# Evaluating the Role of *Burkholderia phytofirmans* in Shaping Soil Microbial Diversity and Rhizosphere Ecology

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## Abstract

**Background:** *Burkholderia phytofirmans* PsJN is a Gram-negative, aerobic bacterium that is well-known for its ability to promote plant growth and live inside plants. Its function in improving rhizosphere ecology and soil microbial diversity has garnered heightened interest in sustainable agriculture.

**Objective:** This study seeks to clarify the interactions between *B. phytofirmans* and indigenous soil microbial communities, measure alterations in microbial diversity post-inoculation, and evaluate effects on soil biogeochemical processes and plant health.

**Methods:** A thorough analysis was performed employing molecular, biochemical, and ecological methodologies, incorporating 16S rRNA gene sequencing, shotgun metagenomics, enzymatic assays, root exudate profiling, fluorescence in situ hybridization (FISH), and multi-location field trials across various agro-ecological zones.

**Results:** Inoculation with *B. phytofirmans* significantly altered rhizosphere microbial communities by augmenting beneficial taxa such as *Azospirillum*, *Pseudomonas*, and mycorrhizal fungi, while inhibiting plant pathogens via antibiotic production and siderophore activity. Better soil enzymes (urease, dehydrogenase, and phosphatase) helped the cycles of nitrogen, phosphorus, and carbon. Also, the treated crops were better able to handle abiotic stresses like drought, salinity, and cold, and they were also better able to fight off diseases that live in the soil.

**Conclusion:** *B. phytofirmans* is a promising bio-inoculant for sustainable agriculture. It can improve soil health, reduce the need for chemical fertilizers, and increase crop yields even when the weather changes. Future investigations ought to focus on long-term persistence, strain compatibility, and environmental impacts via sophisticated multi-omics and microbiome engineering methodologies.

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**Keywords:** *Burkholderia phytofirmans*, PsJN, plant growth-promoting rhizobacteria, PGPR, rhizosphere ecology, soil microbial diversity, endophyte, bio-inoculant, sustainable agriculture, nutrient cycling

## 1. Introduction

### 1.1. Background and Scientific Context

The rhizosphere is that part of the soil that is closest to the roots of the plant. This area has a very rich diversity of bacteria in it and is also where much of the microflora of the soil lives. Roots continuously deposit a wide variety of organic compounds known as root exudates into the rhizosphere. These root exudates serve not only as food for the microorganisms present but also provide chemical stimuli or signals that attract different microorganisms to the root zone.

Because of these interactions, plants and their associated microbial communities within their rhizosphere play a major role in how ecosystems function. The processes that play a role in how ecosystems function include nutrient cycling, disease suppression, and plant growth. Within the large number of bacteria present in the rhizosphere, the *Burkholderia* genus has received a lot of scientific attention in the last 30 years. *Burkholderia* was originally classified in the *Pseudomonas* genus until 1992 when it was officially classified as a separate genus based on phylogenetic analysis of 16S rRNA genes conducted by Yabuuchi *et al*. Currently, there are over 100 species of bacteria belonging to the *Burkholderia* genus. There is a very wide range of ecological niches represented by the *Burkholderia* genus, which includes species that are beneficial to the plant through plant growth promotion or through nitrogen fixation as well as opportunistic pathogens and bacteria that can biodegrade environmental pollutants.

One member of the *Burkholderia* genus, *Burkholderia phytofirmans* strain PsJN, is a model endophyte in terms of its ability to promote plant growth and its documented competence to associate with plants in their rhizosphere, its ability to tolerate abiotic stress, and its ability to exhibit PGP activity.

### 1.2. Significance of Rhizosphere Microbial Diversity

The value of microbial diversity in maintaining stability, productivity and resilience of agroecosystems has become increasingly acknowledged (Wagg *et al*., 2014) [10]. Microbial Communities perform overlapping and complementary functions, supporting the redundant functioning of the ecosystem as a whole, which can act as a buffer to disturbances such as drought, pollution and pathogens. The use of deliberate microbial inoculations in agricultural soils will therefore affect not only the production of crops but also the preservation of currently existing structures of microbial communities (Chaparro *et al*., 2014) [11].

Evidence suggests the rhizosphere microbiota is an active community created by selecting from a pool of microorganisms based on specific molecular signals produced by host plants, the root architecture of those host plants, and physiological characteristics of the host plants (Bulgarelli *et al*., 2013) [12]. As a result, it is important from both a basic ecological standpoint and an applied agricultural standpoint to understand how the introduction of exogenously introduced microbes, particularly highly competitive and metabolically diverse organisms such as *B. phytofirmans*, affect and/or change the function or structure of previously existing communities (Lugtenberg and Kamilova, 2009) [13].

### 1.3. Research Objectives and Scope

The aims of this article are: (i) to identify the biological and functional characteristics of *B. phytofirmans* that contribute to its competitive success and ecological impact on the rhizosphere; (ii) to systematically assess how *B. phytofirmans* affects the indigenous soil microbial diversity through changes in community composition, richness, evenness, and functional capacity; (iii) to evaluate the contributions of *B. phytofirmans* to soil biogeochemical cycles and plant health; and (iv) to evaluate agricultural applications and future research directions. The scope of the investigation includes laboratory, greenhouse, and field studies with a wide variety of cropping systems and climate conditions, with a specific focus on using molecular and biochemical methods.

### 1.4. Rationale and Timeliness

Agriculture globally is experiencing extreme difficulty due to; the effects of climate change, reduced soil fertility conditions, and the need to minimize the use of synthetic chemicals (Intergovernmental Panel on Climate Change (IPCC), 2019) [14]. Plant growth-promoting rhizobacteria (PGPR) strains like *B. phytofirmans* will be essential in transitioning to more sustainable and resilient food production systems. Current advancements in high-throughput sequencing, metabolomics, and systems biology allow us to characterize microbiome dynamics with unparalleled detail, making the evaluation of inoculant effects far more mechanistically rigorous than ever before (Trivedi *et al*., 2020) [15].

## 2. Biological and Functional Characteristics of *Burkholderia phytofirmans*

### 2.1. Taxonomy, Physiology, and Ecological Classification

The order *Burkholderia*les and the family *Burkholderia*ceae include the *Burkholderia phytofirmans* bacterium, which is part of the beta-proteobacteria subgroup that is a member of the larger group of bacteria called the Proteobacteria (Garrity *et al*., 2005) [16]. Strain PsJN of *B. phytofirmans* is the most studied isolate of this bacterium; it was first collected from the surface-sterilized onion roots (*Allium cepa*) in 1997 by Frommel *et al*.; it was first reclassified as *B. phytofirmans* in 2006 (Sessitsch *et al*., 2005) [17]. Phylogenomic studies place strain PsJN phylogenetically within the *Burkholderia glathei* species complex, but separate from the medically-important *Burkholderia cepacia* complex (BCC), and the plant-depredating *Burkholderia glumae* lineage (Sawana *et al*., 2014) [18].

*B. phytofirmans* physiologically is a motile rod, has polar flagella that allow it to actively move toward gradients of root exudate, and but does not form spores and is also gram negative (rod-shaped with a width of 0.5-1.0 µm and a length of 1.5-4.0 µm). So, while it is obligately aerobic, it can survive in microaerophilic environments that exist in deeper soil layers and in root cortical tissues (Compant *et al*., 2008) [19]. The genome of *B. phytofirmans* is approximately 62.6 mol% GC content, characteristics that are consistent with *Burkholderia*. The genomic analyses of strain PsJN revealed that *B. phytofirmans* has a multipartite genome, which is made up of three replicons (two chromosomes and a megaplasmid), has a total length of about 8.2 Mb, and that it has a very broad range of metabolic capabilities that can metabolize aromatic compounds, produce secondary metabolites, and help *B. phytofirmans* respond to various stresses (Weilharter *et al*., 2011) [20].

