-related species, were improved.

Throughout the experiment, transient positive impacts of bacterial treatment were noted on well-known genera such as *Arthrobacter, Clostridium, Hymenobacter, Massilia, Methylobacterium, Paenibacillus, Sphingomonas*, and *Staphylococcus*. Finally, 13 genera containing biocontrol-related species were promoted at the end of the experiment, including *Bacillus, Cellulomonas, Corynebacterium, Kineococcus, Methylobacterium, Micromonospora, Mycolicibacterium, Nakamurella, Nocardioides, Pseudomonas, Rhodococcus, Sphingomonas* and *Sporosarcina*. *Pseudomonas synxantha* was the only species from the *Pseudomonas* genus to be boosted by the bacterial spray, observed at 42 days post-treatment.

This summary underscores the complex and varied impacts of chemical and CF10PS3 treatments on microbial communities, highlighting the species-specific responses.

8.5 Discussion

In this study, the bacterial candidate biocontrol agent *P. sivasensis* CF10PS3 and a chemical treatment comprising fluxapyroxad and mefentrifluconazole were studied for their effects on the wheat phyllosphere microbiome diversity, using a buffer as a negative control. Effective colonization of the bacterial strain was confirmed by *in silico* qPCR, indicated by an initial sharp increase in its specific marker ("T" SNP) post-application (Supplementary Fig. S-5). Although its level gradually declined, it remained above the baseline established by the buffer-treated plots, suggesting a sustained but reduced presence. Our metagenomic approach enabled us to profile the wheat phyllosphere microbial community down to the species level and describe the impacts of both chemical and bacterial treatments on these species.

8.5.1 Microbial community profile

At the beginning of this study, members of the Phyla Pseudomonadota (53%), Bacillota (16.5%), Actinomycetota (16.4%), Bacteroidota

(4.7%), and Cyanobacteriota (6.5%) represented together 97% of the total diversity. The major bacteria at the genus level were *Bacillus, Clostridium, Frigoribacterium, Gloeocapsopsis, Hymenobacter, Massilia, Nocardioides,* and *Pseudomonas*. Given the seven-week span of our sampling, we anticipated natural modifications in the microbial community independent of the treatments. Indeed, a shift to other major genera was observed by the end of the sampling campaign and included *Curtobacterium, Erwinia, Frigoribacterium, Hymenobacter, Massilia, Pantoea, Pseudomonas, Rathayibacter,* and *Sphingomonas*. This seasonal microbiome modification is widely documented in numerous other studies (Copeland et al., 2015; Stone and Jackson, 2016; Carlström et al., 2019; Toju et al., 2019; Janakiev et al., 2020; Zhang et al., 2022; Howe et al., 2023; Li et al., 2023) Although widely reported as important members of phyllospheric microbiomes, *Methylobacterium* and *Burkholderia* were found to be present to a limited extent in this study, with less than 1 percent of reads associated to these genera.

8.5.2 Interactions and responses to bacterial application

The significance of plant microbiota in biocontrol research has often been underestimated, yet it is pivotal both for the integration of biocontrol agents (BCAs) and for the ecological shifts these agents induce within the microbial community (Massart et al., 2015). The interactions between beneficial microorganisms introduced and the native microbial populations typically vary with environmental conditions, emphasizing the need for microbial ecology research to improve the integrative efficacy of BCAs (Mendes et al., 2013). Additionally, as the stability of BCAs post-application is influenced by several factors, including the host microbiota and its environment, formulating BCAs effectively to sustain adequate population densities for disease control could be an interesting leverage (De Clercq et al., 2003; Lahlali et al., 2009; Sare et al., 2021).

Our results showed that although numerous perturbations in microbial populations were noted following the application of CF10PS3, few were enduring. For instance, *Nakamurella sp.* and *Limnochorda pilosa* exhibited temporary depletion that subsided after 14 days, whereas *Rubrivivax gelatinosus* demonstrated promoted abundance starting two days post-treatment and persisting for 14 days. Throughout the experiment, transient positive impacts of bacterial treatment were observed on well-known genera such as *Arthrobacter*, *Clostridium*, *Hymenobacter*, *Massilia*, *Methylobacterium*, *Paenibacillus*, *Sphingomonas*, and *Staphylococcus*. These changes remind the shifts in diversity and composition seen in the strawberry fruit microbiome influenced by the BCA *Metschnikowia fructicola*, including the enrichment of potentially beneficial genera *Bacillus*, *Methylobacterium*, *Rhizobium* and *Sphingomonas*

(Zhimo et al., 2021).

Sporosarcina thermotolerans and *Clostridium chauvoei* also displayed enhanced population from two days post-application and sustained these benefits throughout the study period. This suggests that while the introduction of BCAs can disrupt the native microbial ecosystem, the effects vary significantly between species, with some adapting or recovering over time and others remaining depleted or enhanced.

These findings align with previous observations where the introduction of *Pseudomonas*, such as *P. corrugata* CCR80 in the pepper rhizosphere, altered the bacterial community composition (Sang and Kim, 2012). *Pseudomonas spp.* DSMZ13134 transiently modified the barley rhizosphere microbiota for up to three weeks post-treatment (Buddrus-Schiemann et al., 2010), and similar transient modifications were noted with *P. fluorescens* strains 2P24 and CPF10 on the cucumber rhizosphere, with differences vanishing after eight weeks (Yin et al., 2013).

Furthermore, the response to BCAs varies depending on specific microbial communities and environmental conditions. For instance, *Bacillus amyloliquefaciens* and *Beauveria bassiana* had negligible impacts on bacterial diversity one month post-treatment (Sylla et al., 2013), yet a positive quantitative response of Bacilliales following BCA application was evident. In our study, several Bacilliales members, including *Paenibacillus*, *Sporosarcina*, and *Staphylococcus*, were favored following CF10PS3 treatment. This increase in Bacilliales, along with a decrease in Enterobacterales (among which *Erwinia* is found), was also observed by Cernava et al. (2019). Additionally, an increase in *Paenarthrobacter* population following BCA application was noted, a genus recently distinguished from *Arthrobacter* (Busse, 2016), which also benefited from BCA application (Yang et al., 2020).

In this study, a significant impact of *P. sivasensis* CF10PS3 introduction on other *Pseudomonas* members was only evident at 42 days post-treatment. *Pseudomonas* species were affected either positively (with a logfold change value of a taxa attributed to *P. synxantha* of 1.50) or negatively (with a logfold change value of a taxa attributed to *P. coleopteorum* of -0.78), additionally indicating a species-specific effect. *Pantoea agglomerans* was impacted by a log fold change of -0.98. In a comparative study of seed to seedling microbiomes, these two species demonstrated robust initial colonization capabilities. However, they faced challenges in maintaining their dominance in the new seedling environment, potentially due to competitive interactions with other microbes (Arnault et al., 2024). This could indicates their poor persistence in adapting to changing environmental conditions, potentially explaining their behaviour observed here.

In terms of plant health, inferred from the number of biocontrol-

related genera, the CF10PS3 treatment proved more beneficial than the chemical treatment. This is evidenced by the enhancement of 13 genera containing biocontrol-related species with the bacterial treatment, compared to only 4 genera with the chemical treatment at the end of the study. However, this is a simplified view of plant health and does not consider commensal interactions.

8.5.3 Effects of chemical fungicide on microbial dynamics

Mefentrifluconazole belongs to the triazoles class, which primarily inhibit the ergosterol synthesis crucial for fungal cell membranes. SDHIs, including fluxapyroxad, block the fungal mitochondrial respiration. Following fungal population reduction with fungicide treatment, some bacterial groups may suffer due to their specific nutritional dependencies on fungi (Velez et al., 2018), sensitivity to chemical changes, or direct sensitivity to the chemical itself (Andreolli et al., 2023). These trends seem species-dependent, as for instance *Sphingomonas*, a typically dominant genus, has been shown to be evenly distributed between chemically treated plots and untreated ones, indicating its resilience (Köberl et al., 2020; Chen et al., 2021). However, the use of iprodione has been shown to decrease populations of *Corynebacterium* and *Arthrobacter* (Katsoula et al., 2020) while *Pseudomonas* faces a decline in abundance with increasing pesticide concentrations, and the bacterial genera *Flavobacterium*, *Massilia*, and *Methylobacterium* experience altered abundance profiles under pesticide stress (Chen et al., 2021). *Methylobacterium* bacteria were particularly susceptible to the impacts of tebuconazole (Han et al., 2021), and this molecule also negatively impacted *Sphingomonas* (Baćmaga et al., 2022). *Bradyrhizobium* is a member of the Alpha-proteobacteria intrinsically associated with plants. This class often decreases in numbers with pesticide exposure (Köberl et al., 2020; Cheng et al., 2021). *Clostridium* has also been reported as depleted by several fungicides (Ma et al., 2021).

Fungicide broader ecological impacts can indirectly benefit certain bacterial groups in the microbiome (Nair et al., 2019; Lloyd et al., 2021). Indeed, bacteria and fungi are in harsh competition for nutrients in the environment (Mille-Lindblom et al., 2006; Wang and Kuzyakov, 2024). By reducing competitive fungal populations, these fungicides can decrease competition for resources and can change substrate availability, which can enhance bacterial growth. Additionally, the alteration of microhabitat conditions, such as pH (Akinnifesi et al., 2006; Crini et al., 2017), and the reduction of antagonistic metabolites, can create favorable niches for specific bacteria adapted to these new environmental conditions (Čaušević et al., 2024). This combination of reduced com-

petition and altered environmental conditions can lead to the proliferation of certain bacterial groups that are otherwise suppressed in the presence of robust fungal activity. This response is species-dependent, as shown by Pekkonen et al. (2013) with oligotroph versus copiotroph behaviours. In our study, some taxa from the copiotroph *Rhodococcus* genus showed an increased abundance after treatment. In an other study, such enhancement of *Rhodococcus* was correlated with the degradation of chemicals like difenoconazole (Zhang et al., 2021b). Tebuconazole has also been noted to increase *Rhodococcus* as well as the relative number of bacteria from the *Arthrobacter* genus (Baćmaga et al., 2022). Pyraclostrobin, another fungicide, increases *Bacillus* and *Paenibacillus* which have shown the capability to degrade the molecule (Nair et al., 2019). Gamma-proteobacteria, characterized by their fast growth, are believed to recolonize the phyllosphere more quickly than other microbes after pesticide treatment, effectively filling the vacated niches during the recovery phase (Whipps et al., 2008b). *Erwinia*, a member of the Gamma-proteobacteria, could exemplify this resilience. The Bacteroidetes group, among which *Hymenobacter* is found, showed increasing abundance in iprodione-treated samples (Katsoula et al., 2020), and *Planococcus* strains have been isolated from pesticide-contaminated soils (Gaur et al., 2020).

Specific fungicides can have dual effects on specific bacterial groups. For example, Pyraclostrobin increases *Bacillus* and *Paenibacillus* (Nair et al., 2019) as mentioned earlier, but has also been found to deplete *Exiguobacterium* (Zhang et al., 2019), illustrating the complex and varied impacts of chemical fungicides on microbial communities. The impact of fungicides on bacterial populations is highly variable, and depends on each group's ecological role, adaptability to environmental changes, resistance to chemical effects, and the chemical molecule considered.

8.5.4 Overall microbiome response

While the bacterial and chemical treatments impacted specific microbial species, they did not broadly alter the overall structure of the microbial community as indicated by alpha and beta diversity indices (Supplementary Fig. S-4). The results revealed that alpha diversity was initially stable, then decreased as the season advanced, a trend likely shaped by both intrinsic plant characteristics and external environmental factors such as temperature, desiccation and UV exposure (Segarra et al., 2015b; Daranas et al., 2018b). Chemical and bacterial treatments showed minimal impact on this initial stability, possibly due to favorable conditions for microbial colonization (Perazzolli et al., 2014; Copeland et al., 2015; Darlison et al., 2019; Stone and Jackson, 2021; Zhang et al., 2022). Despite a reduction in alpha diversity towards the end of the season, beta diversity remained stable, reflecting a con-

sistent overall microbial community structure (Copeland et al., 2015; Stone and Jackson, 2021). This consistency underscores a resilient core microbiome able to adapt to environmental shifts (Zhang et al., 2022). The analyses of alpha and beta diversity showed no notable differences across treatments and controls (Supplementary Fig. S-4). This indicates that these indices, relevant for overall effects, are less indicative in studies where major impacts are observable at fine taxonomic resolutions. Indeed, specific species populations were either depleted or enriched significantly, which was not mirrored in the diversity indices.

8.5.5 Detailed taxonomy and study limitations

Taking advantage of the species-level taxonomic attribution with PRONAME, this study highlighted specific taxa promoted or depleted by the *Pseudomonas* introduction in the wheat foliar microbiome and corroborated most of other studies' conclusions to upper taxonomic levels. The sequencing analysis focused exclusively on high-quality duplex reads, facilitated by PRONAME, a robust tool designed for processing complex sequencing data. By prioritizing these reads, a high level of confidence was ensured in our taxonomic classifications down to the species level, which was crucial for detailed resolution required in our study. The 4500 bp 16S-ITS-23S amplicon length used in this analysis covered enough genetic variance to enable accurate species discrimination. An overview of the sequencing results with the number of reads obtained for each duplicated sample is indicated in Supplementary Table S-3. This table illustrates the depth and consistency of sequencing across the samples examined. This study, while providing valuable insights into the impact of *P. sivasensis* CF10PS3 on the phytobiome of wheat, was conducted under specific environmental conditions at a single geographic location, which may limit the generalizability of the findings to other regions or under different environmental scenarios. Comparison with chemical treatment involved one of several plant protection products, composed of two of many active molecules. Additionally, the use of a single wheat cultivar, Chevignon, may not represent the response of other cultivars with different genetic backgrounds, as it is known to deeply influence the microbiome (Schlemper et al., 2017; Urbina et al., 2018; Wei et al., 2019). These factors could influence the microbial dynamics observed and the interaction between the biocontrol agent and the plant microbiome, potentially leading to different outcomes in other settings or with different plant varieties.

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Chapter 9
General discussion

9.1 Discussion

The genus *Pseudomonas* plays a significant role in the wheat microbiome, with various species contributing to plant health and productivity (Preston, 2004; Poncheewin et al., 2022; Mehmood et al., 2023). Notable species include *Pseudomonas aeruginosa*, *P. chlororaphis*, *P. fluorescens*, *P. lurida*, *P. piscium*, *P. orientalis*, *P. poae*, *P. salmasensis*, *P. simiae*, and *P. putida*, known for their association with cereals and biocontrol properties (Kron et al., 2020; Gómez-Lama Cabanás et al., 2022; Ibrahim et al., 2023). These *Pseudomonas* species are known for producing a range of bioactive compounds that enhance plant growth and control pathogens, including antimicrobial compounds, siderophores, and enzymes that may contribute to disease control within the phyllosphere. These bioactive compounds, along with their roles and targets, are summarized in Tables 4-1, 4-2, and 4-3. These compounds have also been extensively reviewed by Höfte (2021) Hofte (2021). Furthermore, some of these species have been shown to induce systemic resistance in plants, enhancing their ability to withstand pathogenic attacks. In our study, we identified numerous *Pseudomonas* species associated with cereals, including *P. tensinigenes*, known to colonize the wheat rhizosphere and produce tensin (Girard et al., 2021); *P. granadensis*, previously isolated from soil (Pascual et al., 2015); *P. germanica*, obtained from *Iris germanica* rhizomes (Atanasov et al., 2022); *P. lactis*, a known soil inhabitant (Havryliuk et al., 2020) and *P. sivasensis*. *P. sivasensis* promotes the growth of canola (Świątczak et al., 2023b) and has been identified in various environments (Duman et al., 2020; Wu et al., 2022; Świątczak et al., 2023a; Xiong et al., 2023), but to the best of our knowledge, it had not been previously associated with cereals despite the fact that the

bacteria was found systematically associated with small grain cereals in this study.

Indeed, among the 444 *Pseudomonas* isolates associated with cereals and obtained from various agricultural contexts, 33 showed antagonist activities against at least three of the four tested pathogens in *in vitro* quantitative tests (*G. graminis* var. *tritici*, *O. yallundae, F. graminearum* and *Z. tritici* (See Chapter 6). *In vitro* characterization might provide insight into their ecological roles, as almost all isolates from heads could control *in vitro* the head-pathogen *Fusarium graminearum*, and almost all leaf isolates could antagonize the leaf-pathogen *Zymoseptoria tritici*, indicating their potential roles in plant defense with various mechanisms, including antagonism (See Chapter 6). *P. sivasensis* and closely related species comprised over 25% of our 33 isolates. Among these, we identified a new species, *Pseudomonas arvensis* sp. nov., which is genetically different of the closely related species while producing similar secondary metabolites. These species are widely associated with cereals, as isolates were collected from all plant compartments and from the first sampling in late March to the last in late June. They were obtained from four fields, irrespective of tillage use and agricultural practices, whether organic or conventional, or the plant host, be it spelt or winter wheat. Given their notable presence in our study and the significance of *Pseudomonas* research in agriculture, it is surprising that *P. sivasensis* and *P. arvensis* sp. nov. had not been previously identified as part of the wheat microbiome. This highlight the fact that most of the bacteria associated with small grain cereals have been mostly looked at as plant pathogens, therefore bypassing major bacterial species associated with the cereal phytobiome. Further research is required to determine whether *P. sivasensis* and *P. arvensis* are unique to Belgium or if they have a broader distribution. In this study, we concentrated primarily on wheat defenses against *Zymoseptoria tritici*. *In vitro* tests showed that the *Pseudomonas sivasensis* strain CF10PS3 can reduce symptoms and pycnidia production by *Z. tritici*, although the precise mechanisms remain unexplored. Strain CF10PS3 is capable of producing cellulase, protease, and siderophore, and has the genetic potential to synthesize viscosin, fragin, APE-Vf, pseudopyronine, and koreenceine (See Chapter 6). While these metabolites have not been directly linked to controlling *Z. tritici*, their involvement is suspected due to the broad activity spectrum of these compounds. Comparative genome analysis revealed a significant difference between the closely related species *P. sivasensis* and *P. arvensis* sp. nov. regarding biocontrol capabilities. Both possess the genetic cluster necessary for producing fragin, a metallophore with antifungal properties (Jenul et al., 2018), but the latter lacks the cluster for koreenceine, with the exception of one strain (DR1PS3) out of four.

9.1.1 Large scale adoption journey

While having numerous pros and benefits, large scale adoption of biocontrol solutions by farmers is still subjected to several steps and obstacles.

The use of Biocontrol Agents (BCAs) represents a fundamental shift in agricultural pest management compared to traditional chemical pesticides. Unlike chemical treatments that focus on reducing specific pathogens, BCAs aim to enhance the overall health of the plant holobiont – the plant and its associated microbial communities. This integrated approach leverages the natural interactions between plants and beneficial microbes to promote plant health, resilience, and productivity. However, this strategy also comes with its own set of drawbacks, limitations, and compromises that must be carefully considered. Chemical pesticides aim to directly target and reduce specific pathogens or pests, providing immediate crop protection by eliminating harmful organisms. However, they can lead to resistance development among pests, negatively impact non-target organisms, and cause environmental contamination. In contrast, biocontrol agents (BCAs) like *Pseudomonas sivasensis* CF10PS3 take a holistic approach by enhancing the overall health and resilience of the plant holobiont. BCAs promote beneficial microbial communities that support plant growth, improve nutrient uptake, and enhance natural defenses, fostering a diverse and balanced microbial community on the plant surface. This approach aims to create a more sustainable and resilient agricultural system.
BCAs offer several benefits, including sustainability by reducing chemical inputs, resistance management by promoting diverse microbial ecosystems, and enhanced plant health, making crops more resilient to various stresses. However, their efficacy is generally considered variable and inconsistent at field level (Nicot et al., 2011). It can vary based on environmental conditions, they act more slowly than chemical pesticides, and they require more sophisticated management practices combined with a thorough knowledge of the plant, BCA and pathogen biology (Elad et al., 2007; Ruocco et al., 2012). Several leverages can be considered to enhance field performance of BCA, as reviewed by Marian and Shimizu (2019). They include strategies such as improving abiotic stress tolerance (Puopolo et al., 2015; Daranas et al., 2018a), incorporating anti-stress protectants (Sui and Liu, 2014), utilizing nutrient provisioning and organic amendments (Yandigeri et al., 2015; Gramisci et al., 2018), combined application of multiple BCAs (Chemeltorit et al., 2017; Jambhulkar et al., 2018) with an optimization of formulation procedures (Segarra et al., 2015a; Angeli et al., 2017; Bejarano et al., 2017). These approaches can help in enhancing the persistence, colonization, and biocontrol efficacy of BCAs under field conditions.
Adopting BCAs involves compromises between immediate pest control and long-term sustainability. Integrated Pest Management (IPM) systems, which can combine BCAs with chemical treatments, offer a bal-

anced approach, allowing BCAs to promote plant and soil health while reserving chemical treatments for emergency interventions. This shift from chemical pesticides to BCAs represents a more integrated and sustainable approach to pest management, focusing on plant holobiont health rather than solely targeting pathogens. To enhance the adoption of BCAs, demonstration trials in diverse agricultural settings are essential to showcase their efficacy and benefits. Training and education programs for farmers on proper application techniques and benefits are crucial, along with ensuring regulatory approval from bodies like the EFSA or EPA. Addressing economic considerations through cost-effective production methods, financial incentives, and cost-benefit analyses can encourage adoption. Increasing awareness through targeted campaigns and success stories can build trust, while extensive research and standardized application guidelines can improve consistency of results. By addressing these steps and obstacles, the adoption of BCAs can be significantly enhanced, leading to more sustainable and resilient agricultural practices.

9.1.2 Durability

One of the benefits offered by BCA and helping for its wider adoption could be its theoritically enhanced durability. The durability of a control method for plant protection is defined by its sustained efficacy over time and space, influenced by the selection pressure it exerts on pest populations and their ability to adapt (Bardin et al., 2021). *Pseudomonas sivasensis* CF10PS3 can also persist in the wheat phyllosphere for up to seven weeks post-application (Chapter 7), highlighting its potential as a long-term biocontrol agent for wheat diseases. This persistence can reduce the need for frequent reapplications, lowering costs and labor for farmers, and supports the practical implementation of biocontrol agents (BCAs) in agriculture as stable alternatives to chemical pesticides. The durability of chemical control methods has been challenged by the frequent emergence of resistance in various plant pathogenic fungi (Brent and Hollomon, 2007). Similarly, resistance to microbial biocontrol agents or their metabolites has been observed in arthropod pests (Siegwart et al., 2015). For example, resistance to *Bacillus thuringiensis* toxins was reported in three pest species several years after its market introduction. However, documented cases of resistance in plant pathogens to microbial bioprotectants are scarce, possibly due to their limited use compared to conventional protection tools (Bardin et al., 2015).
Antibiosis, a mode of action similar to chemical control, is effective but raises concerns about resistance development. Plant pathogens have developed resistance mechanisms to various toxic compounds produced by beneficial microorganisms (Duffy et al., 2003; Raaijmakers et al., 2009). Conversely, resistance to competitive mechanisms seems unlikely, but

some pathogens may exploit nutritional resources more efficiently, affecting the efficacy of microbial bioprotectants (Köhl et al., 2019b). Some bacteria, like those producing lipopeptides, can alter plant leaf surfaces to hinder pathogen attachment and growth, with a direct antimicrobial effect and the ability to induce systemic resistance in plants (Ongena and Jacques, 2008; Falardeau et al., 2013). The development of resistance to lipopeptides appears unlikely due to their biosurfactant properties and the costly mechanisms required for microorganisms to repair membrane damage (Falardeau et al., 2013; Zhao et al., 2017). However, different levels of sensitivity to lipopeptides were observed in *Venturia inaequalis* strains (Desmyttere et al., 2019). Microbial bioprotectants like *Trichoderma* combine multiple modes of action, including hyperparasitism and antibiosis, enhancing their efficacy against various pathogens (Elad, 2000; Harman, 2006; Howell, 2003; Karlsson et al., 2017). *Bacillus subtilis* QST713, for instance, uses antimicrobial metabolites, nutrient competition, and induction of systemic plant resistance to control pathogens such as *Botrytis cinerea* and *Pythium ultimum* (Paulitz and Bélanger, 2001; Lahlali et al., 2013; Levasseur et al., 2005). The durability of biocontrol depends on the specific modes of action involved, with some being more prone to resistance development than others. Studies have shown varying levels of susceptibility to microbial bioprotectants among plant pathogen strains, potentially reducing field efficacy. For example, different sensitivities to phenazine-1-carboxylic acid and 2,4-diacetylphloroglucinol were observed among *Gaeumannomyces graminis* var. *tritici* and *Fusarium oxysporum* strains (Mazzola et al., 1995; Schouten et al., 2004). Monitoring resistance levels before and after treatment can inform on resistance distribution and impact on efficacy. Testing numerous isolates of a target plant pathogen for resistance to a microbial bioprotectant is crucial for estimating resistance emergence risk. Rapid assessments of resistance in large pathogen strain collections, as done for fungicides, should also be applied to BCAs to ensure their long-term efficacy.

The widespread use of microbial bioprotectants in commercial agriculture raises concerns about the potential development of resistant strains, similar to what has occurred with chemical pesticides. To assess this risk, it is necessary to repeatedly expose plant pathogens to microbial bioprotectants or their compounds through experimental evolution, a method also used in human pathology and fungicide research (Cowen, 2001). One study involving the microbial bioprotectant *B. subtilis* CL27 in a greenhouse setting found that after ten treatments, it became ineffective against *B. cinerea* (Li and Leifert, 1994). Another study demonstrated that *B. cinerea* could adapt to pyrrolnitrin, an antibiotic produced by several microbial bioprotectants including *Pseudomonas*, and showed cross-resistance with iprodione, a common fungicide (Ajouz et al., 2010; Fillinger et al., 2012). Although resistance developed, it came with significant fitness costs, such as reduced growth,

aggressiveness, and conidia production, which limited the pathogen's epidemic potential (Ajouz et al., 2011). Understanding the potential for resistance development is crucial for ensuring the long-term effectiveness of microbial bioprotectants. This knowledge helps identify risk factors and select microbial bioprotectants with a lower risk of losing efficacy against target plant pathogens. Major research efforts are still needed to gain detailed knowledge of the modes of action of biocontrol agents. This knowledge will allow optimizing their use in the field and should therefore foster their durability. For example, guidelines could be established for using complex formulations (mixtures of metabolites or microorganisms with different modes of action) or for alternating different products. This could also help ward off potential failures by guiding the screening procedure for new biocontrol agents towards more durable modes of action (Bardin and Siegwart, 2022).

9.1.3 Environmental impacts of repeated introduction of BCA

The introduction of CF10PS3 initially alters the microbial community composition, finally enhancing the abundance of potentially beneficial genera and reducing the prevalence of potentially harmful ones. However, these changes are generally transient and species-specific, with some microbial populations recovering or adapting over time. In the short term, this disruption could improve plant health and disease resistance. Similar patterns are observed with other BCAs, where initial beneficial impacts may stabilize as the ecosystem adjusts, highlighting the importance of understanding these dynamics for effective biocontrol application. Repeated application of BCAs could lead to shifts in the native microbial community structure and function, potentially promoting beneficial microbes that support plant health while suppressing pathogens. However, long-term ecological studies are necessary to fully understand these impacts and to ensure that they do not lead to unintended consequences such as the development of resistance or disruption of native microbial ecosystems. Over time, the consistent presence of BCAs like CF10PS3 might select for microbial communities that are more resilient and supportive of crop health, yet the ecological balance must be monitored to prevent negative outcomes. This general consideration applies to all BCAs, emphasizing the need for sustainable practices and ongoing monitoring. The persistence and environmental impacts of CF10PS3 highlight broader considerations for the use of BCAs. Ensuring that BCAs are compatible with local ecosystems and integrated into comprehensive IPM strategies is essential. This includes rotating BCAs to prevent resistance, monitoring microbial community changes, and conducting long-term impact studies. To maximize the survival and efficacy of CF10PS3, it is crucial to consider environmental conditions

such as humidity, temperature, and rainfall when planning applications. For example, applying CF10PS3 during periods of moderate humidity and temperature can enhance its colonization and persistence on plant surfaces. In regions with heavy rainfall, applications should be timed to avoid wash-off, such as targeting early morning or late afternoon when dew is present but rain is less likely. Seasonal variations also play a role; applying CF10PS3 in spring when plant growth is vigorous can support better establishment and activity compared to late summer applications when conditions might be less favorable. Of course, the timing regarding the targeted pathogen life cycle is obviously important too. Experimenting with different dosages and formulations is essential to find the most effective combination for sustained microbial presence and biocontrol activity. For instance, initial field trials might involve varying the concentration of CF10PS3 from 10^6 to 10^9 CFU/ml to determine the optimal dose that balances efficacy with cost. Formulations that include protective agents, such as glycerol or skim milk powder, can enhance the survival of CF10PS3 during storage and application. Additionally, encapsulating CF10PS3 in biodegradable polymers might protect the bacteria from environmental stressors, gradually releasing them in the phyllosphere and promoting prolonged activity. Assessing the impact of different co-formulants on BCA stability and efficacy is crucial to identify synergistic combinations that enhance performance. For example, combining CF10PS3 with organic amendments like compost tea can provide additional nutrients that support bacterial growth and activity. Another approach could be co-applying CF10PS3 with other agents that produce complementary metabolites, enhancing overall plant health and disease control. Studies could explore the potential synergistic effects of CF10PS3 with natural antifungal compounds like chitosan, which can help suppress a broader range of pathogens while promoting the biocontrol efficacy of CF10PS3.

9.1.4 Persistence of introduced BCA in the environment

The persistence of *P. sivasensis* CF10PS3 in the soil could be an important factor for its effectiveness as a biocontrol agent. This study, which focused on the phyllosphere, provides valuable insights that are applicable to soil environments as well. Whereas the strain was applied on above ground plant parts with foliar spray, bacteria can also reach the soil either by off-target fraction of the treatment or with rain washoff. The persistence of CF10PS3 in the soil might be influenced by various environmental conditions such as temperature, humidity, and soil pH, as well as by competitive interactions with native soil microbes. These factors collectively determine the survival, colonization, and activity of CF10PS3, which in turn impact its efficacy in controlling soil-borne pathogens and promoting plant health. Considering the strain's ability

to control eyespot and take-all disease pathogens in *in vitro* tests, along with its capability to colonize aerial parts of the plants from a simple seed coating, the persistence of the strain in agricultural soils could be beneficial. Studies similar to the one presented in Chapter 8 but focused on the soil would nevertheless be necessary to ensure that such long-term introduction does not have detrimental effects on the microbiome. Monitoring techniques, such as strain-specific qPCR, are essential tools for studying the long-term dynamics and stability of CF10PS3 in the soil. Strain-specific qPCR allows for the precise quantification of CF10PS3 populations, distinguishing them from native *Pseudomonas* strains and other microbes. This technology provides detailed data on the persistence and distribution of CF10PS3 over time, under various environmental conditions and agricultural practices. Such insights are crucial for optimizing application strategies, ensuring that CF10PS3 remains active and effective as a biocontrol agent over extended periods. Extending this understanding to biocontrol agents (BCAs) in general, the principles of persistence and monitoring apply broadly. The effectiveness of any BCA in the soil depends on its ability to establish itself and interact beneficially with the native microbial community. Environmental factors play a significant role; for instance, temperature extremes can reduce microbial activity, while appropriate moisture levels can enhance microbial survival and function. Soil characteristics such as texture, organic matter content, and nutrient availability also influence BCA persistence. By monitoring BCA populations, it is possible to adjust application methods and timings to enhance their persistence and effectiveness. For example, applying BCAs during periods of moderate environmental stress or in conjunction with soil amendments that support microbial life can improve their establishment and longevity. Moreover, integrating BCAs into broader soil health management practices is crucial. Practices such as reduced tillage, organic amendments, and cover cropping can create more favorable conditions for BCAs by enhancing soil structure, moisture retention, and microbial diversity. These practices could not only support the persistence of BCAs but also contribute to overall soil fertility and plant health, creating a more resilient agricultural ecosystem. At the beginning of the field study, we observed an initial composition of the phyllospheric microbial community dominated by major phyla such as *Pseudomonadota*, *Bacillota*, *Actinomycetota*, and *Bacteroidota*, a taxonomic distribution that echoes numerous studies (Kavamura et al., 2021; Chen et al., 2022). When applied to leaves, strain CF10PS3 initially distributed homogeneously across the surface. Confocal imagery revealed that the bacteria quickly migrated to preferential colonization sites such as around stomata, along veins, and at the bases of trichomes. This movement was facilitated by its swimming and swarming abilities, likely enhanced by the production of viscosin, a cyclic lipopeptide that reduces surface tension and promotes bacterial spread and motility (Vacher et al., 2016). Indeed, the connection between lipopeptides and *Pseudomonas* in facilitating

bacterial spread is well-documented (Flamand et al., 1996). Following bacterial introduction, numerous resident species were depleted. This might result from the massive introduction of CF10PS3, triggering ecological countermeasures by both the natural population and the introduced strain. As the introduced bacteria migrated towards preferential sites, they likely encountered other microorganisms already occupying those sites, necessitating the use of CF10PS3's antibiosis machinery for successful colonization. This could involve cellulase, which can help disrupt bacterial biofilms (Kamali et al., 2021), and alkaline protease, which may inactivate antibiotics produced by other bacteria (Anderson et al., 2004). Although lacking a complete operon for pyoverdines production (missing *pvdD* and *pvdJ* genes responsible for NRPSs synthesis (Ghssein and Ezzeddine, 2022)), *in vitro* tests confirmed the strain's capability to produce unidentified siderophores that can significantly impact competitors by inducing iron starvation (Colombowala and Aruna, 2018; Vollenweider et al., 2023). Other compounds potentially produced by the strain could also play a role in this antibiosis against resident phyllospheric bacteria. Their genetic determinants are present in CF10PS3 genomes, but actual production was not assessed. These include viscosin, which has potential antibacterial effects through disrupting bacterial membranes (Alsohim et al., 2014); koreenceine, a polyketide with noted antifungal properties and underexplored antibacterial activity (Riera et al., 2023); pseudopyronine, primarily effective against fungal pathogens, and APE-Vf, used in antifungal biocontrol, which may possess antibacterial properties through mechanisms that need further verification (Nishanth Kumar et al., 2016; Dutta et al., 2020).

Besides this direct antagonism of the introduced strain toward the resident microbiome, the massive introduction could also rapidly decrease resource availability, thereby reducing the number of surviving species (Pekkonen et al., 2013).

Most epiphytes, including CF10PS3 as shown by confocal imagery, survive on leaf surfaces by forming large aggregates where they produce extracellular polymeric substances (EPS), enhancing their hydration and protection against environmental stressors (Morris and Kinkel, 2002; Lindow and Brandl, 2003; Baldotto and Olivares, 2008; Vorholt, 2012). The quorum-sensing (QS) molecule Acyl-homoserine lactone (AHL) plays a crucial role in biofilm formation by regulating EPS production (Chaudhry et al., 2021). The potential ability of strain CF10PS3 to engage in QS and its biofilm formation capability could not only enhance bacterial resistance to antimicrobial compounds and environmental challenges, such as UV radiation, desiccation, temperature fluctuations, or wash off but could also provide a stable environment through the production of biosurfactants like viscosin, which supports cell adhesion and aggregation (Alsohim et al., 2014). These various and comple-

mentary mechanisms of strain CF10PS3 are key factors in its successful colonization observed in the field trial (Nadakuduti et al., 2012; Braga et al., 2016; Ueda et al., 2018; Leveau, 2019; Oso et al., 2019; Streletskii et al., 2019; Flores-Núñez et al., 2020). With the development of specific qPCR probes, we were able to effectively track the strain's presence in the field. Two days after the massive introduction of the bacteria, strain CF10PS3 numbers had dropped, likely due to the reasons explained earlier and as a result of phyllospheric interactions. Remarkably, it showed no significant influence on other *P. sivasensis s.l.* populations naturally present in the phyllosphere (see chapter 7). Inside the microbiome, the situation tended toward an equilibrium between the populations, with several species still depleted but many others now promoted (see chapter 8). Some enhanced species may take advantage of the niche clearing resulting from the bacterial competition (Čaušević et al., 2024). The chemical treatment also brought several direct perturbations in the natural populations following the treatment. Numerous species were depleted while others were promoted, to a lesser extent. In the two weeks following the bacterial treatment with *P. sivasensis* CF10PS3, an equilibrium persisted between the number of promoted or depleted species, culminating in 33 enhanced species and only three still depleted by the end of the sampling period. Despite a decrease in the abundance of CF10PS3, its presence remained higher than in control plots as evidenced by "T" SNP detection through qPCR and in metagenomic reads (See Chapters 7 and 8). By the experiment's conclusion, 13 genera containing biocontrol-related species were enhanced in the bacterial treatment plots, compared to only 4 in those receiving the chemical treatment, reflecting a more beneficial impact on plant health. The sustained influence on specific species despite the reduction in CF10PS3 population suggests that the lasting effects could be attributed either to the residual bacteria, their metabolite production, or a shift in the microbial community dynamics initiated by the treatment. Strategies to enhance the persistence of CF10PS3, such as encapsulation or co-application with supportive nutrients, might confirm the direct role of the bacteria and its metabolites in these observed effects. CF10PS3 caused significant shifts in the abundance of specific microbial species. The biocontrol agent particularly promoted a sustained effect on certain species, unlike the chemical treatment's more transient impact. This underscores the potential of targeted biocontrol strategies to improve desirable microbial traits without disrupting the overall community structure. In this context, microbiome engineering via bacterial inoculation represents a promising strategy for manipulating microbial communities to bolster plant health. While the biocontrol and chemical treatments affected specific microbial populations, as observed at the species level, they did not broadly disrupt the overall microbial community structure, measured by alpha and beta diversity indices. This indicates that these indices, relevant for overall, big-picture effects, are less indicative in studies where major impacts are observable at fine tax-

onomic resolutions. Indeed, we observed numerous species populations either depleted or enhanced, which was not reflected in the diversity indices. *A contrario*, as the season progressed, external factors such as temperature dessication and UV exposure did influence microbial diversity, highlighting the impact of environmental conditions on these communities (Segarra et al., 2015b; Daranas et al., 2018b). Overall, the research illustrates the complex dynamics within the wheat phyllosphere, where introduced biocontrol agents and chemical treatments transiently affect specific microbes without causing widespread disruption to the community, while having an effect on specific species.

9.2 Perspectives

Research in the fields of microbial interactions and microbiome engineering continues to expand rapidly, yet several pivotal questions remain unanswered, and numerous gaps require to be resolved. The complex mechanisms by which CF10PS3 influences adjacent microbial populations—whether through direct competition, niche modification, or biochemical processes like antimicrobial compounds production and quorum sensing—are still not fully understood, necessitating further detailed exploration. While initial steps have been taken to characterize general trends in microbial community shifts following treatment, the specific taxa that are either promoted or suppressed have been identified here. However, their precise ecological roles and implications on plant health have not yet been thoroughly elucidated. This lack of detailed understanding underscores the need for identifying the roles these microbes play within their ecosystems. By examining the functional impacts of the biocontrol agent CF10PS3 on the phyllosphere microbiome, we can begin to unravel how these microbial shifts directly influence plant health. Such insights could help developing targeted interventions that enhance phyllosphere resilience and productivity, moving from mere identification to a comprehensive functional understanding. Another intriguing aspect is the ecological and evolutionary responses of microbial communities to repeated applications of CF10S3. Given indications of CF10PS3's association with the wheat xylem and its exit through hydathodes, suggesting a continuous introduction of the bacteria into the phyllosphere from a seed coating. It raises also the question of how the microbiome might adapt and evolve. Factors such as the production of enzymes like cellulase, which may facilitate the breakdown of plant cell walls and enhance colonization within plant tissues, are crucial to consider (Flint and Bayer, 2008). Additionally, traits like the production of biosurfactants such as viscosin which reduce surface tension and aid in bacterial spread across plant surfaces, certainly play a pivotal role (Alsohim et al., 2014). Genes related to motility, such as those encoding flagella, and nutrient acquisition mechanisms like siderophore produc-

tion for iron uptake, are probably important in determining how effectively CF10PS3 colonizes the plant. Moreover, the ability of CF10PS3 to form biofilms could be critical for bacterial adherence and plant colonization (Mina et al., 2019). Examining the differential microbiome responses with other *Pseudomonas* species, distinct genera, or within microbial consortia could also shed light on strain-specific versus more general responses, as the strain-specific response has been highlighted for gut microbiota (Ma et al., 2023). Enhancing the presence of keystone species on wheat through selective breeding (Gruet et al., 2023), foliar sprays, or via seeds (Johnston-Monje et al., 2021) offers a promising path forward. Establishing the threshold levels necessary for effective biocontrol of *Z. tritici* could take advantage of tools like qPCR to monitor and optimize the field effectiveness of microbial interventions (Barbau-Piednoir et al., 2014; Taylor et al., 2014; Thilakarathna et al., 2022). Additionally, implementing techniques such as viability-qPCR (v-qPCR), a technique using a dye or a compound such as propidium monazide to prevent amplification of dead-cells DNA, could be a significant advancement for accurately assessing viable bacterial populations and refining biocontrol strategies under diverse environmental conditions. This could also open new avenues for the study of co-formulants to improve BCA survival. Microbiome engineering aims to tailor the plant microbiome towards a community structure that enhances essential plant functions. Despite considerable progress and the deployment of complex approaches using biological control agents or plant growth-promoting microbes, applying these strategies in commercial settings has encountered challenges (Glick, 2012). This is likely due to the exclusion of new microbes by more resilient existing microbial communities, whose composition has evolved through intricate multi-faceted interactions with the environment (Hardoim et al., 2015). The response of native microbiota to introduced microbial strains remains poorly understood, and insights from research such as those presented here could help illuminate this issue, particularly in the rhizosphere, which is the most studied plant compartment in this context (Araujo et al., 2019; Afridi et al., 2022; Zhang et al., 2023). Reinhold-Hurek et al. (2015) and Hu et al. (2018) provide comprehensive insights into the mechanisms through which plants influence their microbiomes. Their studies elucidate the various microhabitats within specific niches and how microbial communities in these niches are selectively enriched by the plant. This selective enrichment plays a critical role in enhancing plant nutrient uptake and health, offering insights into the complex plant-microbe interactions that are pivotal for microbiome engineering. Alongside the various drivers of microbial community stability (Shade et al., 2012), Glick (2012) also addresses the practical challenges of implementing these microbiome modifications in agricultural settings, noting the resistance posed by established microbial communities. The plant itself plays a crucial role as many of the molecular signals that activate plant immune responses are remarkably similar, and often identical, across

both pathogenic and beneficial microbes (Rodriguez et al., 2019).

In light of these challenges, the future of microbiome research lies not only in deciphering microbial identities but also their functions. This shift towards function-based strategies will likely be propelled by advancements in shotgun metagenomics, which have already begun to map microbial functions in other contexts like the phyllosphere microbiome of rice (Roman-Reyna et al., 2020). As we continue to unravel these complex microbial networks, the potential for favoring and directing microbiomes to enhance plant development, nutrition, and disease control appears promising (Compant et al., 2019; Hardoim et al., 2015; Lemanceau et al., 2017).

In this work, we introduced a novel application of qPCR probes to distinguish the introduced *P. sivasensis* strain CF10PS3 from naturally occurring strains. This specific detection allows for precise monitoring of the persistence and dynamics of the biocontrol agent (BCA) in the field, which is a significant advancement over traditional methods that could not differentiate between introduced and native populations. The study's findings on the persistence and impact of CF10PS3 on the wheat phyllosphere microbiome are crucial for developing robust biocontrol strategies and enhancing sustainable agricultural practices.

The specific qPCR for CF10PS3 can be applied in various contexts to monitor and optimize biocontrol strategies. In co-formulant studies, this tool allows researchers to study interactions between CF10PS3 and other co-formulants in biocontrol products. By monitoring the population dynamics of CF10PS3, researchers can infer how different co-formulants affect its survival, colonization, and biocontrol efficacy, leading to the development of optimal formulations that enhance the overall effectiveness of biocontrol agents.

For biocontrol strategy optimization, the specific qPCR can determine the optimal timing and concentration for applying CF10PS3 in the field by monitoring its persistence and activity under different environmental conditions. By quantifying CF10PS3 populations over time, the tool helps establish the most effective dosage and frequency for application, ensuring sustained biocontrol activity.

Environmental impact assessments benefit from monitoring CF10PS3 with specific qPCR, as it helps assess its impact on native microbial communities. Understanding these interactions provides insights into the ecological safety and long-term effects of introducing CF10PS3 into agricultural environments. Additionally, regular monitoring can detect any changes in the susceptibility of target pathogens to CF10PS3, providing early warning signs of resistance development.

In mutant studies for metabolite production, the specific qPCR can

monitor the persistence and impact of mutants of CF10PS3 that are deficient in specific metabolites. This helps elucidate the roles of these metabolites in plant-microbe interactions and biocontrol efficacy. By comparing the behavior of wild-type and mutant CF10PS3 strains, researchers can determine how specific metabolites influence microbial community dynamics, pathogen suppression, and overall plant health.

The specific qPCR can also evaluate how different agricultural practices, such as tillage, crop rotation, and organic amendments, affect the persistence and activity of CF10PS3. This information can guide the development of sustainable farming practices that support biocontrol efficacy. Furthermore, integrating the specific qPCR into Integrated Pest Management (IPM) programs allows for the monitoring of CF10PS3 as part of a broader strategy to manage pests and diseases with minimal chemical inputs.

The broader implications and research opportunities of this work include linking CF10PS3 behavior to ecosystem functions. By monitoring CF10PS3 populations and their interactions with other microbial species, researchers can gain insights into the ecosystem functions supported by this biocontrol agent, such as nutrient cycling, pathogen suppression, and plant growth promotion. Additionally, the specific qPCR tool contributes to a deeper understanding of microbial ecology by providing data on the persistence and behavior of introduced strains within native microbial communities. This can inform ecological theories and models related to microbial colonization and competition.

The insights gained from monitoring CF10PS3 can inform the development of next-generation biocontrol agents tailored to specific crops and environmental conditions. By understanding the factors that influence the success of CF10PS3, researchers can design more effective and resilient biocontrol strategies.

Additionnaly to the qPCR tool developped in Chapter 7, we also took advantage of a newly designed tool opening several other perspectives. PRONAME is a user-friendly pipeline for processing long-read Nanopore metabarcoding data, significantly advancing species-level taxonomic identification in microbial ecology and biocontrol research. By leveraging long-read sequencing technology, PRONAME allows for longer sequence reads, capturing entire gene regions and reducing ambiguities in taxonomic assignments. This enhances the resolution of microbial community analyses. PRONAME generates high-quality consensus sequences, reducing errors and biases, and ensuring reliable taxonomic classifications. Its accessible interface allows researchers of varying bioinformatics expertise to easily process and analyze complex sequencing data, making advanced taxonomic identification more accessible. PRONAME integrates with comprehensive microbial databases,

enhancing species-level identifications and facilitating the discovery of novel or rare taxa. It has been effectively used to identify and monitor specific strains of *P. sivasensis* in the wheat phyllosphere, crucial for understanding biocontrol agents' dynamics and impacts. PRONAME provides accurate and detailed microbial community profiles, enabling researchers to explore relationships between microbial diversity, ecosystem functions, and environmental factors, essential for developing sustainable biocontrol strategies. In microbial ecology, it could help study microbial diversity and functions in different ecosystems, aiding in bioremediation and diversity-function studies. In agricultural and plant sciences, it could profile soil microbiomes under different practices and study plant-microbe interactions, aiding in biofertilizer and biopesticide development. In industrial biotechnology, it could identify microbial strains for enzyme production, biofuel generation, and waste degradation, and monitor fermentation processes. In public health and epidemiology, PRONAME could help tracking microbial contamination sources and study antibiotic resistance gene distribution, informing strategies to combat antibiotic-resistant infections.

9.3 Achievements

The work presented here has made several advancements in theoretical knowledge, allowed by advances in two practical aspects. Accomplishments encompass:

- Comprehensive identification and genomic characterization of *Pseudomonas* species within the wheat microbiome, providing insights into their diversity, presence, and ecological roles throughout different wheat growth stages.
- Demonstration of wheat association with *Pseudomonas sivasensis* in Belgium
- Discovery of a new species named *Pseudomonas arvensis* sp. nov., closely related to *Pseudomonas sivasensis*, and associated with wheat.
- Evaluation of *Pseudomonas sivasensis* CF10PS3 for its potential as a biocontrol agent against *Zymoseptoria tritici*, including assessment of its biocontrol traits and effectiveness in suppressing this pathogen. The ability of CF10PS3 to colonize plant leaves from a simple seed coating and to form large aggregates in preferential locations after foliar spray was also highlighted.
- Documentation of the persistence of the introduced strain CF10PS3 in the wheat phyllosphere, despite environmental challenges.

- Identification of the impacts of strain CF10PS3 on natural populations, suggesting that the introduced strain can coexist with the native microbial communities without major disruption, while favoring specific species.

The methodological developments include:

- The development of novel quantitative PCR (qPCR) protocols for the specific detection and quantification of *Pseudomonas sivasensis* CF10PS3, allowing for precise monitoring of its persistence in the phyllosphere over time.
- A GFP-tagged strain of *Pseudomonas sivasensis* CF10PS3
- The creation of an innovative bioinformatic pipeline capable of conducting species-level metagenomic evaluations, to assess the impact of strain CF10PS3 introduction on the native microbial communities.
- Overall, a methodological pipeline to monitor the wheat cereal leaves phytobiome at field level.

Bibliography

Abdalla, M., Hastings, A., Cheng, K., Yue, Q., Chadwick, D., Espenberg, M., Truu, J., Rees, R. M., and Smith, P. (2019). A critical review of the impacts of cover crops on nitrogen leaching, net greenhouse gas balance and crop productivity. *Global Change Biology*, 25(8):2530–2543.

Adam, E., Bernhart, M., Müller, H., Winkler, J., and Berg, G. (2018). The Cucurbita pepo seed microbiome: genotype-specific composition and implications for breeding. *Plant and Soil*, 422(1-2):35–49.

Afonso, L., Gionco-Cano, B., Simionato, A. S., Niekawa, E. T. G., Pega, G. E. A., Taira, L. A., Dealis, M. L., Andreata, M. F. d. L., Chryssafidis, A. L., and Andrade, G. (2021). Microbial bioactive compounds in plant disease management. *Food Security and Plant Disease Management*, pages 37–61.

Afridi, M. S., Ali, S., Salam, A., César Terra, W., Hafeez, A., Sumaira, Ali, B., S. AlTami, M., Ameen, F., Ercisli, S., Marc, R. A., Medeiros, F. H., and Karunakaran, R. (2022). Plant Microbiome Engineering: Hopes or Hypes. *Biology*, 11(12).

Agisha, V. N., Eapen, S. J., Bhai, R. S., and Kumar, A. (2017). Detecting and monitoring endophytic colonization by Pseudomonas putida BP25 in black pepper (Piper nigrum L.) using quantitative real-time PCR. *Journal of Spices and Aromatic Crops*, 26(1):01.

Ait Tayeb, L., Ageron, E., Grimont, F., and Grimont, P. A. (2005). Molecular phylogeny of the genus Pseudomonas based on rpoB sequences and application for the identification of isolates. *Research in Microbiology*, 156(5-6):763–773.

Ajijah, N., Fiodor, A., Dziurzynski, M., Stasiuk, R., Pawlowska, J., Dziewit, L., and Pranaw, K. (2023). Biocontrol potential of Pseudomonas protegens ML15 against Botrytis cinerea causing gray

mold on postharvest tomato (Solanum lycopersicum var. cerasiforme). *Frontiers in Plant Science*, 14.

Ajouz, S., Bardin, M., Nicot, P. C., and El Maâtaoui, M. (2011). Comparison of the development in planta of a pyrrolnitrin-resistant mutant of Botrytis cinerea and its sensitive wild-type parent isolate. *European Journal of Plant Pathology*, 129(1):31–42.

Ajouz, S., Nicot, P. C., and Bardin, M. (2010). Adaptation to pyrrolnitrin in Botrytis cinerea and cost of resistance. *Plant Pathology*, 59(3):556–566.

Akinnifesi, T. A., Asubiojo, O. I., and Amusan, A. A. (2006). Effects of fungicide residues on the physico-chemical characteristics of soils of a major cocoa-producing area of Nigeria. *Science of the Total Environment*, 366(2-3):876–879.

Akutse, K. S., Subramanian, S., Maniania, N. K., Dubois, T., and Ekesi, S. (2020). Biopesticide Research and Product Development in Africa for Sustainable Agriculture and Food Security – Experiences From the International Centre of Insect Physiology and Ecology (icipe). *Frontiers in Sustainable Food Systems*, 4.

Alahmad, A., Decocq, G., Spicher, F., Kheirbeik, L., Kobaissi, A., Tetu, T., Dubois, F., and Duclercq, J. (2019). Cover crops in arable lands increase functional complementarity and redundancy of bacterial communities. *Journal of Applied Ecology*, 56(3):651–664.

Aleklett, K., Leff, J. W., Fierer, N., and Hart, M. (2015). Wild plant species growing closely connected in a subalpine meadow host distinct root-associated bacterial communities. *PeerJ*, 2015(2).

Ali, N., Khan, S., Yao, H., and Wang, J. (2019). Biochars reduced the bioaccessibility and (bio)uptake of organochlorine pesticides and changed the microbial community dynamics in agricultural soils. *Chemosphere*, 224:805–815.

Alimi, M., Soleimani, M., and Taghinasab Phd, M. (2012). Characterization and application of microbial antagonists for control of fusarium head blight of wheat caused by fusarium graminearum using single and mixture strain of antagonistic bacteria on resistance and susceptible cultivars. *African journal of microbiology research*, 6:326–334.

Almario, J., Bruto, M., Vacheron, J., Prigent-Combaret, C., Moënne-Loccoz, Y., and Muller, D. (2017). Distribution of 2,4-diacetylphloroglucinol biosynthetic genes among the Pseudomonas spp. Reveals unexpected Polyphyletism. *Frontiers in Microbiology*, 8(JUN).

Alsohim, A. S., Taylor, T. B., Barrett, G. A., Gallie, J., Zhang, X. X., Altamirano-Junqueira, A. E., Johnson, L. J., Rainey, P. B., and Jackson, R. W. (2014). The biosurfactant viscosin produced by Pseudomonas fluorescensSBW25 aids spreading motility and plant growth promotion. *Environmental Microbiology*, 16(7):2267–2281.

Anand, A., Chinchilla, D., Tan, C., Mène-Saffrané, L., L'haridon, F., and Weisskopf, L. (2020). Contribution of hydrogen cyanide to the antagonistic activity of pseudomonas strains against phytophthora infestans. *Microorganisms*, 8(8):1–10.

Anantharajah, A., Mingeot-Leclercq, M. P., and Van Bambeke, F. (2016). Targeting the Type Three Secretion System in Pseudomonas aeruginosa. *Trends in Pharmacological Sciences*, 37(9):734–749.

Anderson, E. M., Sychantha, D., Brewer, D., Clarke, A. J., Geddes-McAlister, J., and Khursigara, C. M. (2020). Peptidoglycomics reveals compositional changes in peptidoglycan between biofilm- And planktonic-derived Pseudomonas aeruginosa. *Journal of Biological Chemistry*, 295(2):504–516.

Anderson, L. M., Stockwell, V. O., and Loper, J. E. (2004). An extracellular protease of Pseudomonas fluorescens inactivates antibiotics of Pantoea agglomerans. *Phytopathology*, 94(11):1228–1234.

Andreani, N. A., Martino, M. E., Fasolato, L., Carraro, L., Montemurro, F., Mioni, R., Bordin, P., and Cardazzo, B. (2014). Tracking the blue: A MLST approach to characterise the Pseudomonas fluorescens group. *Food Microbiology*, 39:116–126.

Andreolli, M., Lampis, S., Tosi, L., Marano, V., and Zapparoli, G. (2023). Fungicide sensitivity of grapevine bacteria with plant growth-promoting traits and antagonistic activity as non-target microorganisms. *World Journal of Microbiology and Biotechnology*, 39(5):1–15.

Andreote, F. D., De Araújo, W. L., De Azevedo, J. L., Van Elsas, J. D., Da Rocha, U. N., and Van Overbeek, L. S. (2009). Endophytic colonization of potato (Solanum tuberosum L.) by a novel competent bacterial endophyte, Pseudomonas putida strain P9, and its effect on associated bacterial communities. *Applied and Environmental Microbiology*, 75(11):3396–3406.

Angeli, D., Saharan, K., Segarra, G., Sicher, C., and Pertot, I. (2017). Production of Ampelomyces quisqualis conidia in submerged fermentation and improvements in the formulation for increased shelf-life. *Crop Protection*, 97:135–144.

Angelopoulou, D. J., Naska, E. J., Paplomatas, E. J., and Tjamos, S. E. (2014). Biological control agents (BCAs) of verticillium wilt: Influence of application rates and delivery method on plant protection,

triggering of host defence mechanisms and rhizosphere populations of BCAs. *Plant Pathology*, 63(5):1062–1069.

Araujo, R., Dunlap, C., Barnett, S., and Franco, C. M. (2019). Decoding Wheat Endosphere–Rhizosphere Microbiomes in Rhizoctonia solani–Infested Soils Challenged by Streptomyces Biocontrol Agents. *Frontiers in Plant Science*, 10(August).

Arnault, G., Marais, C., Préveaux, A., Briand, M., Poisson, A. S., Sarniguet, A., Barret, M., and Simonin, M. (2024). Seedling microbiota engineering using bacterial synthetic community inoculation on seeds. *FEMS Microbiology Ecology*, 100(4).

Arp, J., Götze, S., Mukherji, R., Mattern, D. J., García-Altares, M., Klapper, M., Brock, D. A., Brakhage, A. A., Strassmann, J. E., Queller, D. C., Bardl, B., Willing, K., Peschel, G., and Stallforth, P. (2018). Synergistic activity of cosecreted natural products from amoebae-associated bacteria. *Proceedings of the National Academy of Sciences of the United States of America*, 115(15):3758–3763.

Asad, M. A. U., Lavoie, M., Song, H., Jin, Y., Fu, Z., and Qian, H. (2017). Interaction of chiral herbicides with soil microorganisms, algae and vascular plants. *Science of The Total Environment*, 580:1287–1299.

Association Française de Normalisation (AFNOR) (2003). Détection et quantification des organismes végétaux génétiquement modifiés et produits dérivés (XP V 0.020-2). Technical report, AFNOR, Saint-Denis La Plaine.

Astaykina, A., Streletskii, R., Maslov, M., Belov, A., Gorbatov, V., and Stepanov, A. (2020). The Impact of Pesticides on the Microbial Community of Agrosoddy-Podzolic Soil. *Eurasian Soil Science*, 53:696–706.

Atanasov, K. E., Galbis, D. M., Gallego, J., Serpico, A., Bosch, M., Altabella, T., and Ferrer, A. (2022). Pseudomonas germanica sp. nov., isolated from Iris germanica rhizomes. *International Journal of Systematic and Evolutionary Microbiology*, 72(3).

Aung, K., Jiang, Y., and He, S. Y. (2018). The role of water in plant–microbe interactions. *Plant Journal*, 93(4):771–780.

Azarbad, H., Bainard, L. D., Agoussar, A., Tremblay, J., and Yergeau, E. (2022). The response of wheat and its microbiome to contemporary and historical water stress in a field experiment. *ISME Communications*, 2(1).

Babin, D., Deubel, A., Jacquiod, S., Sørensen, S. J., Geistlinger, J., Grosch, R., and Smalla, K. (2019). Impact of long-term agricultural management practices on soil prokaryotic communities. *Soil Biology and Biochemistry*, 129:17–28.

Babu, P., Baranwal, D. K., Harikrishna, Pal, D., Bharti, H., Joshi, P., Thiyagarajan, B., Gaikwad, K. B., Bhardwaj, S. C., Singh, G. P., and Singh, A. (2020). Application of Genomics Tools in Wheat Breeding to Attain Durable Rust Resistance. *Frontiers in Plant Science*, 11.

Backer, R., Rokem, J. S., Ilangumaran, G., Lamont, J., Praslickova, D., Ricci, E., Subramanian, S., and Smith, D. L. (2018). Plant growth-promoting rhizobacteria: Context, mechanisms of action, and roadmap to commercialization of biostimulants for sustainable agriculture. *Frontiers in Plant Science*, 871.

Baćmaga, M., Wyszkowska, J., Borowik, A., and Kucharski, J. (2022). Effects of Tebuconazole Application on Soil Microbiota and Enzymes. *Molecules*, 27(21).

Bakker, P. A., Pieterse, C. M., and Van Loon, L. C. (2007). Induced systemic resistance by fluorescent Pseudomonas spp. *Phytopathology*, 97(2):239–243.

Bakker, P. A. H. M., Pieterse, C. M. J., de Jonge, R., and Berendsen, R. L. (2018). The Soil-Borne Legacy. *Cell*, 172:1178–1180.

Baldan, E., Nigris, S., Romualdi, C., D'Alessandro, S., Clocchiatti, A., Zottini, M., Stevanato, P., Squartini, A., and Baldan, B. (2015). Beneficial bacteria isolated from grapevine inner tissues shape arabidopsis Thaliana roots. *PLoS ONE*, 10(10).