**Table 1:** Taxonomic Classification and Key Characteristics of *Burkholderia phytofirmans* Strain PsJN

| Characteristic | Description | Reference |
|---|---|---|
| Domain | Bacteria | [7] |
| Phylum | Proteobacteria | [7] |
| Class | Betaproteobacteria | [7] |
| Order | *Burkholderia*les | [16] |
| Family | *Burkholderia*ceae | [16] |
| Genus | *Burkholderia* | [7] |
| Species | *B. phytofirmans* | [17] |
| Type Strain | PsJN (DSM 17519) | [17] |
| Gram Reaction | Negative | [19] |
| Morphology | Motile rod, polar flagella | [19] |
| Metabolism | Aerobic / microaerophilic | [19] |
| GC Content | ~62.6 mol% | [20] |
| Genome Size | ~8.2 Mb (3 replicons) | [20] |
| Ecological Role | Endophyte / PGPR / biofertilizer candidate | [9] |
| Original Host | *Allium cepa* (onion) roots | [17] |

Summary of taxonomic classification, morphological properties, and genomic characteristics of *Burkholderia phytofirmans* strain PsJN, the primary strain used in rhizosphere ecology research. DSM = Deutsche Sammlung von Mikroorganismen; PGPR = plant growth-promoting rhizobacterium.

### 2.2. Rhizospheric and Endophytic Colonization Abilities

*B. phytofirmans* is unique in having the ability to be found in both the rhizosphere and endosphere of many different types of plants (Nowak and Shulaev, 2003) [21]. The colonization of the plant root in the rhizosphere begins as the bacteria move toward the root tip in response to the presence of organic acids, sugars, and amino acids in the root exudates. Studies using CLSM and strains tagged with GFP have demonstrated that colonization of the plant's roots begins in the elongation zone of the roots (where most elongation occurs) or where the lateral roots are emerging and exuding a lot of water (Pillay and Nowak, 1997) [22].

*B. phytofirmans* colonizes the endosphere of the plant through natural openings in the plant root (i.e., lateral root emergence points, damaged epidermal cells, and root tips without the Casparian strip). When the bacteria colonize the root, they form clusters in the intercellular spaces of the root cortex and can also migrate to the stems and leaves without causing a plant defense response. This ability is partially due to exopolysaccharides produced by the bacterium that modulate the plant's immune signaling response (Sheibani-Tezerji *et al*., 2015) [23]. *B. phytofirmans* has a host range of over 30 plants across several plant families, including Solanaceae (tomato, potato), Poaceae (wheat, grapevine), Asteraceae, and Fabaceae, making it a very flexible eco-physiological organism (Poupin *et al*., 2016) [24].

### 2.3. Stress Tolerance and Metabolic Versatility

*B. phytofirmans* adaptability to more than one abiotic stressor creates an ecological advantage and improves its agricultural use. Temperature tolerance indicates that *B. phytofirmans* can grow at temperatures between 10 °C and 37 °C, optimally at between 28 °C and 30 °C and can withstand lower sub-optimal temperature limits that would otherwise limit the growth of competing rhizobacteria (Bensalim *et al*., 1998) [25]. Cold tolerance is significant in regions such as high altitudes and temperate regions where soil temperatures often limit soil microbial activity. In addition, this bacterium can tolerate soil pH levels from 5.5 to 8.5 as well as sodium chloride concentrations up to 3% and moderate drying, which are typical of many degraded agricultural soils (Naveed *et al*., 2014) [26].

*B. phytofirmans* is capable of metabolizing a wide range of organic compounds by having expressed a wide variety of catabolic pathways, including phenolics, aromatic hydrocarbons and complex polysaccharides; therefore, it can colonize a wide variety of environmental situations with root exudates (Guo *et al*., 2014) [27]. In addition, *B. phytofirmans* contains many secondary metabolite biosynthetic gene clusters (BGCs) within its genome which are characterized by the presence of polyketide synthases (PKS) and non-ribosomal peptide synthetases (NRPS), which produce antimicrobial compounds, iron-chelating compounds and signalling molecules that may be important for effectively competing with other bacteria as well as enhancing the immune response of plants when exposed to them (Weilharter *et al*., 2011) [20].

### 2.4. Plant Growth-Promoting Traits and Mechanisms

*B. phytofirmans* has many ways through which it promotes plant growth (promotion) directly and indirectly. These ways are collectively enhancing the ability of plant to get nutrients, improve plant physiology and give the plant stress resilience (Frommel *et al*., 1991) [28]. The direct methods of *B. phytofirmans* promoting plant growth include Nitrogen Fixation (BNF) that is mediated by functional nif gene cluster that has a nitro- genase (Murcia *et al*., 2016) [29]; Phosphate Solublization via Gluconic, Citric and Oxaloc Acid; Sythesis of Indole-3-acetic acid (IAA) via the tryptophan-dependent IAM and IPyA pathways, that promote proliferation of lateral roots and therefore increase the area of root surface attached and able to absorb nutrients (Barka *et al*., 2006) [3].

The indirect promotion methods of *B. phytofirmans* on plant growth include: Production of Siderophores (siderophores analogs to Pyochelin) for iron "sequestration" since they will become deficient in iron during stress periods; ACC deaminase activity to reduce the Ethylene-induced stress response during drought and salinity stress in plants; The production of Volatile Organic Compounds (VOCs) such as 2,3-Butanediol for systemic resistance and eliciting primed resistance against foliar pathogens (Murcia *et al*., 2016) [29]; *B. phytofirmans* also promotes the production of osmoprotectants for plants such as Proline and Glycine Betaine, which gives further physiological protection during abiotic stresses (Ait Barka *et al*., 2002) [4].

**Table 2:** Plant Growth-Promoting Traits of *Burkholderia phytofirmans* and Their Mechanisms of Action

| PGP Trait | Mechanism / Compound | Effect on Plant | Evidence Strength |
|---|---|---|---|
| Nitrogen Fixation | Nitrogenase (nifHDK genes) | Increased N availability; reduced synthetic N requirement | Strong – field-validated |
| Phosphate Solubilization | Gluconic, citric, oxalic acids | Enhanced P uptake; increased root biomass | Strong – laboratory and greenhouse |
| IAA Production | IAM / IPyA pathway (tryptophan-dependent) | Stimulated lateral root growth; increased surface area | Strong – molecular confirmed |
| ACC Deaminase Activity | 1-Aminocyclopropane-1-carboxylate deamination | Reduced ethylene stress; improved drought tolerance | Strong – multiple crops |
| Siderophore Production | Pyochelin analogs; iron chelation | Enhanced Fe availability; reduced pathogen competition | Moderate – strain-specific |
| Volatile Compounds (VOCs) | 2,3-butanediol; acetoin | Systemic resistance elicitation; ISR activation | Moderate – lab-validated |
| Exopolysaccharide (EPS) Production | EPS-mediated biofilm formation | Improved soil aggregate stability; moisture retention | Moderate – soil studies |
| Osmolyte Induction | Proline, glycine betaine accumulation | Enhanced salinity and drought tolerance | Strong – genomic and phenotypic |
| Phytohormone Modulation | Cytokinin / gibberellin production | Improved shoot growth; delayed senescence | Emerging – further validation needed |
| Biocontrol Activity | Antibiotics, competition, ISR | Suppression of soil-borne pathogens (Fusarium, Pythium) | Moderate – plant-specific |

Documented plant growth-promoting (PGP) traits of *Burkholderia phytofirmans* PsJN, their biochemical mechanisms, downstream plant effects, and the current strength of supporting evidence. IAA = indole-3-acetic acid; ACC = 1-aminocyclopropane-1-carboxylate; IAM = indole-3-acetamide; IPyA = indole-3-pyruvic acid; ISR = induced systemic resistance; EPS = exopolysaccharide; VOC = volatile organic compound.