Baldotto, L. E. B. and Olivares, F. L. (2008). Phylloepiphytic interaction between bacteria and different plant species in a tropical agricultural system. *Canadian Journal of Microbiology*, 54(11):918–931.

Banerjee, S., Walder, F., Büchi, L., Meyer, M., Held, A. Y., Gattinger, A., Keller, T., Charles, R., and van der Heijden, M. G. A. (2019). Agricultural intensification reduces microbial network complexity and the abundance of keystone taxa in roots. *The ISME Journal*, 13:1722–1736.

Barbau-Piednoir, E., Mahillon, J., Pillyser, J., Coucke, W., Roosens, N. H., and Botteldoorn, N. (2014). Evaluation of viability-qPCR detection system on viable and dead Salmonella serovar Enteritidis. *Journal of Microbiological Methods*, 103:131–137.

Bardin, M., Ajouz, S., Comby, M., Lopez-Ferber, M., Graillot, B., Siegwart, M., and Nicot, P. C. (2015). Is the efficacy of biological control against plant diseases likely to be more durable than that of chemical pesticides? *Frontiers in Plant Science*, 6(JULY).

Bardin, M., Pressecq, T., Nicot, P. C., and Bouaoud, Y. (2021). Durability of efficacy of microbial bioprotectants against plant diseases. In *Microbial bioprotectants for plant disease management*, pages 103–122. Burleigh Dodds Science Publishing.

Bardin, M. and Siegwart, M. (2022). Can Pests Develop Resistance to Biocontrol Products? *Extended Biocontrol*, pages 267–272.

Barroso-Bergadà, D., Massot, M., Vignolles, N., D'Arcier, J. F., Chancerel, E., Guichoux, E., Walker, A. S., Vacher, C., Bohan, D. A., Laval, V., and Suffert, F. (2023). Metagenomic Next-Generation Sequencing (mNGS) Data Reveal the Phyllosphere Microbiome of Wheat Plants Infected by the Fungal Pathogen Zymoseptoria tritici. *Phytobiomes Journal*, 7(2):281–287.

Bashir, I., War, A. F., Rafiq, I., Reshi, Z. A., Rashid, I., and Shouche, Y. S. (2022). Phyllosphere microbiome: Diversity and functions. *Microbiological Research*, 254.

Battache, M., Lebrun, M. H., Sakai, K., Soudière, O., Cambon, F., Langin, T., and Saintenac, C. (2022). Blocked at the Stomatal Gate, a Key Step of Wheat Stb16q-Mediated Resistance to Zymoseptoria tritici. *Frontiers in Plant Science*, 13(June):1–14.

Batzer, J. C. and Mueller, D. S. (2020). Soybean fungal Endophytes alternaria and Diaporthe spp. Are differentially impacted by fungicide application. *Plant Disease*, 104(1):52–59.

Bejarano, A., Sauer, U., and Preininger, C. (2017). Design and development of a workflow for microbial spray formulations including decision criteria. *Applied Microbiology and Biotechnology*, 101(19):7335–7346.

Bellameche, F., Pedrazzini, C., Mauch-Mani, B., and Mascher, F. (2020). Efficiency of biological and chemical inducers for controlling Septoria tritici leaf blotch (STB) on wheat (Triticum aestivum L.). *European Journal of Plant Pathology*, 158(1):99–109.

Bengtsson, T., Holefors, A., Liljeroth, E., Hultberg, M., and Andreasson, E. (2015). Biosurfactants Have the Potential to Induce Defence Against Phytophthora infestans in Potato. *Potato Research*, 58(1):83–90.

Berg, G., Grube, M., Schloter, M., and Smalla, K. (2014). Unraveling the plant microbiome: Looking back and future perspectives. *Frontiers in Microbiology*, 5(JUN).

Berg, G., Rybakova, D., Fischer, D., Cernava, T., Vergès, M. C. C., Charles, T., Chen, X., Cocolin, L., Eversole, K., Corral, G. H., Kazou, M., Kinkel, L., Lange, L., Lima, N., Loy, A., Macklin, J. A., Maguin, E., Mauchline, T., McClure, R., Mitter, B., Ryan, M., Sarand, I., Smidt, H., Schelkle, B., Roume, H., Kiran, G. S., Selvin, J., de Souza, R. S. C., Van Overbeek, L., Singh, B. K., Wagner, M., Walsh, A., Sessitsch, A., and Schloter, M. (2020a). Microbiome definition revisited: old concepts and new challenges. *Microbiome*, 8(1).

Berg, G., Rybakova, D., Grube, M., and Köberl, M. (2015). The plant microbiome explored: implications for experimental botany. *Journal of Experimental Botany*, 67:995–1002.

Berg, G. and Smalla, K. (2009). Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. *FEMS Microbiology Ecology*, 68:1–13.

Berg, M. and Koskella, B. (2018). Nutrient- and Dose-Dependent Microbiome-Mediated Protection against a Plant Pathogen. *Current Biology*, 28:2487—-2492.e2483.

Berg, S., Dennis, P. G., Paungfoo-Lonhienne, C., Anderson, J., Robinson, N., Brackin, R., Royle, A., DiBella, L., and Schmidt, S. (2020b). Effects of commercial microbial biostimulants on soil and root microbial communities and sugarcane yield. *Biology and Fertility of Soils*, 56:565–580.

Berger, B., Patz, S., Ruppel, S., Dietel, K., Faetke, S., Junge, H., and Becker, M. (2018). Successful Formulation and Application of Plant Growth-Promoting Kosakonia radicincitans in Maize Cultivation. *BioMed Research International*, 2018.

Bernard, F., Chelle, M., Fortineau, A., Riahi El Kamel, O., Pincebourde, S., Sache, I., and Suffert, F. (2022). Daily fluctuations in leaf temperature modulate the development of a foliar pathogen. *Agricultural and Forest Meteorology*, 322.

Berry, C. L., Nandi, M., Manuel, J., Brassinga, A. K. C., Fernando, W. G., Loewen, P. C., and De Kievit, T. R. (2014). Characterization of the Pseudomonas sp. DF41 quorum sensing locus and its role in fungal antagonism. *Biological Control*, 69:82–89.

Berti, A. D., Greve, N. J., Christensen, Q. H., and Thomas, M. G. (2007). Identification of a biosynthetic gene cluster and the six associated lipopeptides involved in swarming motility of Pseudomonas syringae pv. tomato DC3000. *Journal of Bacteriology*, 189(17):6312–6323.

Biessy, A. and Filion, M. (2018). Phenazines in plant-beneficial Pseudomonas spp.: biosynthesis, regulation, function and genomics. *Environmental Microbiology*, 20(11):3905–3917.

Biessy, A., Novinscak, A., Blom, J., Léger, G., Thomashow, L. S., Cazorla, F. M., Josic, D., and Filion, M. (2019). Diversity of phytobeneficial traits revealed by whole-genome analysis of worldwide-isolated phenazine-producing Pseudomonas spp. *Environmental Microbiology*, 21(1):437–455.

Bill, M., Chidamba, L., Gokul, J. K., and Korsten, L. (2021). Mango endophyte and epiphyte microbiome composition during fruit development and post-harvest stages. *Horticulturae*, 7(11).

Bill, M., Gokul, J. K., Viljoen, F., and Korsten, L. (2022). Fungal microbiome shifts on avocado fruit associated with a combination of postharvest chemical and physical interventions. *Journal of Applied Microbiology*, 133(3):1905–1918.

Birolli, W. G., da Silva, B. F., and Rodrigues-Filho, E. (2020). Biodegradation of the fungicide Pyraclostrobin by bacteria from orange cultivation plots. *Science of the Total Environment*, 746.

Bisht, K. (2023). Temperature-specific adaptations and genetic requirements in a biofilm formed by Pseudomonas aeruginosa. *Frontiers in Microbiology*, 13.

Blin, K., Shaw, S., Augustijn, H. E., Reitz, Z. L., Biermann, F., Alanjary, M., Fetter, A., Terlouw, B. R., Metcalf, W. W., Helfrich, E. J., Van Wezel, G. P., Medema, M. H., and Weber, T. (2023). AntiSMASH 7.0: New and improved predictions for detection, regulation, chemical structures and visualisation. *Nucleic Acids Research*, 51(W1):W46–W50.

Blumer, C. and Haas, D. (2000). Mechanism, regulation, and ecological role of bacterial cyanide biosynthesis. *Archives of Microbiology*, 173(3):170–177.

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., Alexander, H., Alm, E. J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J. E., Bittinger, K., Brejnrod, A., Brislawn, C. J., Brown, C. T., Callahan, B. J., Caraballo-Rodríguez, A. M., Chase, J., Cope, E. K., Da Silva, R., Diener, C., Dorrestein, P. C., Douglas, G. M., Durall, D. M., Duvallet, C., Edwardson, C. F., Ernst, M., Estaki, M., Fouquier, J., Gauglitz, J. M., Gibbons, S. M., Gibson, D. L., Gonzalez, A., Gorlick, K., Guo, J., Hillmann, B., Holmes, S., Holste, H., Huttenhower, C., Huttley, G. A., Janssen, S., Jarmusch, A. K., Jiang, L., Kaehler, B. D., Kang, K. B., Keefe, C. R., Keim, P., Kelley, S. T., Knights, D., Koester, I., Kosciolek, T., Kreps, J., Langille, M. G., Lee, J., Ley, R., Liu, Y. X., Loftfield, E., Lozupone, C., Maher, M., Marotz, C., Martin, B. D., McDonald, D., McIver, L. J., Melnik, A. V., Metcalf, J. L., Morgan, S. C., Morton, J. T., Naimey, A. T., Navas-Molina, J. A., Nothias, L. F., Orchanian, S. B., Pearson, T., Peoples, S. L., Petras, D., Preuss, M. L., Pruesse, E., Rasmussen, L. B., Rivers, A., Robeson, M. S., Rosenthal, P., Segata, N., Shaffer, M., Shiffer, A., Sinha, R., Song, S. J., Spear, J. R., Swafford, A. D., Thompson, L. R., Torres, P. J., Trinh, P., Tripathi, A., Turnbaugh, P. J., Ul-Hasan, S., van der Hooft, J. J., Vargas, F., Vázquez-Baeza, Y., Vogtmann, E., von Hippel, M., Walters, W., Wan, Y., Wang, M., Warren, J., Weber, K. C., Williamson, C. H., Willis, A. D., Xu, Z. Z., Zaneveld, J. R., Zhang, Y., Zhu, Q., Knight, R., and Caporaso, J. G. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology*, 37(8):852–857.

Bonanomi, G., Antignani, V., Capodilupo, M., and Scala, F. (2010). Identifying the characteristics of organic soil amendments that suppress soilborne plant diseases. *Soil Biology and Biochemistry*, 42:136–144.

Bonanomi, G., De Filippis, F., Cesarano, G., La Storia, A., Ercolini, D., and Scala, F. (2016). Organic farming induces changes in soil microbiota that affect agro-ecosystem functions. *Soil Biology and Biochemistry*, 103:327–336.

Bonanomi, G., Lorito, M., Vinale, F., and Woo, S. L. (2018). Organic Amendments, Beneficial Microbes, and Soil Microbiota: Toward a Unified Framework for Disease Suppression. *Annual Review of Phytopathology*, 56:1–20.

Bonaterra, A., Badosa, E., Daranas, N., Francés, J., Roselló, G., and Montesinos, E. (2022). Bacteria as Biological Control Agents of Plant Diseases. *Microorganisms*, 10(9).

Braga, R. M., Dourado, M. N., and Araújo, W. L. (2016). Microbial interactions: ecology in a molecular perspective. *Brazilian Journal of Microbiology*, 47:86–98.

Brent, K. J. and Hollomon, D. W. (2007). Fungicide resistance in crop pathogens: How can it be managed? *FRAC Monograph 1, 2nd edition, Brussels: FRAC.*, page 60.

Briard, B., Bomme, P., Lechner, B. E., Mislin, G. L., Lair, V., Prévost, M. C., Latgé, J. P., Haas, H., and Beauvais, A. (2015). Pseudomonas aeruginosa manipulates redox and iron homeostasis of its microbiota partner Aspergillus fumigatus via phenazines. *Scientific Reports*, 5.

Bricout, A., Morris, C. E., Chandeysson, C., Duban, M., Boistel, C., Chataigné, G., Lecouturier, D., Jacques, P., Leclère, V., and Rochex, A. (2022). The Diversity of Lipopeptides in the Pseudomonas syringae Complex Parallels Phylogeny and Sheds Light on Structural Diversification during Evolutionary History. *Microbiology Spectrum*, 10(6).

Broeders, S., Huber, I., Grohmann, L., Berben, G., Taverniers, I., Mazzara, M., Roosens, N., and Morisset, D. (2014). Guidelines for validation of qualitative real-time PCR methods. *Trends in Food Science and Technology*, 37(2):115–126.

Brown, J. K., Chartrain, L., Lasserre-Zuber, P., and Saintenac, C. (2015). Genetics of resistance to Zymoseptoria tritici and applications to wheat breeding. *Fungal Genetics and Biology*, 79:33–41.

Buddrus-Schiemann, K., Schmid, M., Schreiner, K., Welzl, G., and Hartmann, A. (2010). Root Colonization by Pseudomonas sp. DSMZ 13134 and Impact on the Indigenous Rhizosphere Bacterial Community of Barley. *Microbial Ecology*, 60(2):381–393.

Bulgarelli, D., Rott, M., Schlaeppi, K., Ver Loren van Themaat, E., Ahmadinejad, N., Assenza, F., Rauf, P., Huettel, B., Reinhardt, R., Schmelzer, E., Peplies, J., Gloeckner, F. O., Amann, R., Eickhorst, T., and Schulze-Lefert, P. (2012). Revealing structure and assembly cues for Arabidopsis root-inhabiting bacterial microbiota. *Nature*, 488(7409):91–95.

Bultreys, A. and Gheysen, I. (1999). Biological and molecular detection of toxic lipodepsipeptide-producing Pseudomonas syringae strains and PCR identification in plants. *Applied and Environmental Microbiology*, 65(5):1904–1909.

Bultreys, A. and Gheysen, I. (2000). Production and comparison of peptide siderophores from strains of distantly related pathovars of Pseudomonas syringae and Pseudomonas viridiflava LMG 2352. *Applied and Environmental Microbiology*, 66(1):325–331.

Burch, A. Y., Zeisler, V., Yokota, K., Schreiber, L., and Lindow, S. E. (2014). The hygroscopic biosurfactant syringafactin produced by Pseudomonas syringae enhances fitness on leaf surfaces during fluctuating humidity. *Environmental Microbiology*, 16(7):2086–2098.

Busse, H. J. (2016). Review of the taxonomy of the genus Arthrobacter, emendation of the genus arthrobacter sensu lato, proposal to reclassify selected species of the genus Arthrobacter in the novel genera Glutamicibacter gen. Nov., Paeniglutamicibacter gen. nov., Pseudoglutamicibacter gen. nov., Paenarthrobacter gen. nov. and pseudarthrobacter gen. nov., and emended description of Arthrobacter roseus. *International Journal of Systematic and Evolutionary Microbiology*, 66(1):9–37.

Calderón, C. E., Pérez-García, A., De Vicente, A., and Cazorla, F. M. (2013). The dar genes of pseudomonas chlororaphis PCL1606 are crucial for biocontrol activity via production of the antifungal compound 2-hexyl, 5-propyl resorcinol. *Molecular Plant-Microbe Interactions*, 26(5):554–565.

Calvo, P., Nelson, L., and Kloepper, J. W. (2014). Agricultural uses of plant biostimulants. *Plant and Soil*, 383:3–41.

Cania, B., Vestergaard, G., Suhadolc, M., Mihelič, R., Krauss, M., Fliessbach, A., Mäder, P., Szumełda, A., Schloter, M., and Schulz, S. (2020). Site-Specific Conditions Change the Response of Bacterial Producers of Soil Structure-Stabilizing Agents Such as Exopolysaccharides and Lipopolysaccharides to Tillage Intensity. *Frontiers in Microbiology*, 11:568.

Carlström, C. I., Field, C. M., Bortfeld-Miller, M., Müller, B., Sunagawa, S., and Vorholt, J. A. (2019). Synthetic microbiota reveal priority effects and keystone strains in the Arabidopsis phyllosphere. *Nature Ecology and Evolution*, 3(10):1445–1454.

Carmi, R., Carmeli, S., Levy, E., and Gough, F. J. (1994). (+)-(S)-dihydroaeruginoic acid, an inhibitor of Septoria tritici and other phytopathogenic fungi and bacteria, produced by Pseudomonas fluorescens. *Journal of Natural Products*, 57(9):1200–1205.

Castaldi, S., Masi, M., Sautua, F., Cimmino, A., Isticato, R., Carmona, M., Tuzi, A., and Evidente, A. (2021). Pseudomonas fluorescens showing antifungal activity against macrophomina phaseolina, a severe pathogenic fungus of soybean, produces phenazine as the main active metabolite. *Biomolecules*, 11(11).

Caulier, S., Gillis, A., Colau, G., Licciardi, F., Liépin, M., Desoignies, N., Modrie, P., Legrève, A., Mahillon, J., and Bragard, C. (2018). Versatile Antagonistic Activities of Soil-Borne Bacillus spp. and Pseudomonas spp. against Phytophthora infestans and Other Potato Pathogens. *Frontiers in Microbiology*, Vol 9.

Čaušević, S., Dubey, M., Morales, M., Salazar, G., Sentchilo, V., Carraro, N., Ruscheweyh, H. J., Sunagawa, S., and van der Meer, J. R. (2024). Niche availability and competitive loss by facilitation control proliferation of bacterial strains intended for soil microbiome interventions. *Nature Communications*, 15(1):1–19.

Cernava, T., Chen, X., Krug, L., Li, H., Yang, M., and Berg, G. (2019). The tea leaf microbiome shows specific responses to chemical pesticides and biocontrol applications. *Science of the Total Environment*, 667:33–40.

Cesa-Luna, C., Geudens, N., Girard, L., De Roo, V., Maklad, H. R., Martins, J. C., Höfte, M., and De Mot, R. (2023). Charting the Lipopeptidome of Nonpathogenic Pseudomonas. *mSystems*, 8(1).

Cesarano, G., De Filippis, F., La Storia, A., Scala, F., and Bonanomi, G. (2017). Organic amendment type and application frequency affect crop yields, soil fertility and microbiome composition. *Applied Soil Ecology*, 120:254–264.

Ceyhan SÖNMEZ et al (2017). *Biological control in sustainable agriculture.*, volume 50. Unknown.

Chai, Y., Senay, S., Horvath, D., and Pardey, P. (2022). Multi-peril pathogen risks to global wheat production: A probabilistic loss and investment assessment. *Frontiers in Plant Science*, 13.

Chaparro, J. M., Badri, D. V., and Vivanco, J. M. (2014). Rhizosphere microbiome assemblage is affected by plant development. *ISME Journal*, 8(4):790–803.

Chaudhry, V., Runge, P., Sengupta, P., Doehlemann, G., Parker, J. E., and Kemen, E. (2021). Shaping the leaf microbiota: Plant-microbe-microbe interactions. *Journal of Experimental Botany*, 72(1):36–56.

Chemeltorit, P. P., Mutaqin, K. H., and Widodo, W. (2017). Combining Trichoderma hamatum THSW13 and Pseudomonas aeruginosa BJ10–86: a synergistic chili pepper seed treatment for Phytophthora capsici infested soil. *European Journal of Plant Pathology*, 147(1):157–166.

Chen, J., Sharifi, R., Khan, M. S. S., Islam, F., Bhat, J. A., Kui, L., and Majeed, A. (2022). Wheat Microbiome: Structure, Dynamics, and Role in Improving Performance Under Stress Environments. *Frontiers in Microbiology*, 12.

Chen, L., Xin, X., Zhang, J., Redmile-Gordon, M., Nie, G., and Wang, Q. (2019). Soil Characteristics Overwhelm Cultivar Effects on the Structure and Assembly of Root-Associated Microbiomes of Modern Maize. *Pedosphere*, 29:360–373.

Chen, Q., Xin, Y., and Liu, Z. (2020a). Long-Term Fertilization with Potassium Modifies Soil Biological Quality in K-Rich Soils. *Agronomy*, 10:771.

Chen, S. K. and Edwards, C. A. (2001). A microcosm approach to assess the effects of fungicides on soil ecological processes and plant growth: Comparisons of two soil types. *Soil Biology and Biochemistry*, 33(14):1981–1991.

Chen, T., Nomura, K., Wang, X., Sohrabi, R., Xu, J., Yao, L., Paasch, B. C., Ma, L., Kremer, J., Cheng, Y., Zhang, L., Wang, N., Wang, E., Xin, X. F., and He, S. Y. (2020b). A plant genetic network for preventing dysbiosis in the phyllosphere. *Nature*, 580(7805):653–657.

Chen, X., Krug, L., Yang, M., Berg, G., and Cernava, T. (2020c). Conventional seed coating reduces prevalence of proteobacterial endophytes in Nicotiana tabacum. *Industrial Crops and Products*, 155.

Chen, X., Wicaksono, W. A., Berg, G., and Cernava, T. (2021). Bacterial communities in the plant phyllosphere harbour distinct responders to a broad-spectrum pesticide. *Science of the Total Environment*, 751.

Chen, Y., Wang, J., Yang, N., Wen, Z., Sun, X., Chai, Y., and Ma, Z. (2018). Wheat microbiome bacteria can reduce virulence of a plant pathogenic fungus by altering histone acetylation. *Nature Communications*, 9(1).

Cheng, C., Qi, W., XueWei, L., and YuanYuan, L. (2021). Complete genome sequence of Pseudomonas taiwanensis wrs8, a plant growth-promoting and biotite-weathering strain. *Microbiology Resource Announcements*, 10(18).

Chin-A-Woeng, T. F., Bloemberg, G. V., and Lugtenberg, B. J. (2003). Phenazines and their role in biocontrol by Pseudomonas bacteria. *New Phytologist*, 157(3):503–523.

Chincholkar, S. and Thomashow, L. S. (2013). Microbial phenazines: Biosynthesis, agriculture and health. *Microbial Phenazines: Biosynthesis, Agriculture and Health*, 9783642405:1–248.

Choi, K. H. and Schweizer, H. P. (2006). mini-Tn7 insertion in bacteria with secondary, non-glmS-linked attTn7 sites: Example Proteus mirabilis HI4320. *Nature Protocols*, 1(1):170–178.

Chowdhury, S. P., Hartmann, A., Gao, X. W., and Borriss, R. (2015). Biocontrol mechanism by root-associated Bacillus amyloliquefaciens FZB42 - A review. *Frontiers in Microbiology*, 6(JUL).

Christiansen, L., Alanin, K. S., Christopher, C. B., Olsson, S., Stougaard, P., and Hennessy, R. C. (2020). Fungal-Associated Molecules Induce Key Genes Involved in the Biosynthesis of the Antifungal Secondary Metabolites Nunamycin and Nunapeptin in the Biocontrol Strain Pseudomonas fluorescens In5. *Applied and Environmental Microbiology*, 86(21).

CIMMYT (2020). The cereals imperative of future food systems.

Cloutier, M. L., Murrell, E., Barbercheck, M., Kaye, J., Finney, D., García-González, I., and Bruns, M. A. (2020). Fungal community shifts in soils with varied cover crop treatments and edaphic properties. *Scientific Reports*, 10:6198.

Cole, B. J., Feltcher, M. E., Waters, R. J., Wetmore, K. M., Mucyn, T. S., Ryan, E. M., Wang, G., Ul-Hasan, S., McDonald, M., Yoshikuni, Y., Malmstrom, R. R., Deutschbauer, A. M., Dangl, J. L., and Visel, A. (2017). Genome-wide identification of bacterial plant colonization genes. *PLoS Biology*, 15(9).

Colla, G., Hoagland, L., Ruzzi, M., Cardarelli, M., Bonini, P., Canaguier, R., and Rouphael, Y. (2017). Biostimulant Action of Protein Hydrolysates: Unraveling Their Effects on Plant Physiology and Microbiome. *Frontiers in Plant Science*, 8:2202.

Colla, G., Nardi, S., Cardarelli, M., Ertani, A., Lucini, L., Canaguier, R., and Rouphael, Y. (2015). Protein hydrolysates as biostimulants in horticulture. *Scientia Horticulturae*, 196:28–37.

Colombowala and Aruna (2018). Studies on Optimization of Siderophore Production By Pseudomonas Aeruginosa Azar 11 Isolated From Aquatic Soil and Its Antibacterial Activity. *Online) IJPBS TM —*, 8(October 2018):714–731.

Commission, C. A. (2010). Guidelines on performance criteria and validation of methods for detection, identification and quantification of specific DNA sequences and specific protiens in foods. Technical report, Codex committee on methods of analysis and sampling.

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

Copeland, J. K., Yuan, L., Layeghifard, M., Wang, P. W., and Guttman, D. S. (2015). Seasonal community succession of the phyllosphere microbiome. *Molecular Plant-Microbe Interactions*, 28(3):274–285.

Corder asbl (2023). Estimation quantitative des utilisations de produits phytopharmaceutiques par les différents secteurs d'activité en Wallonie.

Cornelis, P. (2010). Iron uptake and metabolism in pseudomonads. *Applied Microbiology and Biotechnology*, 86(6):1637–1645.

Cowen, L. E. (2001). Predicting the emergence of resistance to antifungal drugs. *FEMS Microbiology Letters*, 204(1):1–7.

Crini, G., Exposito Saintemarie, A., Rocchi, S., Fourmentin, M., Jeanvoine, A., Millon, L., and Morin-Crini, N. (2017). Simultaneous removal of five triazole fungicides from synthetic solutions on activated carbons and cyclodextrin-based adsorbents. *Heliyon*, 3(8):e00380.

Crouzet, J., Arguelles-Arias, A., Dhondt-Cordelier, S., Cordelier, S., Pršić, J., Hoff, G., Mazeyrat-Gourbeyre, F., Baillieul, F., Clément, C., Ongena, M., and Dorey, S. (2020). Biosurfactants in Plant Protection Against Diseases: Rhamnolipids and Lipopeptides Case Study. *Frontiers in Bioengineering and Biotechnology*, 8.

Cui, E., Fan, X., Li, Z., Liu, Y., Neal, A. L., Hu, C., and Gao, F. (2019). Variations in soil and plant-microbiome composition with different quality irrigation waters and biochar supplementation. *Applied Soil Ecology*, 142:99–109.

Cui, X., Harling, R., Mutch, P., and Darling, D. (2005). Identification of N -3-hydroxyoctanoyl-homoserine lactone production in Pseudomonas fluorescens 5064, pathogenic to broccoli, and controlling biosurfactant production by quorum sensing. *European Journal of Plant Pathology*, 111(4):297–308.

D'aes, J., De Maeyer, K., Pauwelyn, E., and Höfte, M. (2010). Biosurfactants in plant-Pseudomonas interactions and their importance to biocontrol. *Environmental Microbiology Reports*, 2(3):359–372.

D'aes, J., Kieu, N. P., Léclère, V., Tokarski, C., Olorunleke, F. E., De Maeyer, K., Jacques, P., Höfte, M., and Ongena, M. (2014). To settle or to move? The interplay between two classes of cyclic lipopeptides in the biocontrol strain Pseudomonas CMR12a. *Environmental Microbiology*, 16(7):2282–2300.

Dai, Y., Yang, F., Zhang, L., Xu, Z., Fan, X., Tian, Y., and Wang, T. (2020a). Wheat-associated microbiota and their correlation with stripe rust reaction. *Journal of Applied Microbiology*, 128(2):544–555.

Dai, Z., Liu, G., Chen, H., Chen, C., Wang, J., Ai, S., Wei, D., Li, D., Ma, B., Tang, C., Brookes, P. C., and Xu, J. (2020b). Long-term nutrient inputs shift soil microbial functional profiles of phosphorus cycling in diverse agroecosystems. *The ISME Journal*, 14:757–770.

Daims, H., Nielsen, J. L., Nielsen, P. H., Schleifer, K. H., and Wagner, M. (2001). In Situ Characterization of Nitrospira-Like Nitrite-Oxidizing Bacteria Active in Wastewater Treatment Plants. *Applied and Environmental Microbiology*, 67(3-12):5273–5284.

Damo, J. L. C., Shimizu, T., Sugiura, H., Yamamoto, S., Agake, S. I., Anarna, J., Tanaka, H., Sugihara, S., Okazaki, S., Yokoyama, T., Yasuda, M., and Ohkama-Ohtsu, N. (2023). The Application of Sulfur Influences Microbiome of Soybean Rhizosphere and Nutrient-Mobilizing Bacteria in Andosol. *Microorganisms*, 11(5).

Daranas, N., Badosa, E., Francés, J., Montesinos, E., and Bonaterra, A. (2018a). Enhancing water stress tolerance improves fitness in biological control strains of Lactobacillus plantarum in plant environments. *PLoS ONE*, 13(1).

Daranas, N., Bonaterra, A., Francés, J., Cabrefiga, J., Montesinos, E., and Badosa, E. (2018b). Monitoring viable cells of the biological control agent Lactobacillus plantarum PM411 in aerial plant surfaces by means of a strain-specific viability quantitative PCR method. *Applied and Environmental Microbiology*, 84(10).

Darlison, J., Mogren, L., Rosberg, A. K., Grudén, M., Minet, A., Liné, C., Mieli, M., Bengtsson, T., Håkansson, Å., Uhlig, E., Becher, P. G., Karlsson, M., and Alsanius, B. W. (2019). Leaf mineral content govern microbial community structure in the phyllosphere of spinach (Spinacia oleracea) and rocket (Diplotaxis tenuifolia). *Science of the Total Environment*, 675:501–512.

Dartnell, L. R., Hunter, S. J., Lovell, K. V., Coates, A. J., and Ward, J. M. (2010). Low-temperature ionizing radiation resistance of deinococcus radiodurans and antarctic dry valley bacteria. *Astrobiology*, 10(7):717–732.

Daw, M. E. (2009). Sustainable Agriculture and Food Security in an Era of Oil Scarcity: Lessons from Cuba. By J. Wright. London: Earthscan (2009), pp. 261, £60.00. ISBN 078-1-84407-572-0. *Experimental Agriculture*, 45(3):378–379.

De Bruijn, I., De Kock, M. J., Yang, M., De Waard, P., Van Beek, T. A., and Raaijmakers, J. M. (2007). Genome-based discovery, structure

prediction and functional analysis of cyclic lipopeptide antibiotics in Pseudomonas species. *Molecular Microbiology*, 63(2):417–428.

De Bruijn, I. and Raaijmakers, J. M. (2009). Diversity and functional analysis of LuxR-type transcriptional regulators of cyclic lipopeptide biosynthesis in Pseudomonas fluorescens. *Applied and Environmental Microbiology*, 75(14):4753–4761.

De Clercq, D., Cognet, S., Pujol, M., Lepoivre, P., and Jijakli, M. H. (2003). Development of a SCAR marker and a semi-selective medium for specific quantification of Pichia anomala strain K on apple fruit surfaces. *Postharvest Biology and Technology*, 29(3):237–247.

De Corato, U. (2020). Soil microbiota manipulation and its role in suppressing soil-borne plant pathogens in organic farming systems under the light of microbiome-assisted strategies. *Chemical and Biological Technologies in Agriculture*, 7(1).

de Vries, W. and Schulte-Uebbing, L. (2019). Required Changes in Nitrogen Inputs and Nitrogen Use Efficiencies To Reconcile Agricultural Productivity With Water and Air Quality Objectives in the Eu-27. In *International Fertiliser Society Conference, Cambridge*, volume 842, pages 1–40.

Debode, F., Caulier, S., Demeter, S., Dubois, B., Gelhay, V., Hulin, J., Muhovski, Y., Ninane, V., Rousseau, G., and Bragard, C. (2024). Roadmap for the integration of environmental microbiomes in risk assessments under efsa's remit. *EFSA Supporting Publications*, 21(2):8602E.

Degrune, F., Boeraeve, F., Dufrêne, M., Cornélis, J.-T., Frey, B., and Hartmann, M. (2019). The Pedological Context Modulates the Response of Soil Microbial Communities to Agroecological Management. *Frontiers in Ecology and Evolution*, 7:261.

Degrune, F., Theodorakopoulos, N., Colinet, G., Hiel, M.-P., Bodson, B., Taminiau, B., Daube, G., Vandenbol, M., and Hartmann, M. (2017). Temporal Dynamics of Soil Microbial Communities below the Seedbed under Two Contrasting Tillage Regimes. *Frontiers in Microbiology*, 8:1127.

Degrune, F., Theodorakopoulos, N., Dufrêne, M., Colinet, G., Bodson, B., Hiel, M.-P., Taminiau, B., Nezer, C., Daube, G., and Vandenbol, M. (2016). No favorable effect of reduced tillage on microbial community diversity in a silty loam soil (Belgium). *Agriculture, Ecosystems and Environment*, 224:12–21.

Deketelaere, S., Tyvaert, L., França, S. C., and Hofte, M. (2017). Desirable traits of a good biocontrol agent against Verticillium wilt. *Frontiers in Microbiology*, 8(JUL).

Delitte, M., Caulier, S., Bragard, C., and Desoignies, N. (2021). Plant Microbiota Beyond Farming Practices: A Review .

Desmyttere, H., Deweer, C., Muchembled, J., Sahmer, K., Jacquin, J., Coutte, F., and Jacques, P. (2019). Antifungal activities of bacillus subtilis lipopeptides to two venturia inaequalis strains possessing different tebuconazole sensitivity. *Frontiers in Microbiology*, 10(OCT).

Detheridge, A. P., Brand, G., Fychan, R., Crotty, F. V., Sanderson, R., Griffith, G. W., and Marley, C. L. (2016). The legacy effect of cover crops on soil fungal populations in a cereal rotation. *Agriculture, Ecosystems and Environment*, 228:49–61.

Déziel, E., Lépine, F., Milot, S., and Villemur, R. (2003). rhlA is required for the production of a novel biosurfactant promoting swarming motility in Pseudomonas aeruginosa: 3-(3-hydroxyalkanoyloxy)alkanoic acids (HAAs), the precursors of rhamnolipids. *Microbiology*, 149(8):2005–2013.

Dinelli, G., Bonetti, A., Marotti, I., Minelli, M., Busi, S., and Catizone, P. (2007). Root exudation of diclofop-methyl and triasulfuron from foliar-treated durum wheat and ryegrass. *Weed Research*, 47:25–33.

Döll, P. (2002). Impact of Climate Change and Variability on Irrigation Requirements: A Global Perspective. *Climatic Change*, 54:269–293.

du Jardin, P. (2015). Plant biostimulants: Definition, concept, main categories and regulation. *Scientia Horticulturae*, 196:3–14.

Dubern, J. F., Coppoolse, E. R., Stiekema, W. J., and Bloemberg, G. V. (2008). Genetics and functional characterization of the gene cluster directing the biosynthesis of putisolvin I and II in Pseudomonas putida strain PCL1445. *Microbiology*, 154(7):2070–2083.

Dubern, J. F., Lagendijk, E. L., Lugtenberg, B. J., and Bloemberg, G. V. (2005). The heat shock genes dnaK, dnaJ, and grpE are involved in regulation of putisolvin biosynthesis in Pseudomonas putida PCL1445. *Journal of Bacteriology*, 187(17):5967–5976.

Dubern, J. F., Lugtenberg, B. J., and Bloemberg, G. V. (2006). The ppuI-rsaL-ppuR quorum-sensing system regulates biofilm formation of Pseudomonas putida PCL1445 by controlling biosynthesis of the cyclic lipopeptides putisolvins I and II. *Journal of Bacteriology*, 188(8):2898–2906.

Dubois, B., Debode, F., Hautier, L., Hulin, J., Martin, G. S., Delvaux, A., Janssen, E., and Mingeot, D. (2022). A detailed workflow to develop QIIME2-formatted reference databases for taxonomic analysis of DNA metabarcoding data. *BMC Genomic Data*, 23(1):1–14.

Duffy, B., Schouten, A., and Raaijmakers, J. M. (2003). Pathogen Self-Defense: Mechanisms to Counteract Microbial Antagonism. *Annual Review of Phytopathology*, 41:501–538.

Duman, M., Mulet, M., Saticioglu, I. B., Altun, S., Gomila, M., Lalucat, J., and García-Valdés, E. (2020). Pseudomonas sivasensis sp. nov. isolated from farm fisheries in Turkey. *Systematic and Applied Microbiology*, 43(4).

Durham, D. R., Stewart, D. B., and Stellwag, E. J. (1987). Novel alkaline- and heat-stable serine proteases from alkalophilic Bacillus sp. strain GX6638. *Journal of Bacteriology*, 169(6):2762–2768.

Dutta, S., Yu, S. M., and Lee, Y. H. (2020). Assessment of the contribution of antagonistic secondary metabolites to the antifungal and biocontrol activities of pseudomonas fluorescens nbc275. *Plant Pathology Journal*, 36(5):491–496.

Ebenso, B., Otu, A., Giusti, A., Cousin, P., Adetimirin, V., Razafindralambo, H., Effa, E., Gkisakis, V., Thiare, O., Levavasseur, V., Kouhounde, S., Adeoti, K., Rahim, A., and Mounir, M. (2022). Nature-Based One Health Approaches to Urban Agriculture Can Deliver Food and Nutrition Security. *Frontiers in Nutrition*, 9.

Ehlers, R. U. (2011). Regulation of biological control agents and the EU policy support action REBECA. *Regulation of Biological Control Agents*, pages 3–23.

Elad, Y. (2000). Biological control of foliar pathogens by means of Trichoderma harzianum and potential modes of action. *Crop Protection*, 19:709–714.

Elad, Y. and Kirshner, B. (1993). Survival in the phylloplane of an introduced biocontrol agent (Trichoderma harzianum) and populations of the plant pathogen Botrytis cinerea as modified by abiotic conditions. *Phytoparasitica*, 21(4):303–313.

Elad, Y., Williamson, B., Tudzynski, P., and Delen, N. (2007). Botrytis: Biology, pathology and control. *Botrytis: Biology, Pathology and Control*, pages 1–403.

Ellis, J. G. (2017). Can plant microbiome studies lead to effective biocontrol of plant diseases? *Molecular Plant-Microbe Interactions*, 30(3):190–193.

England, L. S., Hung, L., and Trevors, J. T. (1997). Persistence of Pseudomonas aureofaciens strains and DNA in soil. *Soil Biology and Biochemistry*, 29(9-10):1521–1527.

Estep, L. K., Torriani, S. F., Zala, M., Anderson, N. P., Flowers, M. D., Mcdonald, B. A., Mundt, C. C., and Brunner, P. C. (2015). Emergence and early evolution of fungicide resistance in North American populations of Zymoseptoria tritici. *Plant Pathology*, 64(4):961–971.

European Commission (2020). A Farm to Fork Strategy for a Fair, Healthy and Environmentally-Friendly Food System. Technical report, European Commission.

European Commission (2021). Statistical Factsheet Belgium. *Directorate-General for Agriculture and Rural Development*, June.

Falardeau, J., Wise, C., Novitsky, L., and Avis, T. J. (2013). Ecological and Mechanistic Insights Into the Direct and Indirect Antimicrobial Properties of Bacillus subtilis Lipopeptides on Plant Pathogens. *Journal of Chemical Ecology*, 39(7):869–878.

FAO (2017). The future of food and agriculture: Trends and challenges. https://www.fao.org/3/i6583e/i6583e.pdf. Technical report, Food and Agriculture Organization of the United Nations, Rome.

FAO (2022). The State of Food Security and Nutrition in the World 2022. *The State of Food Security and Nutrition in the World 2022*.

Fedele, G., Brischetto, C., and Rossi, V. (2020). Biocontrol of Botrytis cinerea on Grape Berries as Influenced by Temperature and Humidity. *Frontiers in Plant Science*, 11.

Fernandez, A. L., Sheaffer, C. C., Wyse, D. L., Staley, C., Gould, T. J., and Sadowsky, M. J. (2016). Structure of bacterial communities in soil following cover crop and organic fertilizer incorporation. *Applied Microbiology and Biotechnology*, 100:9331–9341.

Fernández-Calviño, D. and Bååth, E. (2010). Growth response of the bacterial community to pH in soils differing in pH. *FEMS Microbiology Ecology*, 73:149–156.

Ferrarini, E., De Roo, V., Geudens, N., Martins, J. C., and Höfte, M. (2022). Altering in vivo membrane sterol composition affects the activity of the cyclic lipopeptides tolaasin and sessilin against Pythium. *Biochimica et Biophysica Acta - Biomembranes*, 1864(10).

Fierer, N. (2017). Embracing the unknown: Disentangling the complexities of the soil microbiome. *Nature Reviews Microbiology*, 15(10):579–590.

Fierer, N. and Jackson, R. B. (2006). The diversity and biogeography of soil bacterial communities. *Proceedings of the National Academy of Sciences of the United States of America*, 103(3):626–631.

Fierer, N., Lauber, C. L., Ramirez, K. S., Zaneveld, J., Bradford, M. A., and Knight, R. (2012). Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. *The ISME Journal*, 6:1007–1017.

Fierer, N., Schimel, J. P., and Holden, P. A. (2003). Influence of Drying–Rewetting Frequency on Soil Bacterial Community Structure. *Microbial Ecology*, 45:63–71.

Fillinger, S., Ajouz, S., Nicot, P. C., Leroux, P., and Bardin, M. (2012). Functional and structural comparison of Pyrrolnitrin- and Iprodione-induced modifications in the class III histidine-kinase Bos1 of Botrytis cinerea. *PLoS ONE*, 7(8).

Finkel, O. M., Castrillo, G., Herrera Paredes, S., Salas González, I., and Dangl, J. L. (2017). Understanding and exploiting plant beneficial microbes. *Current Opinion in Plant Biology*, 38:155–163.

Finney, D. M., Buyer, J. S., and Kaye, J. P. (2017). Living cover crops have immediate impacts on soil microbial community structure and function. *Journal of Soil and Water Conservation*, 72:361–373.

Fitzpatrick, A. W., Llabrés, S., Neuberger, A., Blaza, J. N., Bai, X. C., Okada, U., Murakami, S., Van Veen, H. W., Zachariae, U., Scheres, S. H., Luisi, B. F., and Du, D. (2017). Structure of the MacAB-TolC ABC-type tripartite multidrug efflux pump. *Nature Microbiology*, 2.

Flaishman, M., Eyal, Z., Voisard, C., and Haas, D. (1990). Suppression of Septoria tritici by Phenazine- or Siderophore-deficient mutants of Pseudomonas. *Current Microbiology*, 20(2):121–124.

Flaishman, M. A., Eyal, Z., Zilberstein, A., Voisard, C., and Haas, D. (1996). Suppression of septoria tritici blotch and leaf rust of wheat by recombinant cyanide-producing strains of Peudomonas putida. *Molecular Plant-Microbe Interactions*, 9(7):642–645.

Flamand, M. C., Pelsser, S., Ewbank, E., and Maraite, H. (1996). Production of syringotoxin and other bioactive peptides by Pseudomonas fuscovaginae. *Physiological and Molecular Plant Pathology*, 48(4):217–231.

Flint, H. J. and Bayer, E. A. (2008). Plant cell wall breakdown by anaerobic microorganisms from the mammalian digestive tract. *Annals of the New York Academy of Sciences*, 1125:280–288.

Flores-Núñez, V. M., Fonseca-García, C., Desgarennes, D., Eloe-Fadrosh, E., Woyke, T., and Partida-Martínez, L. P. (2020). Functional Signatures of the Epiphytic Prokaryotic Microbiome of Agaves and Cacti. *Frontiers in Microbiology*, 10(January):1–13.

Fones, H. and Gurr, S. (2015). The impact of Septoria tritici Blotch disease on wheat: An EU perspective. *Fungal Genetics and Biology*, 79:3–7.

Fones, H. N., Eyles, C. J., Kay, W., Cowper, J., and Gurr, S. J. (2017). A role for random, humidity-dependent epiphytic growth prior to invasion of wheat by Zymoseptoria tritici. *Fungal Genetics and Biology*, 106(July):51–60.

Fournier, B., Pereira Dos Santos, S., Gustavsen, J. A., Imfeld, G., Lamy, F., Mitchell, E. A. D., Mota, M., Noll, D., Planchamp, C., and Heger, T. J. (2020). Impact of a synthetic fungicide (fosetyl-Al and propamocarb-hydrochloride) and a biopesticide (Clonostachys rosea) on soil bacterial, fungal, and protist communities. *Science of The Total Environment*, 738:139635.

Francioli, D., Cid, G., Hajirezaei, M. R., and Kolb, S. (2022). Leaf bacterial microbiota response to flooding is controlled by plant phenology in wheat (Triticum aestivum L.). *Scientific Reports*, 12(1).

Frapolli, M., Pothier, J. F., Défago, G., and Moënne-Loccoz, Y. (2012). Evolutionary history of synthesis pathway genes for phloroglucinol and cyanide antimicrobials in plant-associated fluorescent pseudomonads. *Molecular Phylogenetics and Evolution*, 63(3):877–890.

Frenk, S., Hadar, Y., and Minz, D. (2014). Resilience of soil bacterial community to irrigation with water of different qualities under Mediterranean climate. *Environmental Microbiology*, 16:559–569.

Frenk, S., Hadar, Y., and Minz, D. (2018). Quality of Irrigation Water Affects Soil Functionality and Bacterial Community Stability in Response to Heat Disturbance. *Applied and Environmental Microbiology*, 84:e02087–02017.

Frison, E. A., Cherfas, J., and Hodgkin, T. (2011). Agricultural biodiversity is essential for a sustainable improvement in food and nutrition security. *Sustainability*, 3(1):238–253.

Fukami, J., Nogueira, M. A., Araujo, R. S., and Hungria, M. (2016). Accessing inoculation methods of maize and wheat with Azospirillum brasilense. *AMB Express*, 6(1):1–13.

Galle, M., Carpentier, I., and Beyaert, R. (2013). Structure and Function of the Type III Secretion System of Pseudomonas aeruginosa. *Current Protein and Peptide Science*, 13(8):831–842.

Garland, G., Edlinger, A., Banerjee, S., Degrune, F., García-Palacios, P., Pescador, D. S., Herzog, C., Romdhane, S., Saghai, A., Spor, A., Wagg, C., Hallin, S., Maestre, F. T., Philippot, L., Rillig, M. C., and van der Heijden, M. G. A. (2021). Crop cover is more important than rotational diversity for soil multifunctionality and cereal yields in European cropping systems. *Nature Food*, 2:28–37.

Garrido-Oter, R., Nakano, R. T., Dombrowski, N., Ma, K. W., McHardy, A. C., and Schulze-Lefert, P. (2018). Modular Traits of the Rhizobiales Root Microbiota and Their Evolutionary Relationship with Symbiotic Rhizobia. *Cell Host and Microbe*, 24(1):155–167.e5.

Gaur, V. K., Tripathi, V., Gupta, P., Dhiman, N., Regar, R. K., Gautam, K., Srivastava, J. K., Patnaik, S., Patel, D. K., and Manickam, N. (2020). Rhamnolipids from Planococcus spp. and their mechanism of action against pathogenic bacteria. *Bioresource Technology*, 307.

Geudens, N. and Martins, J. C. (2018). Cyclic lipodepsipeptides from Pseudomonas spp. - Biological Swiss-Army knives. *Frontiers in Microbiology*, 9(AUG).

Geudens, N., Nasir, M. N., Crowet, J. M., Raaijmakers, J. M., Fehér, K., Coenye, T., Martins, J. C., Lins, L., Sinnaeve, D., and Deleu, M. (2017). Membrane Interactions of Natural Cyclic Lipodepsipeptides of the Viscosin Group. *Biochimica et Biophysica Acta - Biomembranes*, 1859(3):331–339.

Geyer, K. M., Altrichter, A. E., Takacs-Vesbach, C. D., Van Horn, D. J., Gooseff, M. N., and Barrett, J. E. (2014). Bacterial community composition of divergent soil habitats in a polar desert. *FEMS Microbiology Ecology*, 89:490–494.

Ghadamgahi, F., Tarighi, S., Taheri, P., Saripella, G. V., Anzalone, A., Kalyandurg, P. B., Catara, V., Ortiz, R., and Vetukuri, R. R. (2022). Plant Growth-Promoting Activity of Pseudomonas aeruginosa FG106 and Its Ability to Act as a Biocontrol Agent against Potato, Tomato and Taro Pathogens. *Biology*, 11(1).

Ghssein, G. and Ezzeddine, Z. (2022). A Review of Pseudomonas aeruginosa Metallophores: Pyoverdine, Pyochelin and Pseudopaline. *Biology*, 11(12).

Girard, L., Geudens, N., Pauwels, B., Höfte, M., Martins, J. C., and De Mot, R. (2022). Transporter Gene-Mediated Typing for Detection and Genome Mining of Lipopeptide-Producing Pseudomonas. *Applied and Environmental Microbiology*, 88(2).

Girard, L., Höfte, M., and Mot, R. D. (2020). Lipopeptide families at the interface between pathogenic and beneficial Pseudomonas-plant interactions. *Critical Reviews in Microbiology*, 46(4):397–419.

Girard, L., Lood, C., Hofte, M., Vandamme, P., Rokni-Zadeh, H., van Noort, V., Lavigne, R., and De Mot, R. (2021). The ever-expanding Pseudomonas genus: Description of 43 new species and partition of the Pseudomonas putida group. *Microorganisms*, 9(8).

Glick, B. R. (2012). Plant Growth-Promoting Bacteria: Mechanisms and Applications. *Scientifica*, 2012:1–15.

Gómez-Lama Cabanás, C., Wentzien, N. M., Zorrilla-Fontanesi, Y., Valverde-Corredor, A., Fernández-González, A. J., Fernández-López, M., and Mercado-Blanco, J. (2022). Impacts of the Biocontrol Strain Pseudomonas simiae PICF7 on the Banana Holobiont: Alteration of Root Microbial Co-occurrence Networks and Effect on Host Defense Responses. *Frontiers in Microbiology*, 13.

Götze, S. and Stallforth, P. (2020). Structure, properties, and biological functions of nonribosomal lipopeptides from pseudomonads. *Natural Product Reports*, 37(1):29–54.

Gramisci, B. R., Lutz, M. C., Lopes, C. A., and Sangorrín, M. P. (2018). Enhancing the efficacy of yeast biocontrol agents against postharvest pathogens through nutrient profiling and the use of other additives. *Biological Control*, 121:151–158.

Gravouil, M. V., Havis, N. D., Hoebe, P. N., and Hoad, S. P. (2016). Changes in Leaf Microbiome Linked To Disease Susceptibility. In *The Dundee Conference: Crop Protection in Northern Britain 2016*.

Green, E. R. and Mecsas, J. (2016). Bacterial secretion systems: An overview. *Virulence Mechanisms of Bacterial Pathogens*, pages 213–239.

Gross, H. and Loper, J. E. (2009). Genomics of secondary metabolite production by Pseudomonas spp. *Natural Product Reports*, 26(11):1408–1446.

Gruet, C., Abrouk, D., Börner, A., Muller, D., and Moënne-Loccoz, Y. (2023). Wheat genome architecture influences interactions with phytobeneficial microbial functional groups in the rhizosphere. *Plant Cell and Environment*, 46(3):1018–1032.

Gu, G., Yin, H. B., Ottesen, A., Bolten, S., Patel, J., Rideout, S., and Nou, X. (2019). Microbiomes in ground water and alternative irrigation water, and spinach microbiomes impacted by irrigation with different types of water. *Phytobiomes Journal*, 3(2):137–147.

Gu, Y., Ma, Y. N., Wang, J., Xia, Z., and Wei, H. L. (2020). Genomic insights into a plant growth-promoting Pseudomonas koreensis strain with cyclic lipopeptide-mediated antifungal activity. *MicrobiologyOpen*, 9(9).

Gu, Y. L., Li, J. Z., Li, Y., Cong, S., Wang, J., Ma, Y. N., and Wei, H. L. (2023). Pseudomonas Cyclic Lipopeptide Medpeptin: Biosynthesis and Modulation of Plant Immunity. *Engineering*, 28:153–165.

Gubry-Rangin, C., Hai, B., Quince, C., Engel, M., Thomson, B. C., James, P., Schloter, M., Griffiths, R. I., Prosser, J. I., and Nicol, G. W. (2011). Niche specialization of terrestrial archaeal ammonia oxidizers. *Proceedings of the National Academy of Sciences*, 108:21206–21211.

Haas, D. and Défago, G. (2005). Biological control of soil-borne pathogens by fluorescent pseudomonads. *Nature Reviews Microbiology*, 3(4):307–319.

Hagemann, M., Ribbeck-Busch, K., Klähn, S., Hasse, D., Steinbruch, R., and Berg, G. (2008). The plant-associated bacterium Stenotrophomonas rhizophila expresses a new enzyme for the synthesis of the compatible solute glucosylglycerol. *Journal of Bacteriology*, 190(17):5898–5906.

Haichar, F. e. Z., Cernava, T., Liu, J., and Timm, C. M. (2021). Editorial: Novel Insights Into the Response of the Plant Microbiome to Abiotic Factors. *Frontiers in Plant Science*, 12.

Hamonts, K., Trivedi, P., Garg, A., Janitz, C., Grinyer, J., Holford, P., Botha, F. C., Anderson, I. C., and Singh, B. K. (2018). Field study reveals core plant microbiota and relative importance of their drivers. *Environmental Microbiology*, 20(1):124–140.

Han, B., Pain, A., and Johnstone, K. (1997). Spontaneous duplication of a 661 bp element within a two-component sensor regulator gene causes phenotypic switching in colonies of Pseudomonas tolaasii, cause of brown blotch disease of mushrooms. *Molecular Microbiology*, 25(2):211–218.

Han, L., Kong, X., Xu, M., and Nie, J. (2021). Repeated exposure to fungicide tebuconazole alters the degradation characteristics, soil microbial community and functional profiles. *Environmental Pollution*, 287.

Hardoim, P. R., Hardoim, C. C. P., van Overbeek, L. S., and van Elsas, J. D. (2012). Dynamics of Seed-Borne Rice Endophytes on Early Plant Growth Stages. *PLOS ONE*, 7:e30438.

Hardoim, P. R., van Overbeek, L. S., Berg, G., Pirttilä, A. M., Compant, S., Campisano, A., Döring, M., and Sessitsch, A. (2015). The Hidden World within Plants: Ecological and Evolutionary Considerations for Defining Functioning of Microbial Endophytes. *Microbiology and Molecular Biology Reviews*, 79(3):293–320.

Harman, G., Khadka, R., Doni, F., and Uphoff, N. (2021). Benefits to Plant Health and Productivity From Enhancing Plant Microbial Symbionts. *Frontiers in Plant Science*, 11.

Harman, G. E. (2006). Overview of mechanisms and uses of Trichoderma spp. *Phytopathology*, 96(2):190–194.

Hartman, K., van der Heijden, M. G. A., Wittwer, R. A., Banerjee, S., Walser, J.-C., and Schlaeppi, K. (2018). Cropping practices manipulate abundance patterns of root and soil microbiome members paving the way to smart farming. *Microbiome*, 6:14.

Hartmann, A., Rothballer, M., and Schmid, M. (2008). Lorenz Hiltner, a pioneer in rhizosphere microbial ecology and soil bacteriology research. *Plant and Soil*, 312(1-2):7–14.

Hartmann, A., Schmid, M., van Tuinen, D., and Berg, G. (2009). Plant-driven selection of microbes. *Plant and Soil*, 321(1-2):235–257.

Hartmann, M., Frey, B., Mayer, J., Mäder, P., and Widmer, F. (2015). Distinct soil microbial diversity under long-term organic and conventional farming. *The ISME Journal*, 9:1177–1194.

Havryliuk, O., Hovorukha, V., Patrauchan, M., Youssef, N. H., and Tashyrev, O. (2020). Draft whole genome sequence for four highly copper resistant soil isolates Pseudomonas lactis strain UKR1, Pseudomonas panacis strain UKR2, and Pseudomonas veronii strains UKR3 and UKR4. *Current Research in Microbial Sciences*, 1:44–52.

He, M., Tian, G., Semenov, A. M., and van Bruggen, A. H. C. (2012). Short-Term Fluctuations of Sugar Beet Damping-Off by Pythium ultimum in Relation to Changes in Bacterial Communities After Organic Amendments to Two Soils. *Phytopathology®*, 102:413–420.

Heeb, S. and Haas, D. (2001). Regulatory roles of the GacS/GacA two-component system in plant-associated and other Gram-negative bacteria. *Molecular Plant-Microbe Interactions*, 14(12):1351–1363.

Hellin, P., Duvivier, M., Heick, T. M., Fraaije, B. A., Bataille, C., Clinckemaillie, A., Legrève, A., Jørgensen, L. N., Andersson, B., Samils, B., Rodemann, B., Berg, G., Hutton, F., Garnault, M., El Jarroudi, M., Couleaud, G., and Kildea, S. (2021). Spatio-temporal distribution of DMI and SDHI fungicide resistance of Zymoseptoria tritici throughout Europe based on frequencies of key target-site alterations. *Pest Management Science*, 77(12):5576–5588.

Hennessy, R. C., Phippen, C. B., Nielsen, K. F., Olsson, S., and Stougaard, P. (2017). Biosynthesis of the antimicrobial cyclic lipopeptides nunamycin and nunapeptin by Pseudomonas fluorescens strain In5 is regulated by the LuxR-type transcriptional regulator NunF. *MicrobiologyOpen*, 6(6).

Hermenau, R., Kugel, S., Komor, A. J., and Hertweck, C. (2020). Helper bacteria halt and disarm mushroom pathogens by linearizing structurally diverse cyclolipopeptides. *Proceedings of the National Academy of Sciences of the United States of America*, 117(38):23802–23806.

Hernandez, R. E., Gallegos-Monterrosa, R., and Coulthurst, S. J. (2020). Type VI secretion system effector proteins: Effective weapons for bacterial competitiveness. *Cellular Microbiology*, 22(9).

Hernandez-Jerez, A. F., Adriaanse, P., Aldrich, A., Berny, P., Coja, T., Duquesne, S., Marinovich, M., Millet, M., Pelkonen, O., Pieper, S., Tiktak, A., Topping, C. J., Wolterink, G., Herman, L., Chiusolo, A., Magrans, J. O., and Widenfalk, A. (2020). Statement on the translocation potential by Pseudomonas chlororaphis MA342 in plants after seed treatment of cereals and peas and assessment of the risk to humans.

Hill, D. S., Stein, J. I., Torkewitz, N. R., Morse, A. M., Howell, C. R., Pachlatko, J. P., Becker, J. O., and Ligon, J. M. (1994). Cloning of genes involved in the synthesis of pyrrolnitrin from Pseudomonas fluorescens and role of pyrrolnitrin synthesis in biological control of plant disease. *Applied and Environmental Microbiology*, 60(1):78–85.

Hinsinger, P., Betencourt, E., Bernard, L., Brauman, A., Plassard, C., Shen, J., Tang, X., and Zhang, F. (2011). P for Two, Sharing a Scarce Resource: Soil Phosphorus Acquisition in the Rhizosphere of Intercropped Species. *Plant Physiology*, 156:1078–1086.

Hobbs, P. R., Sayre, K., and Gupta, R. (2008). The role of conservation agriculture in sustainable agriculture. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 363:543–555.

Hockett, K. L., Burch, A. Y., and Lindow, S. E. (2013). Thermo-Regulation of Genes Mediating Motility and Plant Interactions in Pseudomonas syringae. *PLoS ONE*, 8(3).

Hofte, M. (2021). The use of Pseudomonas spp. as bacterial biocontrol agents to control plant diseases. *BURLEIGH DODDS SERIES IN AGRICULTURAL SCIENCE*, pages 301–374.

Horner, A., Browett, S. S., and Antwis, R. E. (2019). Mixed-Cropping Between Field Pea Varieties Alters Root Bacterial and Fungal Communities. *Scientific Reports*, 9:16953.

Howe, A., Stopnisek, N., Dooley, S. K., Yang, F., Grady, K. L., and Shade, A. (2023). Seasonal activities of the phyllosphere microbiome of perennial crops. *Nature Communications*, 14(1):1–16.

Howell, C. (2003). Mechanisms Employed by Trichoderma Species in the Biological Control of Plant Diseases: The History and Evolution of Current Concepts. *Plant Disease*, 87(1):4–10.

Howell, C. R. (1980). Suppression of Pythium ultimum -Induced Damping-Off of Cotton Seedlings by Pseudomonas fluorescens and its Antibiotic, Pyoluteorin . *Phytopathology*, 70(8):712.

Hu, H.-W., Zhang, L.-M., Dai, Y., Di, H.-J., and He, J.-Z. (2013). pH-dependent distribution of soil ammonia oxidizers across a large geographical scale as revealed by high-throughput pyrosequencing. *Journal of Soils and Sediments*, 13:1439–1449.

Hu, L. et al. (2018). Root exudate metabolites drive plant–soil feedbacks on growth and defense by shaping the rhizosphere microbiota. *Nat. Commun. 9*, 9(2738).

Hu, W., Gao, Q., Hamada, M. S., Dawood, D. H., Zheng, J., Chen, Y., and Ma, Z. (2014). Potential of Pseudomonas chlororaphis subsp. aurantiaca Strain Pcho10 as a biocontrol agent against Fusarium graminearum. *Phytopathology*, 104(12):1289–1297.

Huang, C. J., Pauwelyn, E., Ongena, M., Debois, D., Leclère, V., Jacques, P., Bleyaert, P., and Höfte, M. (2015). Characterization of cichopeptins, new phytotoxic cyclic lipodepsipeptides produced by Pseudomonas cichorii SF1-54 and their role in bacterial midrib rot disease of lettuce. *Molecular Plant-Microbe Interactions*, 28(9):1009–1022.