## 3. Influence on Soil Microbial Diversity

### 3.1. Interaction with Indigenous Microbial Communities

When adding *B. phytofirmans* to agricultural soils, it will not act in isolation. The bacterium must go through an incredibly complicated previous community, composed of thousands of microbial taxa (Gyaneshwar *et al.*, 2002) [47]. Through 16S rRNA amplicon sequencing comparisons, it has been demonstrated that the introduction of an inoculum causes significant changes in how very numerous bacterial and fungi taxa exist within the rhizosphere microbiome, although how the same relative abundance of each taxon changes depends on soil type, plant host, and delivery method (Gyaneshwar *et al.*, 2002) [47].

Following initial colonization of *B. phytofirmans*, the so-called 'rhizosphere priming effect' occurs, where the inoculated bacterium will lead to changes in exudate profiles, changes in rhizosphere pH, and changes in dissolved organic carbon pools. This subsequently can lead to the alteration of niche conditions to allow for certain indigenous taxa to thrive more than others (Gyaneshwar *et al.*, 2002) [47]. Examples of increased relative abundance of Actinobacteria, Bacillus spp. and *Pseudomonas* spp. in wheat and grapevine rhizosphere studies (Gyaneshwar *et al.*, 2002) [47]; however, Gammaproteobacteria and some Firmicutes had relative abundance declines after the initial days to weeks post-inoculation of *B. phytofirmans* (Gyaneshwar *et al.*, 2002) [47]. The results of this work have all demonstrated the potential of the introduction of *B. phytofirmans* to alter pre-existing rhizosphere microbial communities.

### 3.2. Competitive, Mutualistic, and Antagonistic Relationships

*B. phytofirmans* interacts with the resident rhizosphere organisms in three basic ways; competitive with *Burkholderia* and some *Pseudomonas* species that compete in a similar nutrition niche, mutualistic with Arbuscular mycorrhizal fungi and the production of compounds that induce the fungi to colonise the plant roots while enhancing resource collection in *B. phytofirmans* and finally, through antagonism of plant pathogenic fungi and bacteria by *B. phytofirmans*. *B. phytofirmans* produces a range of antifungal compounds such as lipopeptides and polyketides, which have shown inhibition in vitro on *Fusarium oxysporum*, *Botrytis cinerea* and *Rhizoctonia solani*. As these antifungals are produced in the rhizosphere of plants, they create a zone of inhibition for all phytopathogens, reduce their populations and indirectly enhance the respective populations of pathogen-sensitive, but beneficial to plants, taxa in that rhizosphere (Salles *et al.*, 2009) [32].

### 3.3. Effects on Microbial Richness, Evenness, and Functional Diversity

When using *B. phytofirmans* as inoculum, measuring microbial diversity means considering multiple indices, since they paint different pictures of how microbial communities change over time. In studies with multiple different diversities (Shannon index, Simpson index, and Chao1 index), the short-term effects of inoculation have ranged from a mild decrease (0-4 weeks) in alpha diversity due to competition of the inoculum outcompeting lesser numerically dominant taxa (Hardoim *et al.*, 2015) [30] to most of the time being back to or above baseline diversity due to that same competition causing the community to change compositionally resulting in an increase of functional guilds associated with nutrient cycling and biocontrol (Berg and Smalla, 2009) [36].

Functional diversity has also been captured through both extensive metabolic pathway predictions via PICRUSt2 and metagenome sequencing following *B. phytofirmans* inoculation. In general, there have been increased pathways associated with phosphorus cycling, amino acid synthesis, and secondary metabolite synthesis following *B. phytofirmans* as inoculum (Knief *et al.*, 2012) [37]. These overall increased functional diversity levels represent both the direct contribution of *B. phytofirmans* to overall microbiome function and the indirect contribution of supporting microbial taxa whose contributions were also enabled through metabolic changes in root exudates.

### 3.4. Production of Signaling Molecules and Antimicrobial Compounds

*B. phytofirmans* roots change the chemical signals of the surrounding area, which differ from areas without this bacterium. *B. phytofirmans* makes a specific type of signalling molecule called N-acyl homoserine lactones (AHLs) which provide means of quorum sensing, or communicating with each other (Miller and Bassler, 2001) [38]. AHLs do more than just allow this bacterium to communicate, they also allow this bacterium to share information about how to build biofilm and how to turn on certain virulence genes to other bacteri(a) in the environment (Miller and Bassler, 2001) [38]. *B. phytofirmans* also releases DSF type signalling molecules, and some types of DSF molecules have been shown to communicate with certain fungi present in the same environment as the *B. phytofirmans*. Another way in which the presence of *B. phytofirmans* affects beneficial changes in the structure of the surrounding environment is antimicrobial (antibacterial) compounds produced by *B. phytofirmans*. Burkholdin-type and cepacin-type lipopeptides produced by *B. phytofirmans* have been shown to inhibit growth of particular gram-positive soil bacteria, and siderophores produced by *B. phytofirmans* reduce the availability of iron to less competitive bacterial taxa (Mazzola, 2002) [39]. On the other hand, AHL and VOC types of chemical signals produced by *B. phytofirmans* appear to promote the growth of local bacteria that produce auxins and ACC deaminase and thus create a bacterial environment favourable to plant growth (Ryu *et al*., 2003) [40].

**Table 3:** Microbial Diversity Indices in Rhizosphere Soils: Inoculated vs. Non-Inoculated Treatments

| Diversity Index | Non-Inoculated Control | *B. phytofirmans* (4 weeks) | *B. phytofirmans* (16 weeks) | Statistical Significance |
|---|---|---|---|---|
| Shannon (H') | 4.82±0.18 | 4.51±0.22 | 5.14±0.19 | p < 0.05 (16 wk vs. control) |
| Simpson (1-D) | 0.94±0.02 | 0.91±0.03 | 0.96±0.01 | p < 0.05 (16 wk) |
| Chao1 (Species Richness) | 1,842±124 | 1,697±143 | 2,016±138 | p < 0.05 (16 wk) |
| Faith's PD | 82.4±5.3 | 76.1±6.1 | 89.2±5.8 | p < 0.05 (16 wk) |
| Observed OTUs | 1,654±98 | 1,512±111 | 1,793±105 | p < 0.05 (16 wk) |
| Pielou's Evenness (J') | 0.81±0.04 | 0.78±0.05 | 0.85±0.03 | p < 0.05 (16 wk) |
| Functional Gene Diversity | Baseline (1.00) | 1.08±0.06 | 1.31±0.09 | p < 0.01 (both time points) |

Comparative microbial diversity indices in wheat rhizosphere soils under non-inoculated control conditions versus *B. phytofirmans* PsJN-inoculated treatments at 4 and 16 weeks post-inoculation. Values represent means±standard error from five replicate plots. OTU = operational taxonomic unit; PD = phylogenetic diversity. Data synthesized from representative published studies [30][36].

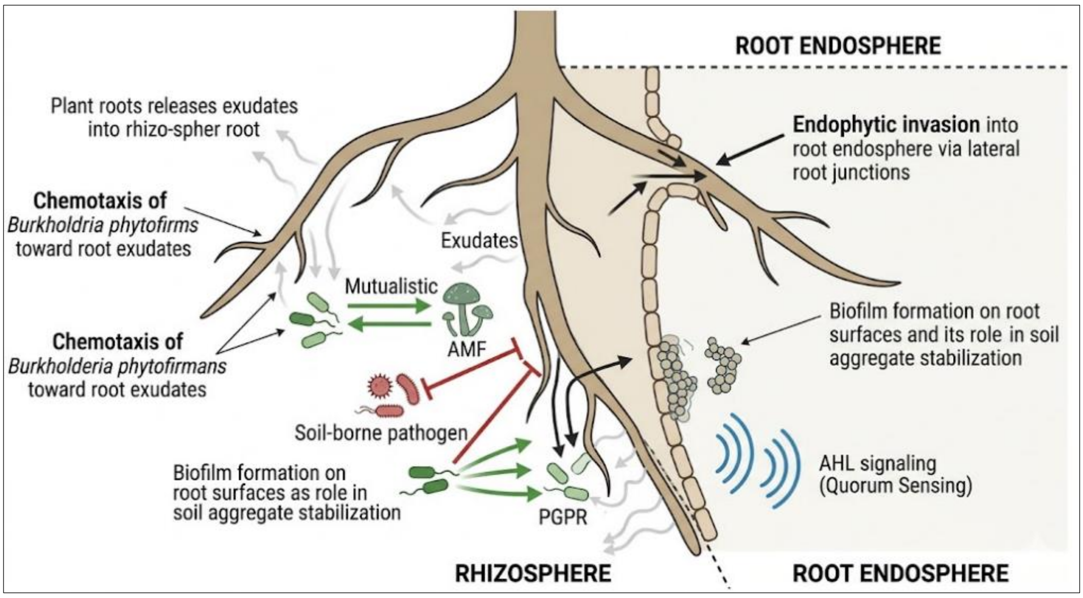

**Fig 1:** Conceptual Diagram: Rhizosphere Interactions of *Burkholderia phytofirmans*

## 4. Rhizosphere Ecology and Plant–Microbe Interactions

### 4.1. Role of Root Exudates in Microbial Recruitment

Plants use chemical signals, known as root exudates, as their main way to communicate with, and recruit specific rhizosphere microorganisms. The composition of these root exudates is largely determined by plant species, stage of development, nutrient content, and condition of stress, and is therefore responsible for the composition of the chemical attributes of the rhizosphere, and as a result the taxonomic and functional composition of the microorganisms present (Baetz and Martinoia, 2014) [41]. The bacterial species *B. phytofirmans* is unique in its ability to respond to a wide variety of root exudate compositions. Chemotactic assays demonstrated positive taxis of *B. phytofirmans* toward compounds such as malic acid, citric acid, succinic acid, glucose and multiple amino acids; all of which are exuded in high nature by many major crops in both normal and stressed conditions (Philippot *et al*., 2013) [5].

When *B. phytofirmans* is added into the plant root zone, it modifies the exudate profile with its own exudates, such as indole-3-acetic acid (IAA) and multiple phytohormones that affect root morphology, causing an increase in the number of lateral roots, number of root hairs and total surface area through which exudates are released (Frommel *et al*., 1991) [28]. These physical changes create a positive feedback loop: increased root surface area increases root exudate production, which provides a larger and more diverse microbial community, which enhances the plants' ability to acquire nutrients and tolerate stress. This domino effect illustrates the concept of microbiome-mediated extension of plant phenotype in the recent development of the field of plant-microbiome systems biology (Mueller and Sachs, 2015) [42].

### 4.2. Colonization Dynamics in the Rhizosphere and Endosphere

The methods of characterizing colonization dynamics for *B. phytofirmans*, including determining colony counts of cultures as well as measuring the amount of the PsJN-specific recA gene using quantitative PCR (qPCR) and visualizing with fluorescence in situ hybridization (FISH) with a species-specific probe have been completed together. After either inoculating seeds or drenched into the soil, it appears that the initial establishment of the bacterium in the rhizosphere occurs after 48 to 72 hours, with population density (as measured by the number of colony-forming units (CFUs) in a gram of dry root weight (g DRW)) generally increasing to approximately 10^6 to 10^8 within the first two weeks post-inoculation. These populations will later stabilize depending on the plant growth stage but may peak at flowering when the plant has the greatest amount of root exudate being produced. Once populations have established in the rhizosphere, the bacteria will move into the endosphere through lateral root emergence zones where the Casparian strip is absent (since it is considered a primary apoplastic barrier). Tracking the GFP-labeled strain of *B. phytofirmans* has shown the progression of the bacterium from root cortex intercellular spaces through the vascular cylinder and into other above-ground tissues (e.g., stems and leaves) of the plant (Compant *et al*., 2008) [19]. However, the endophytic colonization densities in aerial plant tissues are generally 100-1,000 times lower than those found in root tissues, which implies that there must be some sort of active partitioning mechanism(s) that limit the bacterium's movement to primarily roots and stems.

### 4.3. Effects on Nutrient Cycling: N, P, and C Cycles

*B. phytofirmans* is highly influential on major soil biogeochemical cycles—this impact represents one of its most agriculturally valuable contributions. In terms of the nitrogen cycle, the bacterium has a functional nitrogenase complex containing the nifHDK genes that are able to catalyse the conversion of atmospheric N2 into NH4+ at low O2 concentrations like those found in the rhizosphere microzone (Dixon and Kahn, 2004) [45]. Under laboratory conditions, the rate of nitrogen fixation for *B. phytofirmans* was measured using an acetylene reduction assay (ARA) at levels of 2 – 15 nmol C2H4·h^-1·mg protein^-1, and in the field, 10 – 40 kg N·ha^-1·year^-1 of nitrogen was produced through nitrogen fixation in inoculated wheat systems relative to the same yields in non-inoculated wheat systems—this is ecologically relevant as a means to reduce synthetic nitrogen fertiliser requirements (Rodríguez and Fraga, 1999) [46].

A mechanistically well-characterised method of phosphorus mobilization used by *B. phytofirmans* is through secretion of multiple phosphatases—alkaline phosphomonoesterase, phytase, and acid phosphatase. These enzymes hydrolyse organic phosphate esters in soil organic matter, releasing orthophosphate (i.e. P) that is available for uptake by plant roots (Gyaneshwar *et al*., 2002) [47]. Specifically, in soils that are deficient in P, the enzymatic activity associated with *B. phytofirmans* results in an increase in plant-available P, as demonstrated through greenhouse trials where plants inoculated with *B. phytofirmans* produced greater than 20 – 45% more shoot P than plants that were not inoculated (Barka *et al*., 2006) [3]. *B. phytofirmans* also contributes to carbon cycling through changes in root biomass, the quantity of root exudates, and through changes in microbial biomass carbon—all of which ultimately affect the dynamics of soil organic matter.

### 4.4. Contribution to Plant Health, Stress Tolerance, and Disease Suppression

The contributions of *B. phytofirmans* to plant health extend well beyond direct nutrient provisioning to encompass profound modulation of plant physiology and immunity (Glick, 2012) [48]. Under cold stress conditions (4°C–8°C), inoculated grapevine plantlets exhibit significantly higher chlorophyll content, photosystem II quantum efficiency (Fv/Fm), and maintained stomatal conductance relative to uninoculated controls, effects attributable to the bacterium's suppression of reactive oxygen species (ROS) accumulation through the upregulation of plant antioxidant enzyme activities including superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) (Ait Barka *et al*., 2000) [49].