Huang, L., Chen, M. M., Wang, W., Hu, H. B., Peng, H. S., Xu, Y. Q., and Zhang, X. H. (2011). Enhanced production of 2-hydroxyphenazine in Pseudomonas chlororaphis GP72. *Applied Microbiology and Biotechnology*, 89(1):169–177.

Hulot, J. and Hiller, N. (2021). Exploring the benefits of biocontrol for sustainable agriculture – A literature review on biocontrol in light of the European Green Deal. *Institute for European Environmental Policy*.

Hungria, M., Campo, R. J., Souza, E. M., and Pedrosa, F. O. (2010). Inoculation with selected strains of Azospirillum brasilense and A. lipoferum improves yields of maize and wheat in Brazil. *Plant and Soil*, 331(1):413–425.

Ibrahim, E., Nasser, R., Hafeez, R., Ogunyemi, S. O., Abdallah, Y., Khattak, A. A., Shou, L., Zhang, Y., Ahmed, T., Atef Hatamleh, A., Abdullah Al-Dosary, M., M. Ali, H., Luo, J., and Li, B. (2023). Biocontrol Efficacy of Endophyte Pseudomonas poae to Alleviate Fusarium Seedling Blight by Refining the Morpho-Physiological Attributes of Wheat. *Plants*, 12(12).

Inceoğlu, Ö., Salles, J. F., van Overbeek, L., and van Elsas, J. D. (2010). Effects of Plant Genotype and Growth Stage on the Betaproteobacterial Communities Associated with Different Potato Cultivars in Two Fields. *Applied and Environmental Microbiology*, 76:3675–3684.

Inderbitzin, P., Ward, J., Barbella, A., Solares, N., Izyumin, D., Burman, P., Chellemi, D. O., and Subbarao, K. V. (2018). Soil Microbiomes Associated with Verticillium Wilt-Suppressive Broccoli and Chitin Amendments are Enriched with Potential Biocontrol Agents. *Phytopathology®*, 108:31–43.

Islam, M. A., Nain, Z., Alam, M. K., Banu, N. A., and Islam, M. R. (2018). In vitro study of biocontrol potential of rhizospheric pseudomonas aeruginosa against fusarium oxysporum f. Sp. cucumerinum. *Egyptian Journal of Biological Pest Control*, 28(1):1–11.

Ivanova, A. A., Zhelezova, A. D., Chernov, T. I., and Dedysh, S. N. (2020). Linking ecology and systematics of acidobacteria: Distinct habitat preferences of the Acidobacteriia and Blastocatellia in tundra soils. *PLoS ONE*, 15(3).

Jacoby, R., Peukert, M., Succurro, A., Koprivova, A., and Kopriva, S. (2017). The Role of Soil Microorganisms in Plant Mineral Nutrition—Current Knowledge and Future Directions. *Frontiers in Plant Science*, 8:1617.

Jahanshah, G., Yan, Q., Gerhardt, H., Pataj, Z., Lämmerhofer, M., Pianet, I., Josten, M., Sahl, H. G., Silby, M. W., Loper, J. E., and Gross, H. (2019). Discovery of the cyclic lipopeptide gacamide a by genome mining and repair of the defective gaca regulator in pseudomonas fluorescens pf0-1. *Journal of Natural Products*, 82(2):301–308.

Jambhulkar, P. P., Sharma, P., Manokaran, R., Lakshman, D. K., Rokadia, P., and Jambhulkar, N. (2018). Assessing synergism of combined applications of Trichoderma harzianum and Pseudomonas fluorescens to control blast and bacterial leaf blight of rice. *European Journal of Plant Pathology*, 152(3):747–757.

Jamil, S., Shahzad, R., Ahmad, S., Fatima, R., Zahid, R., Anwar, M., Iqbal, M. Z., and Wang, X. (2020). Role of Genetics, Genomics, and Breeding Approaches to Combat Stripe Rust of Wheat. *Frontiers in Nutrition*, 7.

Janakiev, T., Dimkić, I., Bojić, S., Fira, D., Stanković, S., and Berić, T. (2020). Bacterial communities of plum phyllosphere and characterization of indigenous antagonistic Bacillus thuringiensis R3/3 isolate. *Journal of Applied Microbiology*, 128(2):528–543.

Jasim, B., Anish, M. C., Shimil, V., Jyothis, M., and Radhakrishnan, E. K. (2015). Studies on Plant Growth Promoting Properties of Fruit-Associated Bacteria from Elettaria cardamomum and Molecular Analysis of ACC Deaminase Gene. *Applied Biochemistry and Biotechnology*, 177:175–189.

Jasim, B., Mathew, J., and Radhakrishnan, E. K. (2016). Identification of a novel endophytic Bacillus sp. from Capsicum annuum with highly efficient and broad spectrum plant probiotic effect. *Journal of Applied Microbiology*, 121:1079–1094.

Jensen, D. F., Karlsson, M., Sarrocco, S., and Vannacci, G. (2016). Biological Control Using Microorganisms as an Alternative to Disease Resistance. *Plant Pathogen Resistance Biotechnology*, pages 341–363.

Jenul, C., Sieber, S., Daeppen, C., Mathew, A., Lardi, M., Pessi, G., Hoepfner, D., Neuburger, M., Linden, A., Gademann, K., and Eberl, L. (2018). Biosynthesis of fragin is controlled by a novel quorum sensing signal. *Nature Communications*, 9(1).

Ji, R., Dong, G., Shi, W., and Min, J. (2017). Effects of liquid organic fertilizers on plant growth and rhizosphere soil characteristics of chrysanthemum. *Sustainability*, 9:841.

Johnston-Monje, D., Gutiérrez, J. P., and Lopez-Lavalle, L. A. B. (2021). Seed-Transmitted Bacteria and Fungi Dominate Juvenile Plant Microbiomes. *Frontiers in Microbiology*, 12(October).

Kaduskar, R. D., Scala, G. D., Al Jabri, Z. J., Arioli, S., Musso, L., Oggioni, M. R., Dallavalle, S., and Mora, D. (2017). Promysalin is a salicylate-containing antimicrobial with a cell-membrane-disrupting mechanism of action on Gram-positive bacteria. *Scientific Reports*, 7(1).

Kahl, L. J., Eckartt, K. N., Morales, D. K., Price-Whelan, A., and Dietrich, L. E. (2022). Light/Dark and Temperature Cycling Modulate Metabolic Electron Flow in Pseudomonas aeruginosa Biofilms. *mBio*, 13(4).

Kamali, E., Jamali, A., Izanloo, A., and Ardebili, A. (2021). In vitro activities of cellulase and ceftazidime, alone and in combination against Pseudomonas aeruginosa biofilms. *BMC Microbiology*, 21(1):1–10.

Kamilova, F., Validov, S., Azarova, T., Mulders, I., and Lugtenberg, B. (2005). Enrichment for enhanced competitive plant root tip colonizers selects for a new class of biocontrol bacteria. *Environmental Microbiology*, 7(11):1809–1817.

Karlen, D. L., Wollenhaupt, N. C., Erbach, D. C., Berry, E. C., Swan, J. B., Eash, N. S., and Jordahl, J. L. (1994). Crop residue effects on soil quality following 10-years of no-till corn. *Soil and Tillage Research*, 31:149–167.

Karlsson, M., Atanasova, L., Jensen, D. F., and Zeilinger, S. (2017). Necrotrophic mycoparasites and their genomes. *The Fungal Kingdom*, pages 1005–1026.

Karlsson Green, K., Stenberg, J. A., and Lankinen, Å. (2020). Making sense of Integrated Pest Management (IPM) in the light of evolution. *Evolutionary Applications*, 13(8):1791–1805.

Katsoula, A., Vasileiadis, S., Sapountzi, M., and Karpouzas, D. G. (2020). The response of soil and phyllosphere microbial communities to repeated application of the fungicide iprodione: accelerated biodegradation or toxicity? *FEMS Microbiology Ecology*, 96.

Kavamura, V. N., Mendes, R., Bargaz, A., and Mauchline, T. H. (2021). Defining the wheat microbiome: Towards microbiome-facilitated crop production. *Computational and Structural Biotechnology Journal*, 19:1200–1213.

Kecskés, M. L., Choudhury, A. T., Casteriano, A. V., Deaker, R., Roughley, R. J., Lewin, L., Ford, R., and Kennedy, I. R. (2016). Effects of bacterial inoculant biofertilizers on growth, yield and nutrition of rice Australia. *Journal of Plant Nutrition*, 39(3):377–388.

Keesing, F., Belden, L. K., Daszak, P., Dobson, A., Harvell, C. D., Holt, R. D., Hudson, P., Jolles, A., Jones, K. E., Mitchell, C. E., Myers, S. S., Bogich, T., and Ostfeld, R. S. (2010). Impacts of biodiversity on the emergence and transmission of infectious diseases. *Nature*, 468:647–652.

Kennedy, N. A., Walker, A. W., Berry, S. H., Duncan, S. H., Farquarson, F. M., Louis, P., and Thomson, J. M. (2014). The Impact of Different DNA Extraction Kits and Laboratories upon the Assessment of Human Gut Microbiota Composition by 16S rRNA Gene Sequencing. *PLOS ONE*, 9:e88982.

Khattari, Z., Al-Abdullah, T., Maghrabi, M., Khasim, S., Roy, A., and Fasfous, I. (2015). Interaction study of lipopeptide biosurfactant viscosin with DPPC and cholesterol by langmuir monolayer technique. *Soft Materials*, 13(4):254–262.

Kildea, S., Ransbotyn, V., Khan, M. R., Fagan, B., Leonard, G., Mullins, E., and Doohan, F. M. (2008). Bacillus megaterium shows potential for the biocontrol of septoria tritici blotch of wheat. *Biological Control*, 47(1):37–45.

Kim, N., Zabaloy, M. C., Guan, K., and Villamil, M. B. (2020). Do cover crops benefit soil microbiome? A meta-analysis of current research. *Soil Biology and Biochemistry*, 142:107701.

Kirner, S., Hammer, P. E., Hill, D. S., Altmann, A., Fischer, I., Weislo, L. J., Lanahan, M., Van Pee, K. H., and Ligon, J. M. (1998). Functions encoded by pyrrolnitrin biosynthetic genes from Pseudomonas fluorescens. *Journal of Bacteriology*, 180(7):1939–1943.

Kitten, T., Kinscherf, T. G., McEvoy, J. L., and Willis, D. K. (1998). A newly identified regulator is required for virulence and toxin production in Pseudomonas syringae. *Molecular Microbiology*, 28(5):917–929.

Kloepper, J. W., Leong, J., Teintze, M., and Schroth, M. N. (1980). Enhanced plant growth by siderophores produced by plant growth-promoting rhizobacteria. *Nature*, 286:885–886.

Knight, R., Vrbanac, A., Taylor, B. C., Aksenov, A., Callewaert, C., Debelius, J., Gonzalez, A., Kosciolek, T., McCall, L.-I., McDonald,

D., Melnik, A. V., Morton, J. T., Navas, J., Quinn, R. A., Sanders, J. G., Swafford, A. D., Thompson, L. R., Tripathi, A., Xu, Z. Z., Zaneveld, J. R., Zhu, Q., Caporaso, J. G., and Dorrestein, P. C. (2018). Best practices for analysing microbiomes. *Nature Reviews Microbiology*, 16:410–422.

Köberl, M., Wagner, P., Müller, H., Matzer, R., Unterfrauner, H., Cernava, T., and Berg, G. (2020). Unraveling the Complexity of Soil Microbiomes in a Large-Scale Study Subjected to Different Agricultural Management in Styria. *Frontiers in Microbiology*, 11.

Koch, B., Nielsen, T. H., Sørensen, D., Andersen, J. B., Christophersen, C., Molin, S., Givskov, M., Sørensen, J., and Nybroe, O. (2002). Lipopeptide production in Pseudomonas sp. strain DSS73 is regulated by components of sugar beet seed exudate via the Gac two-component regulatory system. *Applied and Environmental Microbiology*, 68(9):4509–4516.

Köhl, J., Kolnaar, R., and Ravensberg, W. J. (2019a). Mode of action of microbial biological control agents against plant diseases: Relevance beyond efficacy. *Frontiers in Plant Science*, 10.

Köhl, J., Kolnaar, R., and Ravensberg, W. J. (2019b). Mode of action of microbial biological control agents against plant diseases: Relevance beyond efficacy. *Frontiers in Plant Science*, 10.

Kopecky, J., Samkova, Z., Sarikhani, E., Kyselková, M., Omelka, M., Kristufek, V., Divis, J., Grundmann, G. G., Moënne-Loccoz, Y., and Sagova-Mareckova, M. (2019). Bacterial, archaeal and micro-eukaryotic communities characterize a disease-suppressive or conducive soil and a cultivar resistant or susceptible to common scab. *Scientific Reports*, 9:14883.

Kreft, L., Botzki, A., Coppens, F., Vandepoele, K., and Van Bel, M. (2017). PhyD3: A phylogenetic tree viewer with extended phyloXML support for functional genomics data visualization. *Bioinformatics*, 33(18):2946–2947.

Kron, A. S., Zengerer, V., Bieri, M., Dreyfuss, V., Sostizzo, T., Schmid, M., Lutz, M., Remus-Emsermann, M. N., and Pelludat, C. (2020). Pseudomonas orientalis F9 pyoverdine, safracin, and phenazine mutants remain effective antagonists against erwinia amylovora in apple flowers. *Applied and Environmental Microbiology*, 86(8).

Krzyzanowska, D. M., Iwanicki, A., Czajkowski, R., and Jafra, S. (2021). High-Quality Complete Genome Resource of Tomato Rhizosphere Strain Pseudomonas donghuensis P482, a Representative of a Species with Biocontrol Activity against Plant Pathogens. *Molecular Plant-Microbe Interactions*, 34(12):1450–1454.

Ku, T., Liu, Y., Xie, Y., Hu, J., Hou, Y., Tan, X., Ning, X., Li, G., and Sang, N. (2023). Tebuconazole mediates cognitive impairment via the microbe-gut-brain axis (MGBA) in mice. *Environment International*, 173.

Kumar, S., Joshi, D., Pandey, S. C., Debbarma, P., Suyal, D. C., Chaubey, A. K., and Soni, R. (2021). Structure and Functions of Rice and Wheat Microbiome. *Survival Strategies in Coldadapted Microorganisms*, pages 343–356.

Kwak, M.-J., Kong, H. G., Choi, K., Kwon, S.-K., Song, J. Y., Lee, J., Lee, P. A., Choi, S. Y., Seo, M., Lee, H. J., Jung, E. J., Park, H., Roy, N., Kim, H., Lee, M. M., Rubin, E. M., Lee, S.-W., and Kim, J. F. (2018). Rhizosphere microbiome structure alters to enable wilt resistance in tomato. *Nature Biotechnology*, 36(11):1100–1109.

Ladygina, N. and Hedlund, K. (2010). Plant species influence microbial diversity and carbon allocation in the rhizosphere. *Soil Biology and Biochemistry*, 42(2):162–168.

Lagesen, K., Hallin, P., Rødland, E. A., Stærfeldt, H. H., Rognes, T., and Ussery, D. W. (2007). RNAmmer: Consistent and rapid annotation of ribosomal RNA genes. *Nucleic Acids Research*, 35(9):3100–3108.

Lahlali, R., Ezrari, S., Radouane, N., Kenfaoui, J., Esmaeel, Q., El Hamss, H., Belabess, Z., and Barka, E. A. (2022). Biological Control of Plant Pathogens: A Global Perspective. *Microorganisms*, 10(3).

Lahlali, R., Massart, S., De Clercq, D., Serrhini, M. N., Creemers, P., and Jijakli, M. H. (2009). Assessment of Pichia anomala (strain K) efficacy against blue mould of apples when applied pre- or post-harvest under laboratory conditions and in orchard trials. *European Journal of Plant Pathology*, 123(1):37–45.

Lahlali, R., Peng, G., Gossen, B. D., McGregor, L., Yu, F. Q., Hynes, R. K., Hwang, S. F., McDonald, M. R., and Boyetchko, S. M. (2013). Evidence that the biofungicide serenade (Bacillus subtilis) suppresses clubroot on canola via antibiosis and induced host resistance. *Phytopathology*, 103(3):245–254.

Lalucat, J., Gomila, M., Mulet, M., Zaruma, A., and García-Valdés, E. (2022). Past, present and future of the boundaries of the Pseudomonas genus: Proposal of Stutzerimonas gen. Nov. *Systematic and Applied Microbiology*, 45(1).

Lapsansky, E. R., Milroy, A. M., Andales, M. J., and Vivanco, J. M. (2016). Soil memory as a potential mechanism for encouraging sustainable plant health and productivity. *Current Opinion in Biotechnology*, 38:137–142.

Larkin, R. P. and Griffin, T. S. (2007). Control of soilborne potato diseases using Brassica green manures. *Crop Protection*, 26:1067–1077.

Latha, P., Anand, T., Prakasam, V., Jonathan, E. I., Paramathma, M., and Samiyappan, R. (2011). Combining Pseudomonas, Bacillus and Trichoderma strains with organic amendments and micronutrient to enhance suppression of collar and root rot disease in physic nut. *Applied Soil Ecology*, 49:215–223.

Latz, E., Eisenhauer, N., Rall, B. C., Allan, E., Roscher, C., Scheu, S., and Jousset, A. (2012). Plant diversity improves protection against soil-borne pathogens by fostering antagonistic bacterial communities. *Journal of Ecology*, 100:597–604.

Latz, M. A., Jensen, B., Collinge, D. B., and Jørgensen, H. J. (2018). Endophytic fungi as biocontrol agents: elucidating mechanisms in disease suppression. *Plant Ecology and Diversity*, 11(5-6):555–567.

Lauber, C. L., Hamady, M., Knight, R., and Fierer, N. (2009). Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. *Applied and Environmental Microbiology*, 75(15):5111–5120.

Laville, J., Voisard, C., Keel, C., Maurhofer, M., Défago, G., and Haas, D. (1992). Global control in Pseudomonas fluorescens mediating antibiotic synthesis and suppression of black root rot of tobacco. *Proceedings of the National Academy of Sciences of the United States of America*, 89(5):1562–1566.

Leach, J. E., Triplett, L. R., Argueso, C. T., and Trivedi, P. (2017). Communication in the Phytobiome. *Cell*, 169(4):587–596.

LeBlanc, N., Kinkel, L., and Kistler, H. C. (2017). Plant diversity and plant identity influence Fusarium communities in soil. *Mycologia*, 109:128–139.

LeBlanc, N., Kinkel, L. L., and Kistler, H. C. (2015). Soil Fungal Communities Respond to Grassland Plant Community Richness and Soil Edaphics. *Microbial Ecology*, 70:188–195.

Leclère, V., Clément, C., Dorey, S., and Prigent-Combaret, C. (2022). The Role of Microbial Metabolites in Biological Control. *Extended Biocontrol*, pages 137–145.

Lee, H., Gurtowski, J., Yoo, S., Nattestad, M., Marcus, S., Goodwin, S., Richard McCombie, W., and Schatz, M. C. (2016). Third-generation sequencing and the future of genomics. *bioRxiv*, page 48603.

Lee, Y., Choi, O., Kang, B., Bae, J., Kim, S., and Kim, J. (2020). Grey mould control by oxalate degradation using non-antifungal Pseudomonas abietaniphila strain ODB36. *Scientific Reports*, 10(1).

Lefort, V., Desper, R., and Gascuel, O. (2015). FastME 2.0: A comprehensive, accurate, and fast distance-based phylogeny inference program. *Molecular Biology and Evolution*, 32(10):2798–2800.

Lehtovirta-Morley, L. E., Sayavedra-Soto, L. A., Gallois, N., Schouten, S., Stein, L. Y., Prosser, J. I., and Nicol, G. W. (2016). Identifying potential mechanisms enabling acidophily in the ammonia-oxidizing archaeon "Candidatus Nitrosotalea devanaterra". *Applied and Environmental Microbiology*, 82(9):2608–2619.

Lemanceau, P., Barret, M., Mazurier, S., Mondy, S., Pivato, B., Fort, T., and Vacher, C. (2017). Chapter Five - Plant Communication With Associated Microbiota in the Spermosphere, Rhizosphere and Phyllosphere. In Becard, G., editor, *Advances in Botanical Research*, pages 101–133. Academic Press.

Lengrand, S., Pesenti, L., Bragard, C., and Legreve, A. (2024). Bacterial endophytome sources, profile and dynamics—a conceptual framework. *Frontiers in Sustainable Food Systems*, 8:1–16.

Lennon, J. T. and Jones, S. E. (2011). Microbial seed banks: the ecological and evolutionary implications of dormancy. *Nature Reviews Microbiology*, 9:119–130.

Levasseur, M., Tombu, B., and Zambaux, C. (2005). Mieux connaitre Serenade Biofungicide. Fongicide biologique contre le Botrytis de la Vigne. *Phytoma*, 588:52–53.

Leveau, J. H. (2019). A brief from the leaf: latest research to inform our understanding of the phyllosphere microbiome. *Current Opinion in Microbiology*, 49:41–49.

Levy, A., Salas Gonzalez, I., Mittelviefhaus, M., Clingenpeel, S., Herrera Paredes, S., Miao, J., Wang, K., Devescovi, G., Stillman, K., Monteiro, F., Rangel Alvarez, B., Lundberg, D. S., Lu, T. Y., Lebeis, S., Jin, Z., McDonald, M., Klein, A. P., Feltcher, M. E., Rio, T. G., Grant, S. R., Doty, S. L., Ley, R. E., Zhao, B., Venturi, V., Pelletier, D. A., Vorholt, J. A., Tringe, S. G., Woyke, T., and Dangl, J. L. (2018). Genomic features of bacterial adaptation to plants. *Nature Genetics*, 50(1):138–150.

Levy, E., EYAL, Z., CARMELY, S., KASHMAN, Y., and CHET, I. (1989). Suppression of Septoria tritici and Puccinia recondita of wheat by an antibiotic-producing fluorescent pseudomonad. *Plant Pathology*, 38(4):564–570.

Levy, E., GOUGH, F. J., BERLIN, K. D., GUIANA, P. W., and SMITH, J. T. (1992). Inhibition of Septoria tritici and other phytopathogenic fungi and bacteria by Pseudomonas fluorescens and its antibiotics. *Plant Pathology*, 41(3):335–341.

Li, H. and Leifert, C. (1994). Development of resistance in Botryotinia fuckeliana (de Bary) Whetzel against the biological control agent Bacillus subtilis CL27. *Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz*, 101(4):414–418.

Li, W., Estrada-De Los Santos, P., Matthijs, S., Xie, G. L., Busson, R., Cornelis, P., Rozenski, J., and De Mot, R. (2011). Promysalin, a salicylate-containing pseudomonas putida antibiotic, promotes surface colonization and selectively targets other pseudomonas. *Chemistry and Biology*, 18(10):1320–1330.

Li, W., O'Neill, K. R., Haft, D. H., Dicuccio, M., Chetvernin, V., Badretdin, A., Coulouris, G., Chitsaz, F., Derbyshire, M. K., Durkin, A. S., Gonzales, N. R., Gwadz, M., Lanczycki, C. J., Song, J. S., Thanki, N., Wang, J., Yamashita, R. A., Yang, M., Zheng, C., Marchler-Bauer, A., and Thibaud-Nissen, F. (2021). RefSeq: Expanding the Prokaryotic Genome Annotation Pipeline reach with protein family model curation. *Nucleic Acids Research*, 49(D1):D1020–D1028.

Li, X., Wang, C., Zhu, X., Ntoukakis, V., Cernava, T., and Jin, D. (2023). Exploration of phyllosphere microbiomes in wheat varieties with differing aphid resistance. *Environmental Microbiome*, 18(1).

Li, Y., An, J., Dang, Z., Lv, H., Pan, W., and Gao, Z. (2018). Treating wheat seeds with neonicotinoid insecticides does not harm the rhizosphere microbial community. *PLOS ONE*, 13:e0205200.

Licciardello, G., Bertani, I., Steindler, L., Bella, P., Venturi, V., and Catara, V. (2009). The transcriptional activator rfiA is quorum-sensing regulated by cotranscription with the luxI homolog pcol and is essential for plant virulence in Pseudomonas corrugata. *Molecular Plant-Microbe Interactions*, 22(12):1514–1522.

Lienhard, P., Terrat, S., Prévost-Bouré, N. C., Nowak, V., Régnier, T., Sayphoummie, S., Panyasiri, K., Tivet, F., Mathieu, O., Levêque, J., Maron, P.-A., and Ranjard, L. (2014). Pyrosequencing evidences the impact of cropping on soil bacterial and fungal diversity in Laos tropical grassland. *Agronomy for Sustainable Development*, 34:525–533.

Ligon, J. M., Hill, D. S., Hammer, P. E., Torkewitz, N. R., Hofmann, D., Kempf, H., and van Pée, K. (2000). Natural products with antifungal activity from Pseudomonas biocontrol bacteria. *Pest Management Science*, 56(8):688–695.

Lin, H. and Peddada, S. D. (2020). Analysis of compositions of microbiomes with bias correction. *Nature Communications*, 11(1).

Lindow, S. E. and Brandl, M. T. (2003). Microbiology of the phyllosphere. *Applied and Environmental Microbiology*, 69(4):1875–1883.

Ling, N., Zhu, C., Xue, C., Chen, H., Duan, Y., Peng, C., Guo, S., and Shen, Q. (2016). Insight into how organic amendments can shape the soil microbiome in long-term field experiments as revealed by network analysis. *Soil Biology and Biochemistry*, 99:137–149.

Liu, B., Zheng, D., Zhou, S., Chen, L., and Yang, J. (2022). VFDB 2022: A general classification scheme for bacterial virulence factors. *Nucleic Acids Research*, 50(D1):D912–D917.

Liu, W. H., Yue, S. J., Feng, T. T., Li, S., Huang, P., Hu, H. B., Wang, W., and Zhang, X. H. (2021). Characterization and Engineering of Pseudomonas chlororaphis LX24 with High Production of 2-Hydroxyphenazine. *Journal of Agricultural and Food Chemistry*, 69(16):4778–4784.

Liu, Y., Zuo, S., Xu, L., Zou, Y., and Song, W. (2012). Study on diversity of endophytic bacterial communities in seeds of hybrid maize and their parental lines. *Archives of Microbiology*, 194:1001–1012.

Liu, Z., Beskrovnaya, P., Melnyk, R. A., Hossain, S. S., Khorasani, S., O'sullivan, L. R., Wiesmann, C. L., Bush, J., Richard, J. D., and Haney, C. H. (2018). A genome-wide screen identifies genes in rhizosphere-associated pseudomonas required to evade plant defenses. *mBio*, 9(6):1–17.

Lloyd, A. W., Percival, D., Langille, M. G., and Yurgel, S. N. (2023). Changes to Soil Microbiome Resulting from Synergetic Effects of Fungistatic Compounds Pyrimethanil and Fluopyram in Lowbush Blueberry Agriculture, with Nine Fungicide Products Tested. *Microorganisms*, 11(2).

Lloyd, A. W., Percival, D., and Yurgel, S. N. (2021). Effect of fungicide application on lowbush blueberries soil microbiome. *Microorganisms*, 9(7).

Loper, J. E., Henkels, M. D., Shaffer, B. T., Valeriote, F. A., and Gross, H. (2008). Isolation and identification of rhizoxin analogs from Pseudomonas fluorescens Pf-5 by using a genomic mining strategy. *Applied and Environmental Microbiology*, 74(10):3085–3093.

Lu, S. E., Scholz-Schroeder, B. K., and Gross, D. C. (2002). Characterization of the salA, syrF, and syrG regulatory genes located at the right border of the syringomycin gene cluster of Pseudomonas syringae pv. syringae. *Molecular Plant-Microbe Interactions*, 15(1):43–53.

Lu, S. E., Wang, N., Wang, J., Chen, Z. J., and Gross, D. C. (2005). Oligonucleotide microarray analysis of the SalA regulon controlling phytotoxin production by Pseudomonas syringae pv. syringae. *Molecular Plant-Microbe Interactions*, 18(4):324–333.

Lucas, J. A., Hawkins, N. J., and Fraaije, B. A. (2015). The Evolution of Fungicide Resistance. *Advances in Applied Microbiology*, 90:29–92.

Lundberg, D. S., Lebeis, S. L., Paredes, S. H., Yourstone, S., Gehring, J., Malfatti, S., Tremblay, J., Engelbrektson, A., Kunin, V., Rio, T. G. D., Edgar, R. C., Eickhorst, T., Ley, R. E., Hugenholtz, P., Tringe, S. G., and Dangl, J. L. (2012). Defining the core Arabidopsis thaliana root microbiome. *Nature*, 488(7409):86–90.

Lupwayi, N. Z., Harker, K. N., Clayton, G. W., O'Donovan, J. T., and Blackshaw, R. E. (2009). Soil microbial response to herbicides applied to glyphosate-resistant canola. *Agriculture, Ecosystems and Environment*, 129:171–176.

Lupwayi, N. Z., Harker, K. N., Clayton, G. W., Turkington, T. K., Rice, W. A., and O'Donovan, J. T. (2004). Soil microbial biomass and diversity after herbicide application. *Canadian Journal of Plant Science*, 84:677–685.

Luziatelli, F., Ficca, A. G., Colla, G., Svecova, E., and Ruzzi, M. (2016). Effects of a protein hydrolysate-based biostimulant and two micronutrient based fertilizers on plant growth and epiphytic bacterial population of lettuce. In *International Society for Horticultural Science (ISHS)*, pages 43–48, Leuven, Belgium.

Lyu, D., Zajonc, J., Pagé, A., Tanney, C. A., Shah, A., Monjezi, N., Msimbira, L. A., Antar, M., Nazari, M., Backer, R., and Smith, D. L. (2021). Plant holobiont theory: The phytomicrobiome plays a central role in evolution and success. *Microorganisms*, 9(4).

Ma, B., Gavzy, S. J., Saxena, V., Song, Y., Piao, W., Lwin, H. W., Lakhan, R., Lyyathurai, J., Li, L., France, M., Paluskievicz, C., Shirkey, M. W., Hittle, L., Munawwar, A., Mongodin, E. F., and Bromberg, J. S. (2023). Strain-specific alterations in gut microbiome and host immune responses elicited by tolerogenic Bifidobacterium pseudolongum. *Scientific Reports*, 13(1):1–55.

Ma, G., Gao, X., Nan, J., Zhang, T., Xie, X., and Cai, Q. (2021). Fungicides alter the distribution and diversity of bacterial and fungal communities in ginseng fields. *Bioengineered*, 12(1):8043–8056.

Madhaiyan, M., Poonguzhali, S., Saravanan, V. S., Selvapravin, K., Duraipandiyan, V., and Al-Dhabi, N. A. (2017). Pseudomonas sesami sp. nov., a plant growth-promoting Gammaproteobacteria isolated from the rhizosphere of Sesamum indicum L.