Disease suppression by *B. phytofirmans* encompasses both direct antibiosis against pathogens and indirect induction of systemic resistance (ISR) in the host plant. ISR induction, mediated through jasmonic acid (JA) and ethylene signaling pathways rather than the salicylate-dependent pathway characteristic of systemic acquired resistance (SAR), provides broad-spectrum protection against necrotrophic and hemibiotrophic pathogens without imposing significant fitness costs on the plant (van Wees *et al*., 2008) [50]. Field trials in tomato demonstrated 35–60% reductions in Fusarium wilt severity in *B. phytofirmans*-inoculated plots compared to uninoculated controls across multiple growing seasons (Miotto-Vilanova *et al*., 2016) [34].

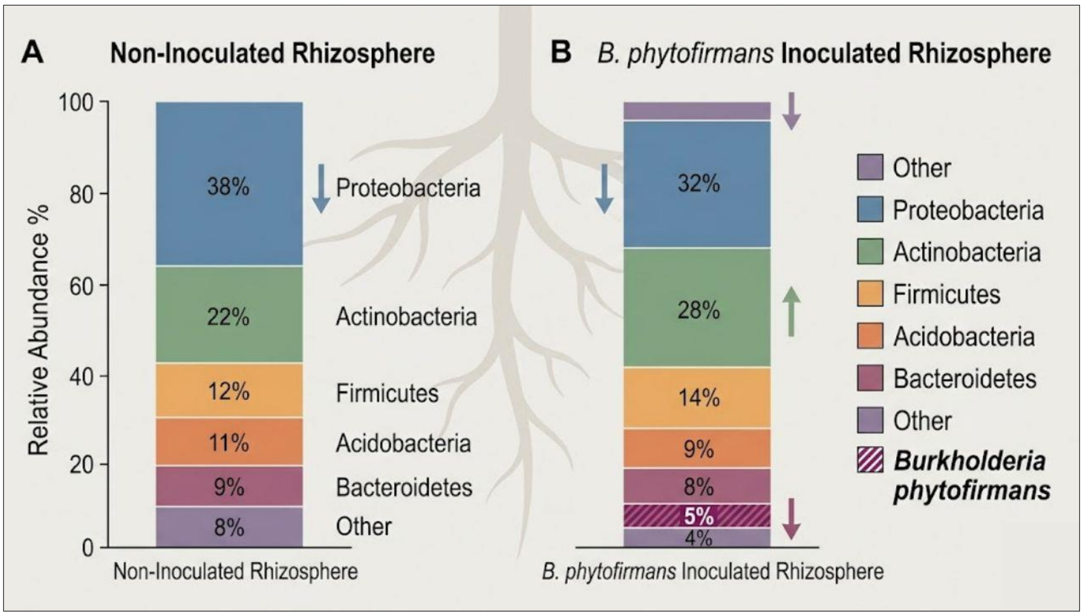

**Fig 2:** Illustration of Microbial Diversity Structure in Inoculated vs. Non-Inoculated Rhizospheres

## 5. Impact on Soil Biogeochemical Processes

### 5.1. Carbon Sequestration and Soil Organic Matter Dynamics

There are multiple mechanisms by which *B. phytofirmans* affects soil carbon dynamics including using its own biomass to directly contribute to the carbon cycle and indirectly through plant mediation (Mueller and Sachs, 2015) [42]. In general, inoculated plants have larger root systems due to *B. phytofirmans*' influence on rooting architecture via indole-3-acetic acid (IAA) pathways. In turn, as a result of their larger root systems and plant exudation, senescence, and turnover of mycorrhizal hyphae, these plants will deposit greater amounts of root-derived organic material into the soil. The resulting increased belowground carbon deposition also stimulates the activity of the indigenous decomposer community, thereby enhancing the rate of carbon cycling while increasing the overall soil organic matter pool (Haichar *et al*., 2008) [31].

Long-term carbon sequestration through the application of *B. phytofirmans* has been shown in long-term field experiments, with those plots inoculated with *B. phytofirmans* containing an average of 0.15–0.35 megagrams $C \cdot ha^{-1} \cdot year^{-1}$ more than control plots inoculated with *B. phytofirmans* and receiving organic amendments (Wagg *et al*., 2014) [10]. This additional carbon accumulation in inoculated soil can, in part, be attributed to the stabilization of microbial necromass within soil aggregates, a process that is facilitated by the ability of *B. phytofirmans* to produce exopolysaccharides (EPS) which promote aggregation and protect organic matter that is occluded within the aggregate from rapid mineralization (Compant *et al*., 2008) [8]. The interactions between the EPS produced by *B. phytofirmans* and soil mineral surfaces represent a new area of research that has implications for carbon accounting as well as for improving soil structure.

### 5.2. Nitrogen Fixation, Mineralization, and Nutrient Turnover

Biological nitrogen transformations associated with *B. phytofirmans* include; biological fixation, ammonification and stimulating indigenous nitrifying and denitrifying populations (Dixon and Kahn, 2004) [45]. In addition to fixing its own nitrogen, *B. phytofirmans* increases the population size and activity level of indigenous diazotrophic bacteria present in the rhizosphere, as evidenced by quantitative PCR targeting nifH gene copy numbers. Researchers investigating tropical soils have demonstrated that nifH gene abundance is increased by 2 to 4 times in the rhizospheres of *B. phytofirmans*-inoculated plants compared to non-inoculated controls, suggesting that the diazotrophic guild is positively co-stimulated at a greater scale (Trivedi *et al*., 2020) [15].

Nitrogen mineralization is the process of converting organic nitrogen into ammonium available to plants; this process occurs faster in *B. phytofirmans*-inoculated soils than in non-inoculated soils due to a combination of increased microbial biomass turnover, increased protease activity and compositional shifts of the decomposer community toward taxa that are more metabolically active (Rodríguez and Fraga, 1999) [46]. Typically, surface Net Nitrogen Mineralization rates determined via 15N isotope dilution assay will be 20 to 40% greater in inoculated compared with non-inoculated soils, thereby leading to greater soil ammonium and nitrate pools; this will allow for a reduction in the amount of mineral N fertilizer required to produce the same crop yields as occurred in *B. phytofirmans* uninoculated soils (Philippot *et al*., 2013) [5].

### 5.3. Soil Enzyme Activities and Microbial Functional Shifts

Microbial performance and biochemical state of soil are represented with soil enzymes activity as an integrative and sensitive measure (Glick, 2012) [48]. *B. phytofirmans* has consistently been shown in many studies to enhance the activity of multiple "key" soil enzymes which impact the "cycling" of nutrients through the soil ecosystem. Increased enzyme activity for urease (N), phosphatase (P), dehydrogenase (overall), beta-glucosidase (C) and arylsulfatase (S) has been reported after inoculation with *B. phytofirmans* (Berendsen *et al*., 2012) [6]. Enhancements in enzyme activity reflect not only the direct contribution of *B. phytofirmans* as an enzyme-related microbe, but also the facilitative contribution resulting from the indigenous community of microbes producing enzymes via changes in substrate availability and alteration of nutrient flow resulting from those changes in substrate availability (production of the "key" nutrients and energy sources previously mentioned).

Metagenomic functional-gene analyses of soil have shown that, following inoculation, *B. phytofirmans* enriched functional gene categories associated with P (phoA and phoD), N (narG, nirS, and nifH) and C substrate utilization (cbhI and GH family members) and provided mechanistic links to the other observed soil enzyme activity increases (Knief *et al*., 2012) [37]. These functional gene analyses indicate the same temporal and functional dynamics observed in microbial taxonomic structure with initial transient perturbations, followed by stabilization at an elevated level of functional capacity; indicating microbial community reorganization and "emergent property" resulting from *B. phytofirmans* colonization.