Mahnert, A., Haratani, M., Schmuck, M., and Berg, G. (2018). Enriching Beneficial Microbial Diversity of Indoor Plants and Their Surrounding Built Environment With Biostimulants. *Frontiers in Microbiology*, 9:2985.

Maleki, M., Mokhtarnejad, L., and Mostafaee, S. (2011). Screening of rhizobacteria for biological control of cucumber root and crown rot caused by Phytophthora drechsleri. *Plant Pathology Journal*, 27(1):78–84.

Manzotti, A., Bergna, A., Burow, M., Jørgensen, H. J. L., Cernava, T., Berg, G., Collinge, D. B., and Jensen, B. (2020). Insights into the community structure and lifestyle of the fungal root endophytes of tomato by combining amplicon sequencing and isolation approaches with phytohormone profiling. *FEMS Microbiology Ecology*, 96.

Marian, M. and Shimizu, M. (2019). Improving performance of microbial biocontrol agents against plant diseases. *Journal of General Plant Pathology*, 85(5):329–336.

Martijn, J., Lind, A. E., Schön, M. E., Spiertz, I., Juzokaite, L., Bunikis, I., Pettersson, O. V., and Ettema, T. J. (2019). Confident phylogenetic identification of uncultured prokaryotes through long read amplicon sequencing of the 16S-ITS-23S rRNA operon. *Environmental Microbiology*, 21(7):2485–2498.

Martínez, J. I., Gómez-Garrido, M., Gómez-López, M. D., Faz, Á., Martínez-Martínez, S., and Acosta, J. A. (2019). Pseudomonas fluorescens affects nutrient dynamics in plant-soil system for melon production. *Chilean Journal of Agricultural Research*, 79(2):223–233.

Martínez-Viveros, O., Jorquera, M. A., Crowley, D. E., Gajardo, G., and Mora, M. L. (2010). Mechanisms and practical considerations involved in plant growth promotion by Rhizobacteria. *Journal of Soil Science and Plant Nutrition*, 10(3):293–319.

Massart, S., Margarita, M. M., and Jijakli, M. H. (2015). Biological control in the microbiome era: Challenges and opportunities. *Biological Control*, 89(October):98–108.

Matchado, M. S., Lauber, M., Reitmeier, S., Kacprowski, T., Baumbach, J., Haller, D., and List, M. (2021). Network analysis methods for studying microbial communities: A mini review. *Computational and Structural Biotechnology Journal*, 19:2687–2698.

Maurhofer, M., Keel, C., Haas, D., and Défago, G. (1994). Pyoluteorin production by Pseudomonas fluorescens strain CHA0 is involved in the suppression of Pythium damping-off of cress but not of cucumber. *European Journal of Plant Pathology*, 100(3-4):221–232.

Mavrodi, D. V., Blankenfeldt, W., and Thomashow, L. S. (2006). Phenazine compounds in fluorescent Pseudomonas spp. biosynthesis and regulation. *Annual Review of Phytopathology*, 44:417–445.

Mavrodi, D. V., Bonsall, R. F., Delaney, S. M., Soule, M. J., Phillips, G., and Thomashow, L. S. (2001). Functional analysis of genes for biosynthesis of pyocyanin and phenazine-1-carboxamide from Pseudomonas aeruginosa PAO1. *Journal of Bacteriology*, 183(21):6454–6465.

Mavrodi, D. V., Mavrodi, O. V., Elbourne, L. D. H., Tetu, S., Bonsall, R. F., Parejko, J., Yang, M., Paulsen, I. T., Weller, D. M., and Thomashow, L. S. (2018). Long-Term Irrigation Affects the Dynamics and Activity of the Wheat Rhizosphere Microbiome. *Frontiers in Plant Science*, 9:345.

Mavrodi, D. V., Parejko, J. A., Mavrodi, O. V., Kwak, Y. S., Weller, D. M., Blankenfeldt, W., and Thomashow, L. S. (2013). Recent insights into the diversity, frequency and ecological roles of phenazines in fluorescent Pseudomonas spp. *Environmental Microbiology*, 15(3):675–686.

Mazzola, M., De Bruijn, I., Cohen, M. F., and Raaijmakers, J. M. (2009). Protozoan-induced regulation of cyclic lipopeptide biosynthesis is an effective predation defense mechanism for Pseudomonas fluorescens. *Applied and Environmental Microbiology*, 75(21):6804–6811.

Mazzola, M., Fujimoto, D. K., Thomashow, L. S., and Cook, R. J. (1995). Variation in sensitivity of Gaeumannomyces graminis to antibiotics produced by fluorescent Pseudomonas spp. and effect on biological control of take-all of wheat. *Applied and Environmental Microbiology*, 61(7):2554–2559.

Mehmood, N., Saeed, M., Zafarullah, S., Hyder, S., Rizvi, Z. F., Gondal, A. S., Jamil, N., Iqbal, R., Ali, B., Ercisli, S., and Kupe, M. (2023). Multifaceted Impacts of Plant-Beneficial Pseudomonas spp. in Managing Various Plant Diseases and Crop Yield Improvement. *ACS Omega*, 8(25):22296–22315.

Meier, U., Bleiholder, H., Buhr, L., Feller, C., Hack, H., Heß, M., Lancashire, P., Schnock, U., Stauß, R., Van den Boom, T., Weber, E., and Zwerger, P. (2009). The BBCH system to coding the phenological growth stages of plants-history and publications. *Journal für Kulturpflanzen*, 61(2):41–52.

Meier-Kolthoff, J. P., Auch, A. F., Klenk, H. P., and Göker, M. (2013). Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinformatics*, 14.

Meier-Kolthoff, J. P., Carbasse, J. S., Peinado-Olarte, R. L., and Göker, M. (2022). TYGS and LPSN: A database tandem for fast and reliable genome-based classification and nomenclature of prokaryotes. *Nucleic Acids Research*, 50(D1):D801–D807.

Meier-Kolthoff, J. P. and Göker, M. (2019). TYGS is an automated high-throughput platform for state-of-the-art genome-based taxonomy. *Nature Communications*, 10(1).

Mejri, S., Siah, A., Coutte, F., Magnin-Robert, M., Randoux, B., Tisserant, B., Krier, F., Jacques, P., Reignault, P., and Halama, P. (2018). Biocontrol of the wheat pathogen Zymoseptoria tritici using cyclic lipopeptides from Bacillus subtilis. *Environmental Science and Pollution Research*, 25(30):29822–29833.

Mendes, L. W., Mendes, R., Raaijmakers, J. M., and Tsai, S. M. (2018). Breeding for soil-borne pathogen resistance impacts active rhizosphere microbiome of common bean. *The ISME Journal*.

Mendes, R., Garbeva, P., and Raaijmakers, J. M. (2013). The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. *FEMS Microbiology Reviews*, 37:634–663.

Mendis, H. C., Thomas, V. P., Schwientek, P., Salamzade, R., Chien, J. T., Waidyarathne, P., Kloepper, J., and De La Fuente, L. (2018). Strain-specific quantification of root colonization by plant growth promoting rhizobacteria Bacillus firmus I-1582 and Bacillus amyloliquefaciens QST713 in non-sterile soil and field conditions. *PLoS ONE*, 13(2).

Mercado-Blanco, J. and Bakker, P. A. (2007). Interactions between plants and beneficial Pseudomonas spp.: Exploiting bacterial traits for crop protection. *Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology*, 92(4):367–389.

Mercado-Blanco, J., Rodríguez-Jurado, D., Hervás, A., and Jiménez-Diaz, R. M. (2004). Suppression of Verticillium wilt in olive planting stocks by root-associated fluorescent Pseudomonas spp. *Biological Control*, 30(2):474–486.

Messing, R. and Brodeur, J. (2018). Current challenges to the implementation of classical biological control. *BioControl*, 63(1):1–9.

Meyer, J. M. (2000). Proverdines: Pigments, siderophores and potential taxonomic markers of fluorescent pseudomonas species. *Archives of Microbiology*, 174(3):135–142.

Micallef, S. A., Channer, S., Shiaris, M. P., and Colón-Carmona, A. (2009). Plant age and genotype impact the progression of bacterial community succession in the Arabidopsis rhizosphere. *Plant Signaling and Behavior*, 4:777–780.

Mille-Lindblom, C., Fischer, H., and Tranvik, L. J. (2006). Antagonism between bacteria and fungi: Substrate competition and a possible tradeoff between fungal growth and tolerance towards bacteria. *Oikos*, 113(2):233–242.

Mina, I. R., Jara, N. P., Criollo, J. E., and Castillo, J. A. (2019). The critical role of biofilms in bacterial vascular plant pathogenesis. *Plant Pathology*, 68(8):1439–1447.

Mishra, J. and Arora, N. K. (2018). Secondary metabolites of fluorescent pseudomonads in biocontrol of phytopathogens for sustainable agriculture. *Applied Soil Ecology*, 125:35–45.

Mitter, B., Pfaffenbichler, N., and Sessitsch, A. (2016). Plant–microbe partnerships in 2020. *Microbial Biotechnology*, 9(5):635–640.

Mönchgesang, S., Strehmel, N., Schmidt, S., Westphal, L., Taruttis, F., Müller, E., Herklotz, S., Neumann, S., and Scheel, D. (2016). Natural variation of root exudates in Arabidopsis thaliana-linking metabolomic and genomic data. *Scientific Reports*, 6:29033.

Moore, W. C. (1952). *'Plant Pathology'*, volume 1. Academic Press, San Diego, CA, 5th edition edition.

Moriya, Y., Itoh, M., Okuda, S., Yoshizawa, A. C., and Kanehisa, M. (2007). KAAS: An automatic genome annotation and pathway reconstruction server. *Nucleic Acids Research*, 35(SUPPL.2).

Morris, C. E. and Kinkel, L. L. (2002). Fifty years of phyllosphere microbiology: Significant contributions to research in related fields. *Phyllosphere Microbiology*, pages 353–363.

Morris, C. E., Lamichhane, J. R., Nikolić, I., Stanković, S., and Moury, B. (2019). The overlapping continuum of host range among strains in the Pseudomonas syringae complex. *Phytopathology Research*, 1(1).

Morrison, C. K., Arseneault, T., Novinscak, A., and Filion, M. (2017). Phenazine-1-carboxylic acid production by pseudomonas fluorescens LBUM636 alters phytophthora infestans growth and late blight development. *Phytopathology*, 107(3):273–279.

Mulet, M., Bennasar, A., Lalucat, J., and García-Valdés, E. (2009). An rpoD-based PCR procedure for the identification of Pseudomonas species and for their detection in environmental samples. *Molecular and Cellular Probes*, 23(3-4):140–147.

Mulet, M., Lalucat, J., and García-Valdés, E. (2010). DNA sequence-based analysis of the Pseudomonas species. *Environmental Microbiology*, 12(6):1513–1530.

Mulet, M., Martínez, M. J., Gomila, M., Dabernig-Heinz, J., Wagner, G. E., Kittinger, C., Zarfel, G., Lalucat, J., and García-Valdés, E. (2023). Genome-Based Species Diversity Assessment in the Pseudomonas chlororaphis Phylogenetic Subgroup and Proposal of Pseudomonas danubii sp. nov. Isolated from Freshwaters, Soil, and Rhizosphere. *Diversity*, 15(5):1–23.

Müller, T. and Ruppel, S. (2014). Progress in cultivation-independent phyllosphere microbiology. *FEMS Microbiology Ecology*, 87(1):2–17.

Müller, T., Ruppel, S., Behrendt, U., Lentzsch, P., and Müller, M. E. (2018). Antagonistic potential of fluorescent pseudomonads colonizing wheat heads against mycotoxin producing alternaria and fusaria. *Frontiers in Microbiology*, 9(SEP).

Munier-Lépinay, E., Mathiron, D., Quéro, A., Khelifa, M., Laclef, S., and Pilard, S. (2023). Pseudomonas PA14H7: Identification and Quantification of the 7-Hydroxytropolone Iron Complex as an Active Metabolite against Dickeya, the Causal Agent of Blackleg on the Potato Plant. *Molecules*, 28(17).

Muñoz-Leoz, B., Garbisu, C., Charcosset, J. Y., Sánchez-Pérez, J. M., Antigüedad, I., and Ruiz-Romera, E. (2013). Non-target effects of three formulated pesticides on microbially-mediated processes in a clay-loam soil. *Science of the Total Environment*, 449:345–354.

Muzio, F. M., Agaras, B. C., Masi, M., Tuzi, A., Evidente, A., and Valverde, C. (2020). 7-hydroxytropolone is the main metabolite responsible for the fungal antagonism of Pseudomonas donghuensis strain SVBP6. *Environmental Microbiology*, 22(7):2550–2563.

Nadakuduti, S. S., Pollard, M., Kosma, D. K., Allen, C., Ohlrogge, J. B., and Barry, C. S. (2012). Pleiotropic phenotypes of the sticky peel mutant provide new insight into the role of CUTIN DEFICIENT2 in epidermal cell function in tomato. *Plant Physiology*, 159(3):945–960.

Nadarajah, K. and Abdul Rahman, N. S. N. (2023). The Microbial Connection to Sustainable Agriculture. *Plants*, 12(12).

Nair, J., Turkington, T. K., Blackshaw, R., Geddes, C. M., Lupwayi, N., Xu, S., and McAllister, T. (2019). Impact of application of foliar fungicide on ensiling properties, feed value, and microbiome of barley silage. *Advances in Silage Research*.

Nallanchakravarthula, S., Mahmood, S., Alström, S., and Finlay, R. D. (2014). Influence of Soil Type, Cultivar and Verticillium dahliae on the Structure of the Root and Rhizosphere Soil Fungal Microbiome of Strawberry. *PLOS ONE*, 9:e111455.

Nautiyal, C. S., Bhadauria, S., Kumar, P., Lal, H., Mondal, R., and Verma, D. (2000). Stress induced phosphate solubilization in bacteria isolated from alkaline soils. *FEMS Microbiology Letters*, 182:291–296.

Nelkner, J., Henke, C., Lin, T. W., Pätzold, W., Hassa, J., Jaenicke, S., Grosch, R., Pühler, A., Sczyrba, A., and Schlüter, A. (2019). Effect of long-term farming practices on agricultural soil microbiome members represented by metagenomically assembled genomes (MAGs) and their predicted plant-beneficial genes. *Genes*, 10(6).

Nettles, R., Watkins, J., Ricks, K., Boyer, M., Licht, M., Atwood, L. W., Peoples, M., Smith, R. G., Mortensen, D. A., and Koide, R. T. (2016). Influence of pesticide seed treatments on rhizosphere fungal and bacterial communities and leaf fungal endophyte communities in maize and soybean. *Applied Soil Ecology*, 102:61–69.

Newman, M. E. J. (2006). Modularity and community structure in networks. *Proceedings of the National Academy of Sciences*, 103:8577–8582.

Newman, M. M., Hoilett, N., Lorenz, N., Dick, R. P., Liles, M. R., Ramsier, C., and Kloepper, J. W. (2016). Glyphosate effects on soil rhizosphere-associated bacterial communities. *Science of the Total Environment*, 543:155–160.

Nguyen, N. H., Smith, D., Peay, K., and Kennedy, P. (2015). Parsing ecological signal from noise in next generation amplicon sequencing. *New Phytologist*, 205:1389–1393.

Nicot, P. C., Bardin, M., Alabouvette, C., Köhl, J., and Ruocco, M. (2011). Potential of biological control based on published research: Protection against plant pathogens of selected crops. *Classical and augmentative biological control against diseases and pests: critical status analysis and review of factors influencing their success*, pages 1–11.

Nielsen, K. M., Johnsen, P. J., Bensasson, D., and Daffonchio, D. (2007). Release and persistence of extracellular DNA in the environment. *Environmental Biosafety Research*, 6(1-2):37–53.

Nielsen, K. M., Smalla, K., and Van Elsas, J. D. (2000). Natural transformation of Acinetobacter sp. strain BD413 with cell lysates of Acinetobacter sp., Pseudomonas fluorescens, and Burkholderia cepacia in soil microcosms. *Applied and Environmental Microbiology*, 66(1):206–212.

Nikolić, I., Stanković, S., Dimkić, I., Berić, T., Stojšin, V., Janse, J., and Popović, T. (2018). Genetic diversity and pathogenicity of Pseudomonas syringae pv. aptata isolated from sugar beet. *Plant Pathology*, 67(5):1194–1207.

Nishanth Kumar, S., Aravind, S. R., Jacob, J., Gopinath, G., Lankalapalli, R. S., Sreelekha, T. T., and Dileep Kumar, B. S. (2016). Pseudopyronine B: A potent antimicrobial and anticancer molecule isolated from a Pseudomonas mosselii. *Frontiers in Microbiology*, 7(AUG).

Nivelle, E., Verzeaux, J., Habbib, H., Kuzyakov, Y., Decocq, G., Roger, D., Lacoux, J., Duclercq, J., Spicher, F., Nava-Saucedo, J.-E., Catterou, M., Dubois, F., and Tetu, T. (2016). Functional response of

soil microbial communities to tillage, cover crops and nitrogen fertilization. *Applied Soil Ecology*, 108:147–155.

Nowak-Thompson, B., Chaney, N., Wing, J. S., Gould, S. J., and Loper, J. E. (1999). Characterization of the pyoluteorin biosynthetic gene cluster of Pseudomonas fluorescens Pf-5. *Journal of Bacteriology*, 181(7):2166–2174.

Nowak-Thompson, B., Hammer, P. E., Hill, D. S., Stafford, J., Torkewitz, N., Gaffney, T. D., Lam, S. T., Molnár, I., and Ligon, J. M. (2003). 2,5-Dialkylresorcinol biosynthesis in Pseudomonas aurantiaca: Novel head-to-head condensation of two fatty acid-derived precursors. *Journal of Bacteriology*, 185(3):860–869.

O'Brien, F. J., Almaraz, M., Foster, M. A., Hill, A. F., Huber, D. P., King, E. K., Langford, H., Lowe, M. A., Mickan, B. S., Miller, V. S., Moore, O. W., Mathes, F., Gleeson, D., Leopold, M., O'Brien, F. J. M., Almaraz, M., Foster, M. A., Hill, A. F., Huber, D. P., King, E. K., Langford, H., Lowe, M. A., Mickan, B. S., Miller, V. S., Moore, O. W., Mathes, F., Gleeson, D., and Leopold, M. (2019). Soil salinity and pH drive soil bacterial community composition and diversity along a lateritic slope in the Avon River Critical Zone Observatory, Western Australia. *Frontiers in Microbiology*, 10(JUL).

O'Brien, P. A. (2017). Biological control of plant diseases. *Australasian Plant Pathology*, 46(4):293–304.

Ofek, M., Hadar, Y., and Minz, D. (2012). Ecology of root colonizing Massilia (Oxalobacteraceae). *PLoS ONE*, 7(7).

Ogata, H., Goto, S., Sato, K., Fujibuchi, W., Bono, H., and Kanehisa, M. (1999). KEGG: Kyoto encyclopedia of genes and genomes. *Nucleic Acids Research*, 27(1):29–34.

Okada, A., Banno, S., Ichiishi, A., Kimura, M., Yamaguchi, I., and Fujimura, M. (2005). Pyrrolnitrin interferes with osmotic signal transduction in Neurospora crassa. *Journal of Pesticide Science*, 30(4):378–383.

Olorunleke, F. E., Kieu, N. P., De Waele, E., Timmerman, M., Ongena, M., and Höfte, M. (2017). Coregulation of the cyclic lipopeptides orfamide and sessilin in the biocontrol strain Pseudomonas sp. CMR12a. *MicrobiologyOpen*, 6(5).

Olorunleke, F. E., Kieu, N. P., and Höfte, M. (2015). Recent Advances in Pseudomonas Biocontrol. *Bacteria-Plant Interactions: Advanced Research and Future Trends*, pages 167–198.

Omoboye, O. O., Geudens, N., Duban, M., Chevalier, M., Flahaut, C., Martins, J. C., Leclère, V., Oni, F. E., and Höfte, M. (2019). Pseudomonas sp. COW3 produces new bananamide-type cyclic lipopep-

tides with antimicrobial activity against Pythium myriotylum and Pyricularia oryzae. *Molecules*, 24(22).

Ondov, B. D., Treangen, T. J., Melsted, P., Mallonee, A. B., Bergman, N. H., Koren, S., and Phillippy, A. M. (2016). Mash: Fast genome and metagenome distance estimation using MinHash. *Genome Biology*, 17(1).

Onet, A., Dincă, L. C., Grenni, P., Laslo, V., Teusdea, A. C., Vasile, D. L., Enescu, R. E., and Crisan, V. E. (2019). Biological indicators for evaluating soil quality improvement in a soil degraded by erosion processes. *Journal of Soils and Sediments*, 19:2393–2404.

Ongena, M. and Jacques, P. (2008). Bacillus lipopeptides: versatile weapons for plant disease biocontrol. *Trends in Microbiology*, 16(3):115–125.

Oni, F. E., Esmaeel, Q., Onyeka, J. T., Adeleke, R., Jacquard, C., Clement, C., Gross, H., Barka, E. A., and Höfte, M. (2022). Pseudomonas Lipopeptide-Mediated Biocontrol: Chemotaxonomy and Biological Activity. *Molecules*, 27(2).

Oni, F. E., Olorunleke, O. F., and Höfte, M. (2019). Phenazines and cyclic lipopeptides produced by Pseudomonas sp. CMR12a are involved in the biological control of Pythium myriotylum on cocoyam (Xanthosoma sagittifolium). *Biological Control*, 129:109–114.

ONT (2023). Medaka: Sequence correction provided by ONT Research.

Onwona-kwakye, M., Plants-paris, K., Keita, K., Lee, J., den Brink, P. J., Hogarh, J. N., and Darkoh, C. (2020). Pesticides decrease bacterial diversity and abundance of irrigated rice fields. *Microorganisms*, 8(3).

Orozco-Mosqueda, M. d. C., Rocha-Granados, M. d. C., Glick, B. R., and Santoyo, G. (2018). Microbiome engineering to improve biocontrol and plant growth-promoting mechanisms. *Microbiological Research*, 208:25–31.

Oso, S., Walters, M., Schlechter, R. O., and Remus-Emsermann, M. N. (2019). Utilisation of hydrocarbons and production of surfactants by bacteria isolated from plant leaf surfaces. *FEMS Microbiology Letters*, 366(6):1–10.

O'Toole, G. A. (2010). Microtiter dish Biofilm formation assay. *Journal of Visualized Experiments*, JoVE(47).

Palojärvi, A., Kellock, M., Parikka, P., Jauhiainen, L., and Alakukku, L. (2020). Tillage System and Crop Sequence Affect Soil Disease Suppressiveness and Carbon Status in Boreal Climate. *Frontiers in Microbiology*, 11.

Pan, Y., Cassman, N., de Hollander, M., Mendes, L. W., Korevaar, H., Geerts, R. H. E. M., van Veen, J. A., and Kuramae, E. E. (2014). Impact of long-term N, P, K, and NPK fertilization on the composition and potential functions of the bacterial community in grassland soil. *FEMS Microbiology Ecology*, 90:195–205.

Pang, Y., Liu, X., Ma, Y., Chernin, L., Berg, G., and Gao, K. (2009). Induction of systemic resistance, root colonisation and biocontrol activities of the rhizospheric strain of Serratia plymuthica are dependent on N-acyl homoserine lactones. *European Journal of Plant Pathology*, 124(2):261–268.

Pantigoso, H. A., Manter, D. K., and Vivanco, J. M. (2018). Phosphorus addition shifts the microbial community in the rhizosphere of blueberry (Vaccinium corymbosum L.). *Rhizosphere*, 7:1–7.

Pascault, N., Ranjard, L., Kaisermann, A., Bachar, D., Christen, R., Terrat, S., Mathieu, O., Lévêque, J., Mougel, C., Henault, C., Lemanceau, P., Péan, M., Boiry, S., Fontaine, S., and Maron, P.-A. (2013). Stimulation of different functional groups of bacteria by various plant residues as a driver of soil priming effect. *Ecosystems*, 16:810–822.

Pascual, J., García-Lopez, M., Bills, G. F., and Genilloud, O. (2015). Pseudomonas granadensis sp. Nov., a new bacterial species isolated from the Tejeda, Almijara and Alhama natural park, Granada, Spain. *International Journal of Systematic and Evolutionary Microbiology*, 65(2):625–632.

Passarelli-Araujo, H., Franco, G. R., and Venancio, T. M. (2022). Network analysis of ten thousand genomes shed light on Pseudomonas diversity and classification. *Microbiological Research*, 254.

Patel, A., Kumar, A., Sheoran, N., Kumar, M., Sahu, K. P., Ganeshan, P., Ashajyothi, M., Gopalakrishnan, S., and Gogoi, R. (2021). Antifungal and defense elicitor activities of pyrazines identified in endophytic Pseudomonas putida BP25 against fungal blast incited by Magnaporthe oryzae in rice. *Journal of Plant Diseases and Protection*, 128(1):261–272.

Paul, S., Paul, B., Khan, M. A., Aggarwal, C., Rathi, M. S., and Tyagi, S. P. (2017). Characterization and evaluation of Bacillus thuringiensis var. kurstaki based formulation for field persistence and insect biocontrol. *Indian Journal of Agricultural Sciences*, 87(4):473–478.

Paulitz, T. C. and Bélanger, R. R. (2001). Biological control in greenhouse systems. *Annual Review of Phytopathology*, 39:103–133.

Pauwelyn, E., Huang, C. J., Ongena, M., Leclère, V., Jacques, P., Bleyaert, P., Budzikiewicz, H., Schäfer, M., and Höfte, M. (2013).

New linear lipopeptides produced by pseudomonas cichorii SF1-54 are involved in virulence, swarming motility, and biofilm formation. *Molecular Plant-Microbe Interactions*, 26(5):585–598.

Pekkonen, M., Ketola, T., and Laakso, J. T. (2013). Resource Availability and Competition Shape the Evolution of Survival and Growth Ability in a Bacterial Community. *PLoS ONE*, 8(9):1–12.

Pellicciaro, M., Padoan, E., Lione, G., Celi, L., and Gonthier, P. (2022). Pyoluteorin Produced by the Biocontrol Agent Pseudomonas protegens Is Involved in the Inhibition of Heterobasidion Species Present in Europe. *Pathogens*, 11(4).

Peñuelas, J. and Terradas, J. (2014). The foliar microbiome. *Trends in Plant Science*, 19(5):278–280.

Perazzolli, M., Antonielli, L., Storari, M., Puopolo, G., Pancher, M., Giovannini, O., Pindo, M., and Pertot, I. (2014). Resilience of the natural phyllosphere microbiota of the grapevine to chemical and biological pesticides. *Applied and Environmental Microbiology*, 80(12):3585–3596.

Pflanze, S., Mukherji, R., Ibrahim, A., Günther, M., Götze, S., Chowdhury, S., Reimer, L., Regestein, L., and Stallforth, P. (2023). Nonribosomal peptides protect Pseudomonas nunensis 4A2e from amoebal and nematodal predation. *Chemical Science*, 14(41):11573–11581.

Piazza, G., Ercoli, L., Nuti, M., and Pellegrino, E. (2019). Interaction Between Conservation Tillage and Nitrogen Fertilization Shapes Prokaryotic and Fungal Diversity at Different Soil Depths: Evidence From a 23-Year Field Experiment in the Mediterranean Area. *Frontiers in Microbiology*, 10:2047.

Pieterse, C. M., Zamioudis, C., Berendsen, R. L., Weller, D. M., Van Wees, S. C., and Bakker, P. A. (2014). Induced systemic resistance by beneficial microbes. *Annual Review of Phytopathology*, 52:347–375.

Pietrarelli, L., Balestra, G. M., and Varvaro, L. (2006). Effects of simulated rain on Pseudomonas syringae pv. tomato populations on tomato plants. *Journal of Plant Pathology*, 88(3):245–251.

Platel, R., Lucau-Danila, A., Baltenweck, R., Maia-Grondard, A., Chaveriat, L., Magnin-Robert, M., Randoux, B., Trapet, P., Halama, P., Martin, P., Hilbert, J. L., Höfte, M., Hugueney, P., Reignault, P., and Siah, A. (2022). Bioinspired Rhamnolipid Protects Wheat Against Zymoseptoria tritici Through Mainly Direct Antifungal Activity and Without Major Impact on Leaf Physiology. *Frontiers in Plant Science*, 13.

Poncheewin, W., van Diepeningen, A. D., van der Lee, T. A., Suarez-Diez, M., and Schaap, P. J. (2022). Classification of the plant-associated lifestyle of Pseudomonas strains using genome properties and machine learning. *Scientific Reports*, 12(1):1–12.

Preston, G. M. (2004). Plant perceptions of plant growth-promoting Pseudomonas. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 359(1446):907–918.

Price-Whelan, A., Dietrich, L. E., and Newman, D. K. (2006). Rethinking 'secondary' metabolism: Physiological roles for phenazine antibiotics. *Nature Chemical Biology*, 2(2):71–78.

Pujol, M., Badosa, E., Manceau, C., and Montesinos, E. (2006). Assessment of the environmental fate of the biological control agent of fire blight, Pseudomonas fluorescens EPS62e, on apple by culture and real-time PCR methods. *Applied and Environmental Microbiology*, 72(4):2421–2427.

Puopolo, G., Palmieri, M. C., Giovannini, O., and Pertot, I. (2015). Impact of temperature on the survival and the biocontrol efficacy of Lysobacter capsici AZ78 against Phytophthora infestans. *BioControl*, 60(5):681–689.

Qi, D., Wieneke, X., Tao, J., Zhou, X., and Desilva, U. (2018). Soil pH is the primary factor correlating with soil microbiome in karst rocky desertification regions in the Wushan County, Chongqing, China. *Frontiers in Microbiology*, 9(MAY).

Quiza, L., Tremblay, J., Pagé, A. P., Greer, C. W., Pozniak, C. J., Li, R., Haug, B., Hemmingsen, S. M., St-Arnaud, M., and Yergeau, E. (2023). The effect of wheat genotype on the microbiome is more evident in roots and varies through time. *ISME Communications*, 3(1).

Raaijmakers, J. M., de Bruijn, I., Nybroe, O., and Ongena, M. (2010). Natural functions of lipopeptides from Bacillus and Pseudomonas: More than surfactants and antibiotics. *FEMS Microbiology Reviews*, 34(6):1037–1062.

Raaijmakers, J. M. and Mazzola, M. (2012). Diversity and natural functions of antibiotics produced by beneficial and plant pathogenic bacteria. *Annual Review of Phytopathology*, 50:403–424.

Raaijmakers, J. M., Paulitz, T. C., Steinberg, C., Alabouvette, C., and Moënne-Loccoz, Y. (2009). The rhizosphere: A playground and battlefield for soilborne pathogens and beneficial microorganisms. *Plant and Soil*, 321(1-2):341–361.

Raaijmakers, J. M., Vlami, M., and de Souza, J. T. (2002). Antibiotic production by bacterial biocontrol agents. *Antonie van Leeuwenhoek,*

International Journal of General and Molecular Microbiology, 81(1-4):537–547.

Rahman, M. D. M., Flory, E., Koyro, H.-W., Abideen, Z., Schikora, A., Suarez, C., Schnell, S., and Cardinale, M. (2018). Consistent associations with beneficial bacteria in the seed endosphere of barley (Hordeum vulgare L.). *Systematic and Applied Microbiology*, 41:386–398.

Ramette, A., Frapolli, M., Saux, M. F. L., Gruffaz, C., Meyer, J. M., Défago, G., Sutra, L., and Moënne-Loccoz, Y. (2011). Pseudomonas protegens sp. nov., widespread plant-protecting bacteria producing the biocontrol compounds 2,4-diacetylphloroglucinol and pyoluteorin. *Systematic and Applied Microbiology*, 34(3):180–188.

Ramirez, K. S., Craine, J. M., and Fierer, N. (2012). Consistent effects of nitrogen amendments on soil microbial communities and processes across biomes. *Global Change Biology*, 18:1918–1927.

Rangasamy, K., Athiappan, M., Devarajan, N., Samykannu, G., Parray, J. A., Aruljothi, K. N., Shameem, N., Alqarawi, A. A., Hashem, A., and Abd_Allah, E. F. (2018). Pesticide degrading natural multidrug resistance bacterial flora. *Microbial Pathogenesis*, 114:304–310.

Rani, L., Thapa, K., Kanojia, N., Sharma, N., Singh, S., Grewal, A. S., Srivastav, A. L., and Kaushal, J. (2021). An extensive review on the consequences of chemical pesticides on human health and environment. *Journal of Cleaner Production*, 283.

Ray, P., Lakshmanan, V., Labbé, J. L., and Craven, K. D. (2020). Microbe to Microbiome: A Paradigm Shift in the Application of Microorganisms for Sustainable Agriculture. *Frontiers in Microbiology*, 11.

Regar, R. K., Gaur, V. K., Bajaj, A., Tambat, S., and Manickam, N. (2019). Comparative microbiome analysis of two different long-term pesticide contaminated soils revealed the anthropogenic influence on functional potential of microbial communities. *Science of the Total Environment*, 681:413–423.

Reinhold-Hurek, B., Bünger, W., Burbano, C. S., Sabale, M., and Hurek, T. (2015). Roots Shaping Their Microbiome: Global Hotspots for Microbial Activity. *Annual Review of Phytopathology*, 53:403–424.

Reiss, E. R. and Drinkwater, L. E. (2018). Cultivar mixtures: a meta-analysis of the effect of intraspecific diversity on crop yield. *Ecological Applications*, 28:62–77.

Richert, K., Brambilla, E., and Stackebrandt, E. (2005). Development of PCR primers specific for the amplification and direct sequencing of gyrB genes from microbacteria, order Actinomycetales. *Journal of Microbiological Methods*, 60(1):115–123.

Ridene, S., Werfelli, N., Mansouri, A., Landoulsi, A., and Abbes, C. (2023). Bioremediation potential of consortium Pseudomonas Stutzeri LBR and Cupriavidus Metallidurans LBJ in soil polluted by lead. *PLoS ONE*, 18(6 June).

Riera, N., Davyt, D., Durán, R., Iraola, G., Lemanceau, P., and Bajsa, N. (2023). An antibiotic produced by Pseudomonas fluorescens CFBP2392 with antifungal activity against Rhizoctonia solani. *Frontiers in Microbiology*, 14.

Ringel, M. T. and Brüser, T. (2018). The biosynthesis of pyoverdines. *Microbial Cell*, 5(10):424–437.

Ritpitakphong, U., Falquet, L., Vimoltust, A., Berger, A., Métraux, J. P., and L'Haridon, F. (2016). The microbiome of the leaf surface of Arabidopsis protects against a fungal pathogen. *New Phytologist*, 210(3):1033–1043.

Rodriguez, P. A. et al. (2019). Systems biology of plant microbiome interactions. *Mol. Plant*, 12.

Roesti, D., Ineichen, K., Braissant, O., Redecker, D., Wiemken, A., and Aragno, M. (2005). Bacteria Associated with Spores of the Arbuscular Mycorrhizal Fungi Glomus geosporum and Glomus constrictum. *Applied and Environmental Microbiology*, 71:6673–6679.

Roman-Reyna, V. . P., Borja, D. ., Quibod, F. N. ., Groen, I. L. ., Alexandrov, S. C. ., Mauleon, N. ., Oliva, R. ., and R. (2020). Characterization of the leaf microbiome from whole-genome sequencing data of the 3000 rice genomes project. *Rice 2020*, 13(72).

Romdhane, S., Spor, A., Busset, H., Falchetto, L., Martin, J., Bizouard, F., Bru, D., Breuil, M.-C., Philippot, L., and Cordeau, S. (2019). Cover Crop Management Practices Rather Than Composition of Cover Crop Mixtures Affect Bacterial Communities in No-Till Agroecosystems. *Frontiers in Microbiology*, 10:1618.

Roquigny, R., Novinscak, A., Arseneault, T., Joly, D. L., and Filion, M. (2018). Transcriptome alteration in Phytophthora infestans in response to phenazine-1-carboxylic acid production by Pseudomonas fluorescens strain LBUM223. *BMC Genomics*, 19(474):(18 June 2018).

Rosselló-Móra, R. and Amann, R. (2015). Past and future species definitions for Bacteria and Archaea. *Systematic and Applied Microbiology*, 38(4):209–216.

Rousk, J., Bååth, E., Brookes, P. C., Lauber, C. L., Lozupone, C., Caporaso, J. G., Knight, R., and Fierer, N. (2010). Soil bacterial and fungal communities across a pH gradient in an arable soil. *The ISME Journal*, 4:1340–1351.

Ruocco, M., Woo, S., Vinale, F., Lanzuise, S., and Lorito, M. (2012). Identified difficulties and conditions for field success of biocontrol. 2. Technical aspects: factors of efficacy. *Classical and augmentative biological control against diseases and pests: critical status analysis and review of factors influencing their success*, pages 45–57.

Ryan, P. R., Dessaux, Y., Thomashow, L. S., and Weller, D. M. (2009). Rhizosphere engineering and management for sustainable agriculture. *Plant and Soil*, 321(1-2):363–383.

Sabu, R., Aswani, R., Jishma, P., Jasim, B., Mathew, J., and Radhakrishnan, E. K. (2019). Plant Growth Promoting Endophytic Serratia sp. ZoB14 Protecting Ginger from Fungal Pathogens. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, 89:213–220.

Sahadevan, N., Radhakrishnan, E. K., and Mathew, J. (2019). Mechanism of Interaction of Endophytic Microbes with Plants. In Verma, S. K. and White, J. J. F., editors, *Seed Endophytes: Biology and Biotechnology*, pages 237–257. Springer International Publishing, Cham.

Samad, A., Antonielli, L., Sessitsch, A., Compant, S., and Trognitz, F. (2017). Comparative genome analysis of the vineyard weed endophyte Pseudomonas viridiflava CDRTc14 showing selective herbicidal activity. *Scientific Reports*, 7(1).

Sang, M. K. and Kim, K. D. (2012). Plant growth-promoting rhizobacteria suppressive to Phytophthora blight affect microbial activities and communities in the rhizosphere of pepper (Capsicum annuum L.) in the field. *Applied Soil Ecology*, 62:88–97.

Santamaria, G., Liao, C., Lindberg, C., Chen, Y., Wang, Z., Rhee, K., Pinto, F. R., Yan, J., and Xavier, J. B. (2022). Evolution and regulation of microbial secondary metabolism. *eLife*, 11.

Santos-González, J. C., Nallanchakravarthula, S., Alström, S., and Finlay, R. D. (2011). Soil, But Not Cultivar, Shapes the Structure of Arbuscular Mycorrhizal Fungal Assemblages Associated with Strawberry. *Microbial Ecology*, 62:25–35.

Santoyo, G., Hernández-Pacheco, C., Hernández-Salmerón, J., and Hernández-León, R. (2017). The role of abiotic factors modulating the plant-microbe-soil interactions: Toward sustainable agriculture. A review. *Spanish Journal of Agricultural Research*, 15(1).

Sapkota, R., Jørgensen, L. N., and Nicolaisen, M. (2017). Spatiotemporal variation and networks in the mycobiome of the wheat canopy. *Frontiers in Plant Science*, 8.

Sare, A. R., Jijakli, M. H., and Massart, S. (2021). Microbial ecology to support integrative efficacy improvement of biocontrol agents for postharvest diseases management. *Postharvest Biology and Technology*, 179(May):111572.

Sasse, J., Martinoia, E., and Northen, T. (2018). Feed Your Friends: Do Plant Exudates Shape the Root Microbiome? *Trends in Plant Science*, 23:25–41.

Sawada, H., Fujikawa, T., Osada, S., and Satou, M. (2021). Pseudomonas cyclaminis Sp. Nov., a pathogen causing bacterial bud blight of cyclamen in Japan. *International Journal of Systematic and Evolutionary Microbiology*, 71(3).

Schalk, I. J., Rigouin, C., and Godet, J. (2020). An overview of siderophore biosynthesis among fluorescent Pseudomonads and new insights into their complex cellular organization. *Environmental Microbiology*, 22(4):1447–1466.

Schebesta, H., Bernaz, N., and Macchi, C. (2020). The European Union farm to fork strategy: Sustainability and responsible business in the food supply chain. *European Food and Feed Law Review*, 15(5):420–427.

Schimel, J. P. (2018). Life in Dry Soils: Effects of Drought on Soil Microbial Communities and Processes. *Annual Review of Ecology, Evolution, and Systematics*, 49:409–432.

Schimel, J. P. and Bennett, J. (2004). Nitrogen mineralization: Challenges of a changing paradigm. *Ecology*, 85:591–602.

Schlatter, D., Kinkel, L., Thomashow, L., Weller, D., and Paulitz, T. (2017). Disease Suppressive Soils: New Insights from the Soil Microbiome. *Phytopathology*, 107:1284–1297.

Schlatter, D. C., Bakker, M. G., Bradeen, J. M., and Kinkel, L. L. (2015). Plant community richness and microbial interactions structure bacterial communities in soil. *Ecology*, 96:134–142.

Schlatter, D. C., Kahl, K., Carlson, B., Huggins, D. R., and Paulitz, T. (2020). Soil acidification modifies soil depth-microbiome relationships in a no-till wheat cropping system. *Soil Biology and Biochemistry*, 149.

Schlemper, T. R., Leite, M. F. A., Lucheta, A. R., Shimels, M., Bouwmeester, H. J., van Veen, J. A., and Kuramae, E. E. (2017). Rhizobacterial community structure differences among sorghum cultivars in different growth stages and soils. *FEMS Microbiology Ecology*, 93.

Schloss, P. D., Jenior, M. L., Koumpouras, C. C., Westcott, S. L., and Highlander, S. K. (2016). Sequencing 16S rRNA gene fragments using the PacBio SMRT DNA sequencing system. *PeerJ*, 4:e1869.

Schmidt, J. E., Kent, A. D., Brisson, V. L., and Gaudin, A. C. M. (2019). Agricultural management and plant selection interactively affect rhizosphere microbial community structure and nitrogen cycling. *Microbiome*, 7:146.

Schmidt, R., Gravuer, K., Bossange, A. V., Mitchell, J., and Scow, K. (2018). Long-term use of cover crops and no-till shift soil microbial community life strategies in agricultural soil. *PLOS ONE*, 13:e0192953.

Schouten, A., Van Den Berg, G., Edel-Hermann, V., Steinberg, C., Gautheron, N., Alabouvette, C., De Vos, C. H., Lemanceau, P., and Raaijmakers, J. M. (2004). Defense responses of Fusarium oxysporum to 2,4-diacetylphloroglucinol, a broad-spectrum antibiotic produced by Pseudomonas fluorescens. *Molecular Plant-Microbe Interactions*, 17(11):1201–1211.

Segarra, G., Puopolo, G., Giovannini, O., and Pertot, I. (2015a). Stepwise flow diagram for the development of formulations of non spore-forming bacteria against foliar pathogens: The case of Lysobacter capsici AZ78. *Journal of Biotechnology*, 216:56–64.

Segarra, G., Puopolo, G., Porcel-Rodríguez, E., Giovannini, O., and Pertot, I. (2015b). Monitoring lysobacter capsici az78 using strain specific qpcr reveals the importance of the formulation for its survival in vineyards. *FEMS Microbiology Letters*, 363(3):1–6.

Serino, L., Reimmann, C., Visca, P., Beyeler, M., Chiesa, V. D., and Haas, D. (1997). Biosynthesis of pyochelin and dihydroaeruginoic acid requires the iron- regulated pchDCBA operon in Pseudomonas aeruginosa. *Journal of Bacteriology*, 179(1):248–257.

Shade, A., Peter, H., Allison, S., Baho, D., Berga, M., Buergmann, H., Huber, D., Langenheder, S., Lennon, J., Martiny, J., Matulich, K., Schmidt, T., and Handelsman, J. (2012). Fundamentals of Microbial Community Resistance and Resilience. *Frontiers in Microbiology*, 3:417.

Sharma, S. B., Sayyed, R. Z., Trivedi, M. H., and Gobi, T. A. (2013). Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. *SpringerPlus*, 2:587.

Shen, Z., Wang, B., Lv, N., Sun, Y., Jiang, X., Li, R., Ruan, Y., and Shen, Q. (2015). Effect of the combination of bio-organic fertiliser with Bacillus amyloliquefaciens NJN-6 on the control of banana Fusarium wilt disease, crop production and banana rhizosphere culturable microflora. *Biocontrol Science and Technology*, 25:716–731.

Shirzad, A., Pazhouhandeh, M., Ahmadabadi, M., and Behboudi, K. (2012). Analysis of cross-talk between Trichoderma atroviride and Pseudomonas fluorescens. *Journal of Plant Pathology*, 94(3):621–628.

Sidorova, T. M., Tomashevich, N. S., Allahverdyan, V. V., Tupertsev, B. S., Kostyukevich, Y. I., and Asaturova, A. M. (2023). New Pseudomonas Bacterial Strains: Biological Activity and Characteristic Properties of Metabolites. *Microorganisms*, 11(8).

Siegwart, M., Graillot, B., Lopez, C. B., Besse, S., Bardin, M., Nicot, P. C., and Lopez-Ferber, M. (2015). Resistance to bio-insecticides or how to enhance their sustainability: A review. *Frontiers in Plant Science*, 6(JUNE):1–19.

Simberloff, D. (2014). Biological invasions: What's worth fighting and what can be won? *Ecological Engineering*, 65:112–121.

Simonin, M., Dasilva, C., Terzi, V., Ngonkeu, E. L., DIouf, D., Kane, A., Béna, G., and Moulin, L. (2020). Influence of plant genotype and soil on the wheat rhizosphere microbiome: Evidences for a core microbiome across eight African and European soils. *FEMS Microbiology Ecology*, 96(6).

Singh, H., Du, J., Trinh, H., Won, K. H., Yang, J. E., Yin, C. S., Kook, M. C., and Yi, T. H. (2016). Nocardioides albidus sp. Nov., an actinobacterium isolated from garden soil. *International Journal of Systematic and Evolutionary Microbiology*, 66(1):371–378.

Sivakumar, N., Sathishkumar, R., Selvakumar, G., Shyamkumar, R., and Arjunekumar, K. (2020). *Phyllospheric Microbiomes: Diversity, Ecological Significance, and Biotechnological Applications*. Springer, Cham.

Sobrero, P. M., Muzlera, A., Frescura, J., Jofré, E., and Valverde, C. (2017). A matter of hierarchy: activation of orfamide production by the post-transcriptional Gac-Rsm cascade of Pseudomonas protegens CHA0 through expression upregulation of the two dedicated transcriptional regulators. *Environmental Microbiology Reports*, 9(5):599–611.

Sommermann, L., Geistlinger, J., Wibberg, D., Deubel, A., Zwanzig, J., Babin, D., Schlüter, A., and Schellenberg, I. (2018). Fungal community profiles in agricultural soils of a long-term field trial under different tillage, fertilization and crop rotation conditions analyzed by high-throughput ITS-amplicon sequencing. *PLOS ONE*, 13:e0195345.

Song, C., Aundy, K., van de Mortel, J., and Raaijmakers, J. M. (2014). Discovery of new regulatory genes of lipopeptide biosynthesis in Pseudomonas fluorescens. *FEMS Microbiology Letters*, 356(2):166–175.

Song, C., van der Voort, M., van de Mortel, J., Hassan, K. A., Elbourne, L. D., Paulsen, I. T., Loper, J. E., and Raaijmakers, J. M. (2015a). The Rsm regulon of plant growth-promoting Pseudomonas fluorescens SS101: Role of small RNAs in regulation of lipopeptide biosynthesis. *Microbial Biotechnology*, 8(2):296–310.

Song, C., van der Voort, M., van de Mortel, J., Hassan, K. A., Elbourne, L. D., Paulsen, I. T., Loper, J. E., and Raaijmakers, J. M. (2015b). The Rsm regulon of plant growth-promoting Pseudomonas fluorescens SS101: Role of small RNAs in regulation of lipopeptide biosynthesis. *Microbial Biotechnology*, 8(2):296–310.

Song, H.-K., Song, W., Kim, M., Tripathi, B. M., Kim, H., Jablonski, P., and Adams, J. M. (2017). Bacterial strategies along nutrient and time gradients, revealed by metagenomic analysis of laboratory microcosms. *FEMS Microbiology Ecology*, 93.

Sonnleitner, E. and Haas, D. (2011). Small RNAs as regulators of primary and secondary metabolism in Pseudomonas species. *Applied Microbiology and Biotechnology*, 91(1):63–79.

Souza, R. C., Cantão, M. E., Vasconcelos, A. T. R., Nogueira, M. A., and Hungria, M. (2013). Soil metagenomics reveals differences under conventional and no-tillage with crop rotation or succession. *Applied Soil Ecology*, 72:49–61.

Srivastava, P. K., Singh, V. P., Singh, A., and Chauhan, D. K. (2020). *Pesticides in Crop Production*. Wiley Online Library.

Srour, A. Y., Ammar, H. A., Subedi, A., Pimentel, M., Cook, R. L., Bond, J., and Fakhoury, A. M. (2020). Microbial Communities Associated With Long-Term Tillage and Fertility Treatments in a Corn-Soybean Cropping System. *Frontiers in Microbiology*, 11:1363.

Statbel (2023). Hausse de la production des céréales d'hiver et de printemps.

Stempfhuber, B., Engel, M., Fischer, D., Neskovic-Prit, G., Wubet, T., Schöning, I., Gubry-Rangin, C., Kublik, S., Schloter-Hai, B., Rattei, T., Welzl, G., Nicol, G. W., Schrumpf, M., Buscot, F., Prosser, J. I., and Schloter, M. (2015). pH as a Driver for Ammonia-Oxidizing Archaea in Forest Soils. *Microbial Ecology*, 69:879–883.

Stolovitzky, G. and Cecchi, G. (1996). Efficiency of DNA replication in the polymerase chain reaction. *Proceedings of the National Academy of Sciences of the United States of America*, 93(23):12947–12952.

Stone, B. W. and Jackson, C. R. (2016). Biogeographic Patterns Between Bacterial Phyllosphere Communities of the Southern Magnolia (Magnolia grandiflora) in a Small Forest. *Microbial Ecology*, 71(4):954–961.

Stone, B. W. and Jackson, C. R. (2021). Seasonal Patterns Contribute More Towards Phyllosphere Bacterial Community Structure than Short-Term Perturbations. *Microbial Ecology*, 81(1):146–156.

Storck, V., Nikolaki, S., Perruchon, C., Chabanis, C., Sacchi, A., Pertile, G., Baguelin, C., Karas, P. A., Spor, A., Devers-Lamrani, M., Papadopoulou, E. S., Sibourg, O., Malandain, C., Trevisan, M., Ferrari, F., Karpouzas, D. G., Tsiamis, G., and Martin-Laurent, F. (2018). Lab to field assessment of the ecotoxicological impact of chlorpyrifos, isoproturon, or tebuconazole on the diversity and composition of the soil bacterial community. *Frontiers in Microbiology*, 9(JUN).

Streletskii, R. A., Kachalkin, A. V., Glushakova, A. M., Yurkov, A. M., and Demin, V. V. (2019). Yeasts producing zeatin. *PeerJ*, 2019(2):1–16.

Sui, Y. and Liu, J. (2014). Effect of glucose on thermotolerance and biocontrol efficacy of the antagonistic yeast Pichia guilliermondii. *Biological Control*, 74:59–64.

Sułowicz, S. and Piotrowska-Seget, Z. (2016). Response of microbial communities from an apple orchard and grassland soils to the first-time application of the fungicide tetraconazole. *Ecotoxicology and Environmental Safety*, 124:193–201.

Sun, L., Dong, Y., Zhou, Y., Yang, M., Zhang, C., Rao, Z., and Zhang, X.-e. (2004). Crystallization and preliminary X-ray studies of methyl parathion hydrolase from Pseudomonas sp. WBC-3. *Acta Crystallographica Section D*, 60:954–956.

Sun, T., Miao, J., Saleem, M., Zhang, H., Yang, Y., and Zhang, Q. (2020). Bacterial compatibility and immobilization with biochar improved tebuconazole degradation, soil microbiome composition and functioning. *Journal of Hazardous Materials*, 398.

Świątczak, J., Kalwasińska, A., and Brzezinska, M. S. (2023a). Plant growth–promoting rhizobacteria: Peribacillus frigoritolerans 2RO30 and Pseudomonas sivasensis 2RO45 for their effect on canola growth under controlled as well as natural conditions. *Frontiers in Plant Science*, 14(January):1–13.

Świątczak, J., Kalwasińska, A., Szabó, A., and Swiontek Brzezinska, M. (2023b). Pseudomonas sivasensis 2RO45 inoculation alters the taxonomic structure and functioning of the canola rhizosphere microbial community. *Frontiers in Microbiology*, 14.

Sylla, J., Alsanius, B. W., Krüger, E., Reineke, A., Strohmeier, S., and Wohanka, W. (2013). Leaf microbiota of strawberries as affected by biological control agents. *Phytopathology*, 103(10):1001–1011.

Takeuchi, K., Noda, N., Katayose, Y., Mukai, Y., Numa, H., Yamada, K., and Someya, N. (2015). Rhizoxin analogs contribute to the biocontrol activity of a newly isolated Pseudomonas strain. *Molecular Plant-Microbe Interactions*, 28(3):333–342.

Tambong, J. T. and Höfte, M. (2001). Phenazines are involved in biocontrol of Pythium myriotylum on cocoyam by Pseudomonas aeruginosa PNA1. *European Journal of Plant Pathology*, 107(5):511–521.

Tan, W., Wang, J., Bai, W., Qi, J., and Chen, W. (2020). Soil bacterial diversity correlates with precipitation and soil pH in long-term maize cropping systems. *Scientific Reports*, 10:6012.

Tao, X., Zhang, H., Gao, M., Li, M., Zhao, T., and Guan, X. (2020). Pseudomonas species isolated via high-throughput screening significantly protect cotton plants against verticillium wilt. *AMB Express*, 10(1).

Taylor, M. J., Bentham, R. H., and Ross, K. E. (2014). Limitations of Using Propidium Monoazide with qPCR to Discriminate between Live and Dead Legionella in Biofilm Samples . *Microbiology Insights*, 7:MBI.S17723.

Tejada, M., Benítez, C., Gómez, I., and Parrado, J. (2011). Use of biostimulants on soil restoration: Effects on soil biochemical properties and microbial community. *Applied Soil Ecology*, 49:11–17.

Tétard-Jones, C. and Edwards, R. (2016). Potential roles for microbial endophytes in herbicide tolerance in plants. *Pest Management Science*, 72:203–209.

The Brussels Times (2024). Food security Belgian farmers dedicate more land to cereals.

Thilakarathna, S. H., Stokowski, T., and Chui, L. (2022). An Improved Real-Time Viability PCR Assay to Detect Salmonella in a Culture-Independent Era. *International Journal of Molecular Sciences*, 23(23).

Thomloudi, E. E., Tsalgatidou, P. C., Baira, E., Papadimitriou, K., Venieraki, A., and Katinakis, P. (2021). Genomic and metabolomic insights into secondary metabolites of the novel bacillus halotolerans hil4, an endophyte with promising antagonistic activity against gray mold and plant growth promoting potential. *Microorganisms*, 9(12).

Tian, B., Zhu, M., Pei, Y., Ran, G., Shi, Y., and Ding, J. (2022). Climate warming alters the soil microbial association network and role of keystone taxa in determining wheat quality in the field. *Agriculture, Ecosystems and Environment*, 326.

Tian, L., Zhang, W., Zhou, G. D., Li, S., Wang, Y., Yang, B., Bai, T., Fan, H., He, P., and Zheng, S. J. (2023). A biological product of Bacillus amyloliquefaciens QST713 strain for promoting banana plant growth and modifying rhizosphere soil microbial diversity and community composition. *Frontiers in Microbiology*, 14.

Tidd, H., Rudd, J. J., Ray, R. V., Bryant, R., and Kanyuka, K. (2023). A large bioassay identifies Stb resistance genes that provide broad resistance against Septoria tritici blotch disease in the UK. *Frontiers in Plant Science*, 13.

Tilman, D., Cassman, K. G., Matson, P. A., Naylor, R., and Polasky, S. (2002). Agricultural sustainability and intensive production practices. *Nature*, 418:671–677.

Toju, H., Okayasu, K., and Notaguchi, M. (2019). Leaf-associated microbiomes of grafted tomato plants. *Scientific Reports*, 9(1):1–11.

Toju, H., Peay, K. G., Yamamichi, M., Narisawa, K., Hiruma, K., Naito, K., Fukuda, S., Ushio, M., Nakaoka, S., Onoda, Y., Yoshida, K., Schlaeppi, K., Bai, Y., Sugiura, R., Ichihashi, Y., Minamisawa, K., and Kiers, E. T. (2018). Core microbiomes for sustainable agroecosystems. *Nature Plants*, 4(5):247–257.

Torabi, E., Wiegert, C., Guyot, B., Vuilleumier, S., and Imfeld, G. (2020). Dissipation of S-metolachlor and butachlor in agricultural soils and responses of bacterial communities: Insights from compound-specific isotope and biomolecular analyses. *Journal of Environmental Sciences (China)*, 92:163–175.

Trippe, K., McPhail, K., Armstrong, D., Azevedo, M., and Banowetz, G. (2013). Pseudomonas fluorescens SBW25 produces furanomycin, a non-proteinogenic amino acid with selective antimicrobial properties. *BMC Microbiology*, 13(1).

Trivedi, P., Leach, J. E., Tringe, S. G., Sa, T., and Singh, B. K. (2020). Plant–microbiome interactions: from community assembly to plant health. *Nature Reviews Microbiology*, 18(11):607–621.

Turner, T. R., James, E. K., and Poole, P. S. (2013). The plant microbiome. *Genome Biology*, 14(6).

Tyler, H. L. (2019). Bacterial community composition under long-term reduced tillage and no till management. *Journal of Applied Microbiology*, 126:1797–1807.

Ueda, H., Kurose, D., Kugimiya, S., Mitsuhara, I., Yoshida, S., Tabata, J., Suzuki, K., and Kitamoto, H. (2018). Disease severity enhancement by an esterase from non-phytopathogenic yeast Pseudozyma antarctica and its potential as adjuvant for biocontrol agents. *Scientific Reports*, 8(1):1–12.

Urbina, H., Breed, M. F., Zhao, W., Lakshmi Gurrala, K., Andersson, S. G. E., Ågren, J., Baldauf, S., and Rosling, A. (2018). Specificity in Arabidopsis thaliana recruitment of root fungal communities from soil and rhizosphere. *Fungal Biology*, 122:231–240.

Vacher, C., Hampe, A., Porté, A. J., Sauer, U., Compant, S., and Morris, C. E. (2016). The Phyllosphere: Microbial Jungle at the Plant-Climate Interface. *Annual Review of Ecology, Evolution, and Systematics*, 47:1–24.

Vacheron, J., Desbrosses, G., Bouffaud, M. L., Touraine, B., Moënne-Loccoz, Y., Muller, D., Legendre, L., Wisniewski-Dyé, F., and Prigent-Combaret, C. (2013). Plant growth-promoting rhizobacteria and root system functioning. *Frontiers in Plant Science*, 4(SEP).

Van Der Heijden, M. G. A., Bardgett, R. D., and Van Straalen, N. M. (2008). The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology Letters*, 11:296–310.

van Dijk, E. L., Jaszczyszyn, Y., Naquin, D., and Thermes, C. (2018). The Third Revolution in Sequencing Technology. *Trends in Genetics*, 34:666–681.

van Elsas, J. D., Chiurazzi, M., Mallon, C. A., Elhottovà, D., Krištůfek, V., and Salles, J. F. (2012). Microbial diversity determines the invasion of soil by a bacterial pathogen. *Proceedings of the National Academy of Sciences*, 109:1159–1164.

Van Gestel, M., Merckx, R., and Vlassak, K. (1993). Microbial biomass responses to soil drying and rewetting: The fate of fast- and slow-growing microorganisms in soils from different climates. *Soil Biology and Biochemistry*, 25:109–123.

Van Overbeek, L. and Van Elsas, J. D. (2008). Effects of plant genotype and growth stage on the structure of bacterial communities associated with potato (Solanum tuberosum L.). *FEMS Microbiology Ecology*, 64:283–296.

Vandenkoornhuyse, P., Quaiser, A., Duhamel, M., Le Van, A., and Dufresne, A. (2015). The importance of the microbiome of the plant holobiont. *New Phytologist*, 206(4):1196–1206.

Vaughn, V. L. and Gross, D. C. (2016). Characterization of salA, syrF, and syrG genes and attendant regulatory networks involved in plant pathogenesis by pseudomonas syringae pv. syringae B728a. *PLoS ONE*, 11(3).

Velez, P., Espinosa-Asuar, L., Figueroa, M., Gasca-Pineda, J., Aguirre-von Wobeser, E., Eguiarte, L. E., Hernandez-Monroy, A., and Souza, V. (2018). Nutrient dependent cross-kingdom interactions: Fungi and bacteria from an oligotrophic desert oasis. *Frontiers in Microbiology*, 9(AUG):1–15.

Verginer, M., Siegmund, B., Cardinale, M., Müller, H., Choi, Y., Míguez, C. B., Leitner, E., and Berg, G. (2010). Monitoring the plant

epiphyte Methylobacterium extorquens DSM 21961 by real-time PCR and its influence on the strawberry flavor. *FEMS Microbiology Ecology*, 74(1):136–145.