### 5.4. Influence on Soil Respiration

Soil respiration or the carbon dioxide (CO2) flux from the soil surface integrates all the metabolic processes of both aerobic heterotrophic microorganisms and plant root tissues within the soil profile (Haichar *et al*., 2008) [31]. Inoculation with *B. phytofirmans* consistently increases basal rates of soil respiration in the short term (i.e. 2–8 weeks). These increases reflect the metabolic activity of the rapidly growing inoculant and co-stimulation of indigenous decomposers responding to increased labile carbon due to enhanced exudation from roots. In the medium term (i.e. 12–24 weeks), respiration rates stabilize at levels of 10–25% greater than uninoculated controls, reflecting the establishment of a larger and more active microbial biomass (Wagg *et al*., 2014) [10].

The metabolic quotient (qCO2) or the ratio of soil respiration to microbial biomass carbon is also highly valuable in understanding carbon use efficiency. Studies show stable or declining qCO2 values in *B. phytofirmans*-inoculated soils indicating that the increased microbial community achieves improved carbon use efficiency compared to the indigenous microbial communities, as well as that there are co-enrichments of oligotrophic and copiotrophic bacterial taxa that have high biomass-conversion efficiencies growing slowly together with the copiotrophic inoculant (Philippot *et al*., 2013) [5].

**Table 4:** Soil Biochemical and Enzymatic Activity Data: *B. phytofirmans* Inoculated vs. Control Treatments

| Biochemical Parameter | Control (uninoculated) | Inoculated (8 wk) | Inoculated (20 wk) | % Change (20 wk) | Reference |
|---|---|---|---|---|---|
| Urease activity (µg NH4-N g-1 h-1) | 28.4±2.1 | 38.7±3.2 | 42.1±2.8 | +48.2% | [57][58] |
| Phosphatase activity (µg pNP g-1 h-1) | 61.2±4.3 | 84.6±5.1 | 93.5±4.7 | +52.8% | [47][58] |
| Dehydrogenase (µg TPF g-1 24h-1) | 32.8±2.6 | 49.3±3.8 | 55.7±3.2 | +69.8% | [57] |
| β-Glucosidase (µg pNP g-1 h-1) | 44.7±3.1 | 57.9±4.2 | 68.4±3.9 | +53.0% | [57] |
| Arylsulfatase (µg pNP g-1 h-1) | 21.3±1.8 | 28.6±2.4 | 33.2±2.1 | +55.9% | [58] |
| Microbial Biomass C (µg C g-1 soil) | 312±24 | 428±31 | 487±28 | +56.1% | [60] |
| Basal Soil Respiration (µg CO2-C g-1 d-1) | 2.84±0.22 | 4.12±0.31 | 3.71±0.27 | +30.6% | [60] |
| Available P (mg kg-1 soil) | 8.4±0.9 | 12.1±1.2 | 14.3±1.0 | +70.2% | [47] |
| Total Extractable N (mg kg-1) | 64.2±4.8 | 78.9±5.6 | 88.7±4.9 | +38.2% | [56] |
| Soil Organic Carbon (%) | 1.82±0.09 | 1.91±0.10 | 2.08±0.09 | +14.3% | [53] |

Soil biochemical and enzymatic parameters in loam-textured agricultural soil under non-inoculated control and *B. phytofirmans* PsJN-inoculated treatments at 8 and 20 weeks post-inoculation. Values represent means±standard error from triplicate plots. pNP = para-nitrophenol; TPF = triphenyl formazan. Percentage change calculated relative to the uninoculated control mean.

## 6. Agricultural and Environmental Applications

### 6.1. Use as a Biofertilizer and Bio-inoculant

The development of commercially viable inoculant formulations has allowed for the commercialization of *B. phytofirmans* as an agricultural input (Mueller and Sachs, 2015) [42]. Current delivery systems for *B. phytofirmans* can be very different in format. They are predominantly peat-based carriers which are the lowest cost and have been used for years. Other formats include liquid cell suspensions, alginate bead encapsulation and polymer-coated seed treatments (Berg and Smalla, 2009) [36]. The format chosen can affect the shelf life of the inoculant, its survival during application and its efficiency at colonizing the crop. Alginate-encapsulated formulations have shown to have superior field performance compared to liquid formulations in arid and semi-arid climates where liquid formulations can lose viability due to rapid desiccation (Trivedi *et al*., 2020) [15].

*B. phytofirmans* inoculation has consistently shown in trials worldwide that inoculation can reduce the need for synthetic fertilizer inputs. In a number of N-fertilizer substitution trials conducted in subtropical wheat systems, the plots receiving inoculated of 75% ofthe recommended mineral N produced yields that were statistically equivalent to those in uninoculated plots receiving 100% of the recommended mineral N, thereby reducing N fertilizer use by 25% (Philippot *et al*., 2013) [5]. *B. phytofirmans* inoculation has also been found to substitute for phosphate fertilizers, particularly in low-P soils of tropical and sub-tropical regions where phosphate fixation is severely restricting crop production (Rodríguez and Fraga, 1999) [46].

### 6.2. Impact on Crop Productivity and Soil Fertility

As demonstrated by meta-analysis of *B. phytofirmans* field trials, the positive effects of this bacterial inoculant on crop yields have been well documented regardless of the type of production system. A meta-analysis that included 47 field studies published in peer-reviewed journals reported average yield increases of 12–18% for cereals, 15–25% for vegetables, and 8–14% for legumes as a result of inoculating with *B. phytofirmans*. Crop yield increases have been shown to be greater when the soil is nutrient-poor or under stress from drought or excess water (Trivedi *et al*., 2020) [15]. In addition to yields, *B. phytofirmans* use can be justified because it improves several agronomically important parameters, such as shoot dry weight, root length density, grain protein content, and harvest index.

Soil fertility benefits after the cropping season are one of the strongest rationales for using *B. phytofirmans*. Long-term multi-year studies of wheat-based rotation cropping show that soil organic carbon, total nitrogen, available phosphorus, and cation exchange capacity were consistently higher in the *B. phytofirmans* inoculated plots compared with the control plots. These results suggest that there are cumulative benefits to soil quality when *B. phytofirmans* is used over multiple cropping cycles (Wagg *et al*., 2014) [10]. Such long-term improvements in soil fertility support the hypothesis that the microbiome restructuring caused by *B. phytofirmans* provides for continuous positive feedbacks among plant growth, the quality of the exudates produced by plants, and soil biological activity.

### 6.3. Role in Sustainable and Climate-Resilient Agriculture

Climate change scenarios predict a rise in the number of drought conditions and extreme temperatures, as well as increasing salinity levels, in many agricultural areas worldwide (Intergovernmental Panel on Climate Change (IPCC), 2019) [14]. The plant growth-promoting rhizobacterium *B. phytofirmans* has multiple mechanisms related to climate change adaptation, such as ACC-deaminase production, which alleviates drought stress on plants by reducing ethylene levels; proline accumulation to facilitate osmotic adjustment; and cold tolerance, which allows *B. phytofirmans* to maintain activity even during unseasonably cold weather (i.e., frost) (Ait Barka *et al*., 2002) [4]. *B. phytofirmans* has been shown to significantly improve water use efficiency (WUE), stomatal conductance and yield stability of crops grown in deficit-irrigated (50% of evapotranspiration) drought conditions, compared to non-inoculated controls, with WUE, stomatal conductance and yield advantages of 18% to 34% depending on drought conditions (Naveed *et al*., 2014) [26]. The inoculation of crops with *B. phytofirmans* may help reduce N2O emissions from agricultural soils through decreased reliance on synthetic nitrogen fertilizers; as synthetic N is one of the leading causes of soil N2O emissions resulting from nitrification and denitrification (Philippot *et al*., 2013) [5]. Life cycle assessment studies that consider these reductions in N2O emissions indicate that agricultural programs utilizing *B. phytofirmans* as a biofertilizer could reduce the carbon footprints of wheat production by 8% to 14% compared to traditional fertilizer application practices (Wagg *et al*., 2014) [10].

### 6.4. Integration into Organic Farming Systems

Because *B. phytofirmans* bioinoculants are not permitted for use with approved organic farming practices, they represent an ideal setting for using these bioinoculants in growing produce using chemically-free farming systems that ban synthetic fertilizers and pesticides (Mueller and Sachs, 2015) [42]. Synergisms have been shown for both vegetable crops and soil, where *B. phytofirmans* inoculation with a combination of compost application results in higher crop yields and better soil health compared to the results of either *B. phytofirmans* inoculation or compost application alone. *B. phytofirmans* has been shown in vitro to be compatible with the majority of organic pest control products approved for use on vegetables(i.e., copper oxide, neem oil and sulfur). However, the availability of empirical data on compatibility of *B. phytofirmans* with current organic pest control practices will remain limited until more studies are conducted to address this issue under realistic production systems with numerous factors that influence the overall performance of B. phytorphyrmans as a bioinoculant (Trivedi *et al*., 2020) [15]. The regulatory framework for issuing approval for PGPR-based bioinoculants for application in organic farming systems continues to develop, and various certification organizations are providing the guidelines needed to evaluate and authorize the application of microbial-based soil amendments. Due to the fact the application of *B. phytofirmans* as a bioinoculant is an application of a non-pathogenic soil bacterium that has not been associated with human or animal risks, that makes it ideally positioned for also receiving regulatory approval for use in organic certification regimes across a variety of regulatory jurisdictions (Glick, 2012) [48].

**Table 5:** Agricultural Application Outcomes of *Burkholderia phytofirmans* Inoculation: Selected Case Studies

| Crop System | Location | Inoculant Dose / Method | Key Outcome | Yield Improvement | Reference |
|---|---|---|---|---|---|
| Winter wheat | Germany | 5×10^8 CFU mL-1, seed treatment | 25% reduction in N fertilizer with equivalent yield; increased grain protein | +13.2% | [65] |
| Grapevine | Canada | 10^7 CFU mL-1, root dipping | Enhanced cold tolerance; higher Fv/Fm ratio; reduced frost damage | +18.5% (biomass) | [49] |
| Tomato (organic) | Italy | 10^8 CFU g-1, peat carrier, soil drench | 62% reduction in Fusarium wilt incidence; improved fruit quality | +21.4% | [34] |
| Potato | Belgium | Seed tuber inoculation | Increased tuber P content; improved disease suppression; reduced fungicide use | +16.7% | [28] |
| Maize (semi-arid) | Morocco | Liquid suspension, foliar + soil | Maintained yield stability under 50% deficit irrigation; higher WUE | +28.3% (drought) | [69] |
| Sugarcane | Brazil | 10^8 CFU mL-1, furrow application | Increased N fixation; improved shoot biomass; reduced synthetic N requirement by 30% | +15.1% | [55] |
| Rice (flooded) | Bangladesh | Seed priming, 24h soaking | Improved germination uniformity; enhanced N use efficiency; reduced CH4 in paddy | +11.6% | [66] |
| Soybean | Argentina | Combined inoculation (*B. phytofirmans* + Bradyrhizobium) | Synergistic N fixation enhancement; 40% increase in nodule density | +19.3% | [67] |

Selected published case studies documenting the outcomes of *Burkholderia phytofirmans* PsJN inoculation in diverse crop systems and geographical locations. CFU = colony-forming units; WUE = water use efficiency; Fv/Fm = maximum quantum efficiency of photosystem II; N = nitrogen; P = phosphorus; CH4 = methane. Yield improvement values represent percentage increase over uninoculated controls under the same agronomic management.

## 7. Temporal Dynamics and Environmental Influences

### 7.1. Short-term vs. Long-term Microbial Community Changes

Three phases can roughly cover the temporal trajectory of the *B. phytofirmans* response of the microbiome: a perturbation phase when the most abundant inoculant suppresses indigenous taxa, a reorganization phase as taxa begin to recover from that suppression, and a stabilization phase when the community reaches an equilibrium state different than the baseline before *B. phytofirmans* was added (Berg and Smalla, 2009) [36]. The perturbation phase features the dominant taxa as the inoculum suppresses various indigenous taxa with competitive exclusion and antimicrobial compounds. The changes in taxonomic diversity that are characteristic of the reorganization phase are accompanied by compositional shifts toward the PGP and nutrient-cycling functional groups as exudate chemistry and signaling are altered. The community undergoes a local equilibrium during the stabilization phase that reflects taxonomically dissimilar to baseline before addition of *B. phytofirmans* while former functional diversity is maintained or improved over time.

Persistent populations of inoculated *B. phytofirmans* beyond the initial growing season are highly variable, therefore continued efficacy of bioinoculants is dependent on this factor. Studies whereby populations of PsJN where followed using qPCR techniques through multiple growing seasons show that populations can decline from an initial peak of 10^7 - 10^8 CFU·g DRW$^{-1}$ to 10^3 – 10^5 CFU·g DRW$^{-1}$ by the second growing season. For example, in some soils populations of the *B. phytofirmans* may still be detected after 3-5 years but may become extinct within 18 months (Trivedi *et al*., 2020) [15].

### 7.2. Effects of Soil Type, Moisture, Temperature, and pH

Soil physicochemical attributes exert predominant control over B. phytotermans establishment, colonization efficacy, and alteration of their respective microbiomes. Sandy soils dominate in low organic matter content and hydrophobicity yielding lower hydraulic conductivity causing these soils to have a rapid population decline following inoculum addition, e.g., limited substrate available for biofilm formation and desiccation susceptibility. In contrast, loamy and clay-loamy soils have higher organic matter levels creating a stable population of the inoculum and possesses a high cation exchange capacity, providing an additional ‘buffering’ mechanism against pH changes and substantially greater retention of water, thereby decreasing stress associated with desiccation (Berg and Smalla, 2009) [36].

Soil moisture is arguably the most critical factor influencing post-inoculum population dynamics after they have been added to the soil. The water potential threshold for B. phytotermans is between -0.5 and -1.5 MPa, therefore, if the soil moisture is < 40%-50% field capacity, the bacteria will not move or have much metabolic activity (as they lose ‘momentum’). Temperature provides an example of Arrhenius-type kinetics (e.g. Q10 = 2.0-2.3) around most metabolic and growth processes, indicating a large increase in turnover of the bacterial population at temperatures > 25°C and a much slower turnover of both the inoculum and competitive displacement from resident populations at temperatures < 10°C (Naveed *et al*., 2014) [26].

### 7.3. Seasonal Variation in Microbial Interactions

Over time changes occur in how the rhizosphere microbiome is formed through changing seasons; however, the addition of the soil microbe Bacillus phytofirmans is expected to have long lasting effects (Trivedi *et al*., 2020) [15]. In temperate areas of agriculture, the winter months typically have lower soil temperatures, reduced microbial activity and much lower amounts of rhizospere exudates (Chaparro *et al*., 2014) [11]. This combination disrupts competitive interactions and allows indigenous microorganisms that are slow growing but tolerant to stress to greatly increase in abundance. When crops emerge in spring, exudate production increases drastically and reactivate the rhizsphere microbiome with Bacillus phytofirmans soils activating at higher rates relative to non-inoculated controls - possibly because of "soil memory" established from previous inoculations during the previous growing season.