Viana, F., Peringathara, S. S., Rizvi, A., and Schroeder, G. N. (2021). Host manipulation by bacterial type III and type IV secretion system effector proteases. *Cellular Microbiology*, 23(11).

Vollenweider, V., Rehm, K., Chepkirui, C., Pérez-Berlanga, M., Polymenidou, M., Piel, J., Bigler, L., and Kümmerli, R. (2023). Antimicrobial activity of iron-depriving pyoverdines against human opportunistic pathogens. *biorxiv*.

Volpicella, M., Costanza, A., Palumbo, O., Italiano, F., Claudia, L., Placido, A., Picardi, E., Carella, M., Trotta, M., and Ceci, L. R. (2014). Rhodobacter sphaeroides adaptation to high concentrations of cobalt ions requires energetic metabolism changes. *FEMS Microbiology Ecology*, 88(2):345–357.

Vorholt, J. A. (2012). Microbial life in the phyllosphere. *Nature Reviews Microbiology*, 10(12):828–840.

Vorholt, J. A., Vogel, C., Carlström, C. I., and Müller, D. B. (2017). Establishing Causality: Opportunities of Synthetic Communities for Plant Microbiome Research. *Cell Host and Microbe*, 22:142–155.

Wakelin, S. A., Macdonald, L. M., Rogers, S. L., Gregg, A. L., Bolger, T. P., and Baldock, J. A. (2008). Habitat selective factors influencing the structural composition and functional capacity of microbial communities in agricultural soils. *Soil Biology and Biochemistry*, 40:803–813.

Walitang, D. I., Kim, K., Madhaiyan, M., Kim, Y. K., Kang, Y., and Sa, T. (2017). Characterizing endophytic competence and plant growth promotion of bacterial endophytes inhabiting the seed endosphere of Rice. *BMC Microbiology*, 17:209.

Wan, Y., Liu, H., Xian, M., and Huang, W. (2021). Biosynthesis and metabolic engineering of 1-hydroxyphenazine in Pseudomonas chlororaphis H18. *Microbial Cell Factories*, 20(1).

Wang, C. and Kuzyakov, Y. (2024). Mechanisms and implications of bacterial – fungal. *ISME Journal*, 18(C).

Wang, D., Yu, J. M., Dorosky, R. J., Pierson, L. S., and Pierson, E. A. (2016a). The phenazine 2-hydroxy-phenazine-1- carboxylic acid promotes extracellular DNA release and has broad transcriptomic consequences in pseudomonas chlororaphis 30-84. *PLoS ONE*, 11(1).

Wang, N., Lu, S. E., Records, A. R., and Gross, D. C. (2006a). Characterization of the transcriptional activators SalA and SyrF, which

are required for syringomycin and syringopeptin production by Pseudomonas syringae pv. syringae. *Journal of Bacteriology*, 188(9):3290–3298.

Wang, N., Lu, S. E., Wang, J., Chen, Z. J., and Gross, D. C. (2006b). The expression of genes encoding lipodepsipeptide phytotoxins by Pseudomonas syringae pv. syringae is coordinated in response to plant signal molecules. *Molecular Plant-Microbe Interactions*, 19(3):257–269.

Wang, X., Liang, C., Mao, J., Jiang, Y., Bian, Q., Liang, Y., Chen, Y., and Sun, B. (2023). Microbial keystone taxa drive succession of plant residue chemistry. *ISME Journal*, 17(5):748–757.

Wang, Y., Fu, F., Li, J., Wang, G., Wu, M., Zhan, J., Chen, X., and Mao, Z. (2016b). Effects of seaweed fertilizer on the growth of Malus hupehensis Rehd. seedlings, soil enzyme activities and fungal communities under replant condition. *European Journal of Soil Biology*, 75:1–7.

Wang, Y. S., Huang, Y. J., Chen, W. C., and Yen, J. H. (2009). Effect of carbendazim and pencycuron on soil bacterial community. *Journal of Hazardous Materials*, 172(1):84–91.

Wang, Z., Li, T., Li, Y., Zhao, D., Han, J., Liu, Y., and Liao, Y. (2020). Relationship between the microbial community and catabolic diversity in response to conservation tillage. *Soil and Tillage Research*, 196:104431.

Wang, Z., Liu, L., Chen, Q., Wen, X., and Liao, Y. (2016c). Conservation tillage increases soil bacterial diversity in the dryland of northern China. *Agronomy for Sustainable Development*, 36:28.

Wang, Z., Liu, L., Chen, Q., Wen, X., Liu, Y., Han, J., and Liao, Y. (2017). Conservation tillage enhances the stability of the rhizosphere bacterial community responding to plant growth. *Agronomy for Sustainable Development*, 37:44.

Washio, K., Lim, S. P., Roongsawang, N., and Morikawa, M. (2010). Identification and characterization of the genes responsible for the production of the cyclic lipopeptide arthrofactin by Pseudomonas sp. MIS38. *Bioscience, Biotechnology and Biochemistry*, 74(5):992–999.

watch, F. (2023). The dark side of grain. *Unknown*.

Webber, M. A., Whitehead, R. N., Mount, M., Loman, N. J., Pallen, M. J., and Piddock, L. J. V. (2015). Parallel evolutionary pathways to antibiotic resistance selected by biocide exposure. *Journal of Antimicrobial Chemotherapy*, 70:2241–2248.

Wei, F., Zhao, L., Xu, X., Feng, H., Shi, Y., Deakin, G., Feng, Z., and Zhu, H. (2019). Cultivar-Dependent Variation of the Cotton Rhizosphere and Endosphere Microbiome Under Field Conditions. *Frontiers in Plant Science*, 10:1659.

Wei, Z., Yang, T., Friman, V.-P., Xu, Y., Shen, Q., and Jousset, A. (2015). Trophic network architecture of root-associated bacterial communities determines pathogen invasion and plant health. *Nature Communications*, 6:8413.

Weiss, S., Xu, Z. Z., Peddada, S., Amir, A., Bittinger, K., Gonzalez, A., Lozupone, C., Zaneveld, J. R., Vázquez-Baeza, Y., Birmingham, A., Hyde, E. R., and Knight, R. (2017). Normalization and microbial differential abundance strategies depend upon data characteristics. *Microbiome*, 5:27.

Weller, D. M., Landa, B. B., Mavrodi, O. V., Schroeder, K. L., De La Fuente, L., Blouin Bankhead, S., Allende Molar, R., Bonsall, R. F., Mavrodi, D. V., and Thomashow, L. S. (2007). Role of 2,4-diacetylphloroglucinol-producing fluorescent Pseudomonas spp. in the defense of plant roots. *Plant Biology*, 9(1):4–20.

Whipps, J. M., Hand, P., Pink, D., and Bending, G. D. (2008a). Phyllosphere microbiology with special reference to diversity and plant genotype. *Journal of Applied Microbiology*, 105(6):1744–1755.

Whipps, J. M., Hand, P., Pink, D. A., and Bending, G. D. (2008b). Human pathogens and the phylloshere. *Advances in applied microbiology*, 64:183–221.

Whipps, J. M., Karen, L., and Cooke, R. C. (1988). Mycoparasitism and plant disease control. *Fungi in biological control systems*, pages 161–187.

White, P. M., Potter, T. L., and Culbreath, A. K. (2010). Fungicide dissipation and impact on metolachlor aerobic soil degradation and soil microbial dynamics. *Science of The Total Environment*, 408:1393–1402.

Wick, R. R. and Holt, K. E. (2022). Polypolish: Short-read polishing of long-read bacterial genome assemblies. *PLoS Computational Biology*, 18(1).

Wick, R. R., Judd, L. M., Cerdeira, L. T., Hawkey, J., Méric, G., Vezina, B., Wyres, K. L., and Holt, K. E. (2021). Trycycler: consensus long-read assemblies for bacterial genomes. *Genome Biology*, 22(1):2021.07.04.451066.

Wick, R. R., Judd, L. M., and Holt, K. E. (2023). Assembling the perfect bacterial genome using Oxford Nanopore and Illumina sequencing. *PLoS Computational Biology*, 19(3).

Winding, A., Binnerup, S. J., and Pritchard, H. (2004). Non-target effects of bacterial biological control agents suppressing root pathogenic fungi. *FEMS Microbiology Ecology*, 47(2):129–141.

Wołejko, E., Jabłońska-Trypuć, A., Wydro, U., Butarewicz, A., and Łozowicka, B. (2020). Soil biological activity as an indicator of soil pollution with pesticides – A review. *Applied Soil Ecology*, 147.

Wood, T. L., Gong, T., Zhu, L., Miller, J., Miller, D. S., Yin, B., and Wood, T. K. (2018). Rhamnolipids from Pseudomonas aeruginosa disperse the biofilms of sulfate-reducing bacteria. *npj Biofilms and Microbiomes*, 4(1).

Wu, D. Q., Ye, J., Ou, H. Y., Wei, X., Huang, X., He, Y. W., and Xu, Y. (2011). Genomic analysis and temperature-dependent transcriptome profiles of the rhizosphere originating strain Pseudomonas aeruginosa M18. *BMC Genomics*, 12.

Wu, J. and Brookes, P. C. (2005). The proportional mineralisation of microbial biomass and organic matter caused by air-drying and rewetting of a grassland soil. *Soil Biology and Biochemistry*, 37:507–515.

Wu, Y., Xiao, S., Qi, J., Gong, Y., and Li, K. (2022). Pseudomonas fluorescens BsEB-1: an endophytic bacterium isolated from the root of Bletilla striata that can promote its growth. *Plant Signaling and Behavior*, 17(1).

Xiong, L., Li, Y., Yu, H., Wei, Y., Li, H., and Ji, X. (2023). Whole genome analysis and cold adaptation strategies of Pseudomonas sivasensis W-6 isolated from the Napahai plateau wetland. *Scientific Reports*, 13(1).

Xu, J., Zhang, Y., Zhang, P., Trivedi, P., Riera, N., Wang, Y., Liu, X., Fan, G., Tang, J., Coletta-Filho, H. D., Cubero, J., Deng, X., Ancona, V., Lu, Z., Zhong, B., Roper, M. C., Capote, N., Catara, V., Pietersen, G., Vernière, C., Al-Sadi, A. M., Li, L., Yang, F., Xu, X., Wang, J., Yang, H., Jin, T., and Wang, N. (2018). The structure and function of the global citrus rhizosphere microbiome. *Nature Communications*, 9(1).

Xu, Y., Wang, G., Jin, J., Liu, J., Zhang, Q., and Liu, X. (2009). Bacterial communities in soybean rhizosphere in response to soil type, soybean genotype, and their growth stage. *Soil Biology and Biochemistry*, 41:919–925.

Yan, Q., Lopes, L. D., Shaffer, B. T., Kidarsa, T. A., Vining, O., Philmus, B., Song, C., Stockwell, V. O., Raaijmakers, J. M., McPhail, K. L., Andreote, F. D., Chang, J. H., and Loper, J. E. (2018). Secondary metabolism and interspecific competition affect accumulation

of spontaneous mutants in the GacS-GacA regulatory system in Pseudomonas protegens. *mBio*, 9(1).

Yan, Z., Xiong, C., Liu, H., and Singh, B. K. (2022). Sustainable agricultural practices contribute significantly to One Health. *Journal of Sustainable Agriculture and Environment*, 1(3):165–176.

Yandigeri, M. S., Malviya, N., Solanki, M. K., Shrivastava, P., and Sivakumar, G. (2015). Chitinolytic Streptomyces vinaceusdrappus S5MW2 isolated from Chilika lake, India enhances plant growth and biocontrol efficacy through chitin supplementation against Rhizoctonia solani. *World Journal of Microbiology and Biotechnology*, 31(8):1217–1225.

Yang, F., Zhang, J., Zhang, H., Ji, G., Zeng, L., Li, Y., Yu, C., Fernando, W. G., and Chen, W. (2020). Bacterial Blight Induced Shifts in Endophytic Microbiome of Rice Leaves and the Enrichment of Specific Bacterial Strains With Pathogen Antagonism. *Frontiers in Plant Science*, 11(July):1–14.

Yang, M.-M., Wen, S.-S., Mavrodi, D. V., Mavrodi, O. V., von Wettstein, D., Thomashow, L. S., Guo, J.-H., and Weller, D. M. (2014). Biological control of wheat root diseases by the CLP-producing strain Pseudomonas fluorescens HC1-07. *Phytopathology*, 104(3):248–256.

Yang, Q., Yan, Q., Zhang, B., Zhang, L. Q., and Wu, X. (2023). Citrate Synthase GltA Modulates the 2,4-Diacetylphloroglucinol Biosynthesis of Pseudomonas fluorescens 2P24 and is Essential for the Biocontrol Capacity. *Journal of Agricultural and Food Chemistry*, 71(31):11892–11901.

Yao, H. and Wu, F. (2010). Soil microbial community structure in cucumber rhizosphere of different resistance cultivars to fusarium wilt. *FEMS Microbiology Ecology*, 72:456–463.

Ye, J., Coulouris, G., Zaretskaya, I., Cutcutache, I., Rozen, S., and Madden, T. L. (2012). Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction. *BMC bioinformatics*, 13:134.

Yin, D., Wang, N., Xia, F., Li, Q., and Wang, W. (2013). Impact of biocontrol agents Pseudomonas fluorescens 2P24 and CPF10 on the bacterial community in the cucumber rhizosphere. *European Journal of Soil Biology*, 59:36–42.

Yoon, S. H., min Ha, S., Lim, J., Kwon, S., and Chun, J. (2017). A large-scale evaluation of algorithms to calculate average nucleotide identity. *Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology*, 110(10):1281–1286.

You, Y., Moreira, B. G., Behlke, M. A., and Owczarzy, R. (2006). Design of LNA probes that improve mismatch discrimination. *Nucleic Acids Research*, 34(8).

Yu, J. M., Wang, D., Pierson, L. S., and Pierson, E. A. (2018). Effect of producing different phenazines on bacterial fitness and biological control in pseudomonas chlororaphis 30-84. *Plant Pathology Journal*, 34(1):44–58.

Yu, K., Stringlis, I. A., van Bentum, S., de Jonge, R., Snoek, B. L., Pieterse, C. M., Bakker, P. A., and Berendsen, R. L. (2021). Transcriptome signatures in pseudomonas simiae wcs417 shed light on role of root-secreted coumarins in arabidopsis-mutualist communication. *Microorganisms*, 9(3):1–15.

Zachow, C., Müller, H., Cardinale, M., Erlacher, A., Grube, M., Tilcher, R., and Berg, G. (2015). The impact of the host genotype on the structure of the seed and rhizosphere microbiome: sugar beet and lettuce as model plants. In Messmer, M., editor, *First EUCARPIA Workshop on Implementing Plant-Microbe Interaction in Plant Breeding, München, Germany, 25 - 26 June 2015*, pages 21–22, Institute of Environmental Biotechnology, Graz University of Technology, Graz, Austria. European Association for Research on Plant Breeding (EUCARPIA).

Zhalnina, K., Dias, R., de Quadros, P. D., Davis-Richardson, A., Camargo, F. A. O., Clark, I. M., McGrath, S. P., Hirsch, P. R., and Triplett, E. W. (2015). Soil pH determines microbial diversity and composition in the park grass experiment. *Microbial Ecology*, 69:395–406.

Zhang, C., Zhou, T., Zhu, L., Juhasz, A., Du, Z., Li, B., Wang, J., Wang, J., and Sun, Y. (2019). Response of soil microbes after direct contact with pyraclostrobin in fluvo-aquic soil. *Environmental Pollution*, 255:113164.

Zhang, H., Song, J., Zhang, Z., Zhang, Q., Chen, S., Mei, J., Yu, Y., and Fang, H. (2021a). Exposure to fungicide difenoconazole reduces the soil bacterial community diversity and the co-occurrence network complexity. *Journal of Hazardous Materials*, 405.

Zhang, J., Cook, J., Nearing, J. T., Zhang, J., Raudonis, R., Glick, B. R., Langille, M. G., and Cheng, Z. (2021b). Harnessing the plant microbiome to promote the growth of agricultural crops. *Microbiological Research*, 245.

Zhang, M., Peng, C., Sun, W., Dong, R., and Hao, J. (2022). Effects of Variety, Plant Location, and Season on the Phyllosphere Bacterial Community Structure of Alfalfa (Medicago sativa L.). *Microorganisms*, 10(10).

Zhang, Q., Liu, Y., Harvey, P. R., Stummer, B. E., Yang, J., and Ji, Z. (2023). Wheat rhizosphere colonization by Bacillus amyloliquefaciens W10 and Pseudomonas protegens FD6 suppress soil and in planta abundance of the sharp eyespot pathogen Rhizoctonia cerealis. *Journal of Applied Microbiology*, 134(5).

Zhang, Q. X., Kong, X. W., Li, S. Y., Chen, X. J., and Chen, X. J. (2020). Antibiotics of Pseudomonas protegens FD6 are essential for biocontrol activity. *Australasian Plant Pathology*, 49(3):307–317.

Zhang, Y., Shen, H., He, X., Thomas, B. W., Lupwayi, N. Z., Hao, X., Thomas, M. C., and Shi, X. (2017). Fertilization Shapes Bacterial Community Structure by Alteration of Soil pH. *Frontiers in Microbiology*, 8:1325.

Zhao, H., Shao, D., Jiang, C., Shi, J., Li, Q., Huang, Q., Rajoka, M. S. R., Yang, H., and Jin, M. (2017). Biological activity of lipopeptides from Bacillus. *Applied Microbiology and Biotechnology*, 101(15):5951–5960.

Zhao, M., Zhang, W., Tian, J., Pei, J., and Xu, X. (2020). N-fertilization drastically reduces phagotrophic protists in different soil types. *Soil Biology and Biochemistry*, 145:107795.

Zhimo, V. Y., Kumar, A., Biasi, A., Salim, S., Feygenberg, O., Toamy, M. A., Abdelfattaah, A., Medina, S., Freilich, S., Wisniewski, M., and Droby, S. (2021). Compositional shifts in the strawberry fruit microbiome in response to near-harvest application of Metschnikowia fructicola, a yeast biocontrol agent. *Postharvest Biology and Technology*, 175(November 2020):111469.

Zhou, L., Höfte, M., and Hennessy, R. C. (2024). Does regulation hold the key to optimizing lipopeptide production in Pseudomonas for biotechnology? *Frontiers in Bioengineering and Biotechnology*, 12.

Zimin, A. V. and Salzberg, S. L. (2020). The genome polishing tool POLCA makes fast and accurate corrections in genome assemblies. *PLoS Computational Biology*, 16(6).

Appendix

Table S-1: Others bioactive molecules produced by *Pseudomonas* spp.

Class	**Metabolite**
Aminoglycoside	Anikasin
Cyclic lipopeptide	Arthrofactin
Cyclic lipopeptide	Bananamides
Cyclic lipopeptide	Massetolide A
Cyclic lipopeptide	Pseudodesmin A
Cyclic lipopeptide	Putisolvin
Cyclic lipopeptide	Viscosin
Cyclic lipopeptide	Viscosinamide A
Cyclic lipopeptide	WLIP
Cyclic lipopeptide	Xantholysin
Cyclic lipopeptide	Poaeamide
Cyclic lipopeptide	Orfamides (ABCDE)
Cyclic lipoundecapeptide	Lokisin
Diazeniumdiolate	Fragin
Enzyme	Oxalate degrading enzym
Lipodepsipeptide	Tolaasin
Polyketide	Koreeceine
Polyketide	Lankacidin
Pyrazolotriazine	Pseudoiodinine
Pyrone	Pseudopyronine
Quinolones	Dihydroaeruginoic acid
Siderophore	Crochelin
Soluble tropolonoid	7-hydroxytropolone

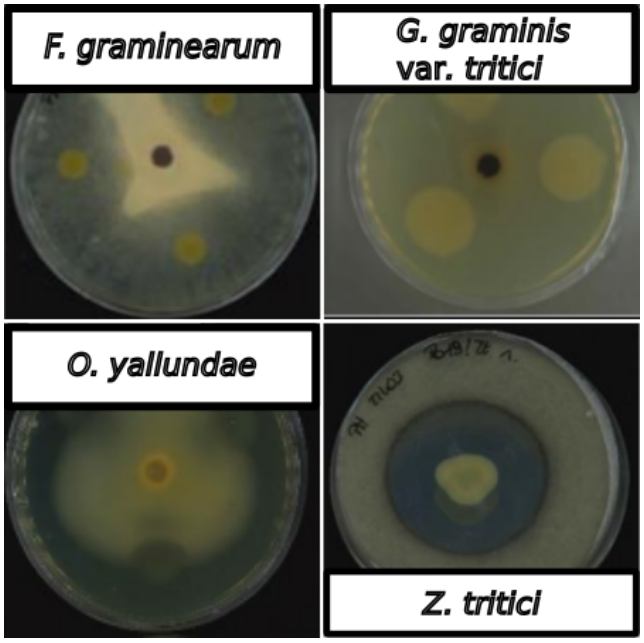

Figure S-1: Exemples of *in vitro* confrontation tests

For in vitro mycelial growth reduction of pathogens, inhibition is measured between bacterial isolates and fungi for Fusarium graminearum, Gaemanomyces graminis var. tritici and Oculimacula yallundae. For Zymoseptoria tritici, a top-agar method is used.

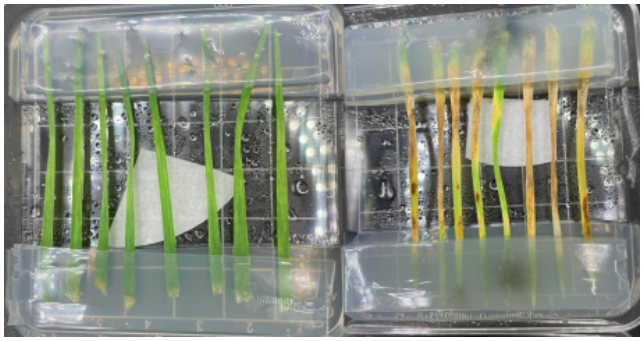

Figure S-2: Detached leaves tests

Illustration of the detached leaves protocol utilized in the study. The leaf ends are encapsulated between two agar slices supplemented with benzimidazole to prevent senescence. The left image shows the negative control, treated only with water, while the right image displays the positive control, treated exclusively with Z. tritici spores. Both images were captured 28 days after treatment.

Table S-2: Number of sequences used in the alignment for probes and primers design and off-target detection of G-probe

Species	Nb of sequences	Nb of strains	Off-target strains
P. arvensis sp. nov.	24	4	/
*P. chlororaphis**	36	7	/
P. cyclaminis	6	1	/
P. fluorescens	92	22	2020WEIHUA ATCC13525 NCTC10038
P. germanica	4	1	/
P. granadensis	4	1	/
P. lactis	11	6	/
P. lurida	15	5	/
P. marginalis	21	5	/
P. orientalis	13	7	/
P. poae	35	11	DSM14936 LMG21465 PA1-8B PA2-1F RE1114 SWRI2 WS5103
P. salmasensis	6	1	/
P. simiae	12	4	/
P. sivasensis	36	8	/
P. tensinigenes	4	1	/
Total	**319**	**84**	**10**

**includes subspecies auranticia, aureofaciens, chlororaphis, piscium and two unspecified.*

Figure S-3: qPCR detection of *P. sivasensis* in field, with meteorological data

Points represent the mean qPCR copies from P. sivasensis CF10PS3 (triangles) or the natural population of P. sivasensis sensu lato (crosses), encompassing closely related species, on flag leaves (a) and second leaves (b). Data are shown for plots (n=4) that were either mock-treated with buffer (green) or treated with CF10PS3 (blue). Error bars indicate standard deviation (SD). For the natural population analysis, data from bacteria-treated and mock-treated plots showed no significant statistical difference and were therefore combined. Each square of the grid represents one day, with the corresponding BBCH stage at sampling indicated on the X-axis. Meteorological data are represented (c) for temperature (curve in red), wind velocity (curve in black), precipitation (bars in blue) on the left Y axis. Relative humidity is represented by the green curve on the right Y axis.

Table S-3: Number of reads generated in each duplicated sample

Treatment	Sampling	Sample1	Sample2
Buffer	BBCH39_0	251685	355773
Buffer	BBCH39_1h	412599	224594
CF10PS3	BBCH39_1h	266221	194977
Buffer	BBCH39_2d	250275	275233
CF10PS3	BBCH39_2d	220216	219650
Buffer	BBCH55_7d	277799	156528
CF10PS3	BBCH55_7d	293216	282216
Chemical	BBCH55_7d	207965	208018
Buffer	BBCH59_14d	264030	272799
CF10PS3	BBCH59_14d	309489	102437
Chemical	BBCH59_14d	201103	226965
Buffer	BBCH81_42d	121745	30362
CF10PS3	BBCH81_42d	279489	249983
Chemical	BBCH81_42d	234114	200209

alpha and beta diversity

In our study, we investigated the effects of a fluxapyroxad and mefentrifluconazole mixture, referred to as the chemical treatment, and *Pseudomonas sivasensis* strain CF10PS3 on the alpha and beta diversity of microbial communities, compared with a buffer control. Alpha diversity indexes, including observed features, Pielou eveness and Shannon entropy, were calculated in QIIME2 (version 2024.2)(Bolyen et al., 2019). Beta diversity was analyzed using Bray-Curtis dissimilarity matrices, with a p-value of <0.05 considered significant. Data were visualized using QIIME view.

Alpha diversity, which assesses the variety and abundance within a single sample, is quantified in our study by two metrics: species richness and evenness. Richness refers to the number of different species present, while evenness measures how similar the abundances of different species are. Our findings revealed that the alpha diversity metrics did not show statistically significant differences between the different treatments and the control over time. However, observable trends indicate changes in these metrics. For instance, the number of observed features (richness) remained relatively stable during the initial 14 days of observation, as depicted in Fig. S-4a. Subsequently, there was a notable decline in both richness and evenness, as seen in the metrics for Pielou's evenness (Fig. S-4b) and Shannon entropy (Fig. S-4c).

Beta diversity, on the other hand, measures the differences between microbial communities across different samples, indicating the variation

in species composition. In our study, beta diversity was assessed through Bray-Curtis distances and illustrated in Fig. S-4d. The results showed no significant changes attributable to either the chemical or CF10PS3 treatments compared to the buffer control. Unlike alpha diversity, beta diversity did not exhibit a decline at the end of the observation period, suggesting that the overall community composition remained consistent across treatments.

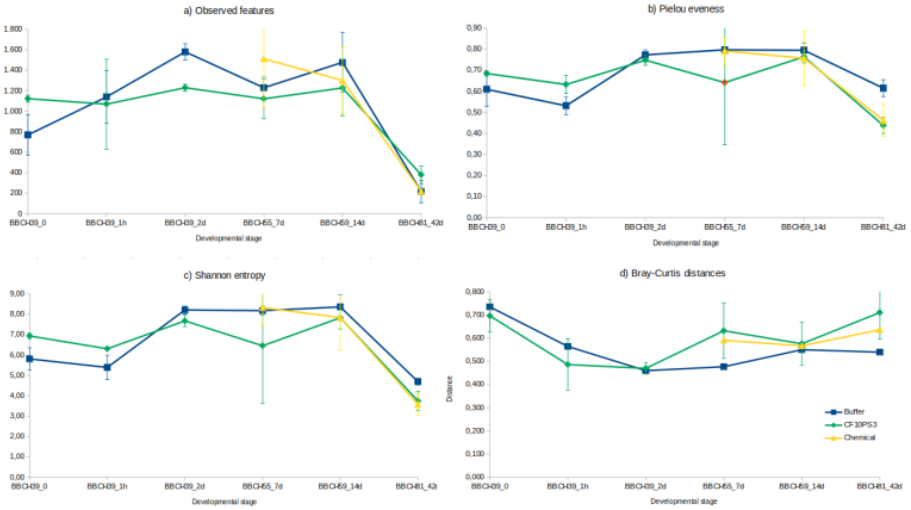

Figure S-4: α and β diversity metrics.

Evolution of a) Observed features, b) Pielou eveness, c) Shannon entropy and d) Bray-Curtis distances metrics during sampling season in CF10PS3 and chemically treated field plots, compared to buffer control.

Monitoring of CF10PS3 after treatment

In a previous work, we developed a qPCR assay specifically designed to detect and monitor the presence of *P. sivasensis* CF10PS3. This was achieved by designing primers and a probe that target a unique thymine (T) single-nucleotide polymorphism (SNP) in the 16S-ITS-23S operon sequence, characteristic of CF10PS3, while other closely related species possess a guanine (G) SNP at this locus. This enables precise differentiation of CF10PS3 from other species and strains. We used qPCR primers and probe sequences to quantify *in silico* the T SNP directly from sequencing reads. Upon application of CF10PS3 to designated field plots, a significant increase in the detection of the T SNP was noted, confirming the successful establishment of CF10PS3 in the treated environments. In contrast, field plots treated with buffer or chemical agents (fluxapyroxad and mefentrifluconazole mixture) did not exhibit any increase in T SNP levels. Following the initial rise in detection, the levels of CF10PS3 gradually decreased over subsequent days and weeks. Despite this decline, the detected levels of CF10PS3 remained consistently above those observed in buffer-treated plots, indicating a sustained presence of CF10PS3, even at reduced concentrations.

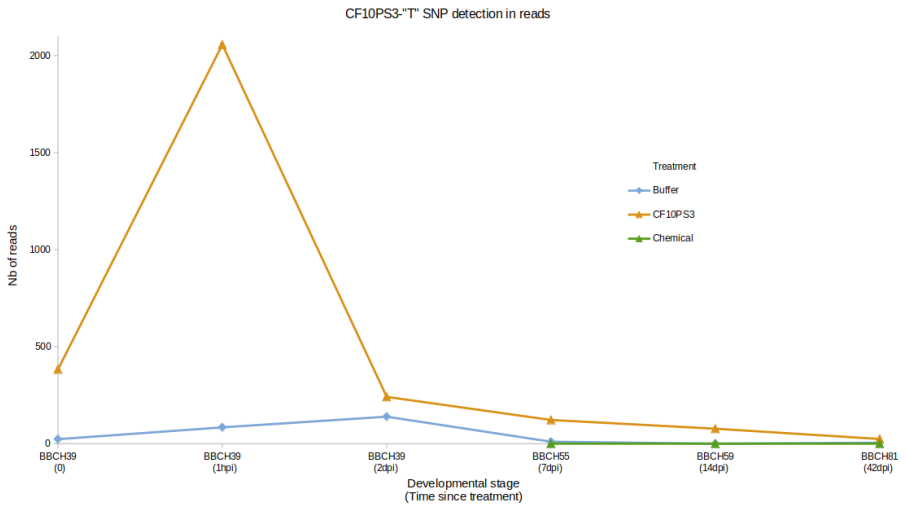

Figure S-5: CF10PS3 detection in field plots.

In silico detection of T SNP specifically associated with P. sivasensis strain CF10PS3 present in sequencing reads.