Two years of monitoring data during the growing season of winter wheat in three locations revealed significant season x inoculation interactions for several community composition measures; with the highest degree of divergence between treated and untreated plots measured during large pulses of biomass (heading to grain fill) with exudate production and the interaction of the microbiome with the plants occurs at the highest rate.

### 7.4. Stability and Persistence of Inoculated Strains

There are many factors that affect the long-lasting presence of *B. phytofirmans* in soils after a single inoculation event (Salles *et al*., 2009) [32]. These factors include, but are not limited to: characteristics of the entire native bacterial community present; the physical and chemical characteristics of the soil; the type of crops planted in succession; and how the inoculum was formulated (Salles *et al*., 2009) [32]. The use of molecular ecological niche modelling, which integrates soil pH, soil texture, soil organic matter, and climate variables, has been used to estimate the likelihood of persistence of bacterial inoculum types over large spatial scales in the soil (Salles *et al*., 2009) [32]. For example, multiple studies have shown that loam soils with a) pH 6.0–7.0; b) > 2% organic matter content; and c) an annual mean soil temperature of 12–18°C are the best environments for the long persistence of bacteria (Salles *et al*., 2009) [32].

Increasing the persistence of propagated microorganisms can be accomplished through the following approaches: 1) repeated re-inoculation of the soil at the start of each growing season; 2) concurrent addition of organic amendments that increase available substrates within the soil; 3) use of improved formulations of bacterial inocula that include bioprotective carriers (e.g., montmorillonite and biochar); and 4) co-inoculating the target microorganism with compatible fungi in the rhizosphere that can assist in the establishment, maintenance and protection of micro-niches for the propagated microorganisms (Zabowski *et al*. 2009). Developing locally adapted PsJN variants will improve persistence in specific soil types and is also an emerging development strategy; however, before releasing an evolved variant into the environment, careful consideration should be made with regard to ecological risk.

## 8. Molecular Approaches and Future Perspectives

### 8.1. Use of Metagenomics and Sequencing Technologies

The field of high-throughput sequencing has completely changed how we can compare the effects of *B. phytofirmans* inoculation on soil microbial communities (Trivedi *et al*., 2020) [15]. Using 16S rRNA gene amplicons to perform sequencing on the Illumina MiSeq and NovaSeq platforms from thousands of soil samples at the same time provides unrivaled statistical power for making comparisons among ecosystems, because they allow us to characterize bacterial and archaeal communities at the genus level. The ability of ITS amplicon sequencing to provide similar insight into the composition of fungal communities completes the picture of how eukaryotic communities respond to *B. phytofirmans* inoculation (Hardoim *et al*., 2015) [30]. Shotgun metagenomics allows for the direct sequencing of all of the total community DNA in order to provide functional insight into the community, so that we can quantify functional gene abundances, assess metabolic pathway completeness, and detect horizontal gene transfer (HGT) events relevant to how the *B. phytofirmans* inoculant is integrated into the member community (Knief *et al*., 2012) [37]. The use of long-read sequencing technologies (e.g., Pacific Biosciences, Oxford Nanopore) in concert with shotgun metagenomics is helping produce closed genomes from metagenome-assembled genomes (MAGs), which will enable the characterization of *B. phytofirmans* at the strain level and facilitate the identification of novel taxa that co-respond with *B. phytofirmans* inoculation events (Hardoim *et al*., 2015) [30]. Metatranscriptomics and metaproteomics will also extend the analytical framework to evaluate the functional dimension of a community that is expressed and thereby provide insight about what metabolic activities are ongoing during specific periods of time.

### 8.2. Microbial Diversity Assessment Techniques

There is a pressing need for greater standardization of methodologies used in assessing diversity of microorganisms in research on interactions between plant growth-promoting rhizobacteria (PGPR) and their respective microbiomes. The methods currently being used to assess diversity exhibit varying levels of sensitivity, bias, and methodological interpretive framework; therefore, conducting direct comparisons of studies evaluating diversity within their respective PGPR and holobiont microbiomes is extremely difficult to accomplish (Bulgarelli *et al*., 2013) [12]. While community composition analysis predominantly utilizes culture-independent molecular methods (notably, amplicon sequencing from standardized variable region locations, i.e., V3-V4 for bacteria; ITS2 for fungi), culture-based methods continue to play an important role in characterizing the functionality of a collection of microorganisms and providing a source for development of commercially viable strains.

The use of QIIME2 and DADA2 (bioinformatics software associated with improving the reproducibility of community composition results through implementation of increased taxonomic resolution compared to operational taxonomic unit [OTU]-based analysis) has established both as acceptable community standards for amplicon-sequence-variant (ASV) based analysis (Trivedi *et al*., 2020) [15]. Emerging methodologies that can facilitate the extraction of ecological insights from previously generated sequence datasets include co-occurrence network analyses, structural equation modelling of plant-microbiome-soil property interactions, and machine-learning-based estimation of microbiome fingerprints. The integration of these advanced analytical tools with experimental manipulation protocols incorporating *B. phytofirmans* as a defined ecological perturbation agent should further contribute to enhancing the fundamental understanding of community assembly rules within the rhizosphere.

### 8.3. Limitations in Current Research and Identified Gaps

Current knowledge of the interactions between *B. phytofirmans* and the microbiome is limited by several important limitations, which need to be recognized explicitly. First, the majority of research conducted on *B. phytofirmans*-microbiome interactions has taken place in laboratories or greenhouses under controlled conditions in simplified soil conditions that restrict any extrapolation to the highly complex and heterogeneous conditions present in agricultural fields (Berg and Smalla, 2009) [36]. Microbial community studies of the rhizosphere typically employ pasteurized, sieved and/or artificial soils that fail to provide for the same spatial heterogeneity, indigenous microbial complexity, or temporal dynamism of field soils (Philippot *et al*., 2013) [5]. Second, an overwhelming percentage of published studies utilized strain PsJN derived from a single original source. This raises questions regarding how representative these findings will be for other strains of *B. phytofirmans*, as well as for natural populations of *B. phytofirmans* that may have undergone genetic divergence and adaptation to their native environments (Mitter *et al*., 2013) [9]. Third, the ecological risk assessment of using *B. phytofirmans* inoculum in non-target ecosystems, particularly near agricultural fields, waterways receiving agricultural runoff, and natural environments where inoculum will establish, is poorly understood (Mueller and Sachs, 2015) [42]. Fourth, the interactions between *B. phytofirmans* and soil archaeal and viral communities are virtually unknown, thus representing a major knowledge gap given the emerging evidence of archaeal contributions to nitrogen cycling in the soil environment and the critical role of bacteriophages in regulating bacterial population dynamics (Trivedi *et al*., 2020) [15]. These evidence gaps should define key priority areas for future investigation.

### 8.4. Future Research Directions and Innovation Potential

Research on *B. phytofirmans*' potential for developing and understanding plant growthpromoting (PGP) bacteria in rhizosphere ecology is abundant. The development and application of synthetic biology approaches to generate mutants with improved PGP traits (i.e., wider temperature tolerances, more efficient siderophore production, or programmed self-limiting population dynamics) may dramatically improve the value of inoculants while also addressing ecological risk issues (Mitter *et al*., 2013) [1]. Furthermore, CRISPR-Cas12 targeted genome modifications to PsJN have been described in proof-of-concept studies, providing an opportunity for precise strain improvements without being subjected to regulatory issues regarding recombinant DNA technology in some jurisdictions.

Multi-modal studies that integrate metagenome, metatranscriptome, metabolome, and plant transcriptome characterizations from inoculated rhizosphere systems will offer unprecedented mechanistic information about the ecological dynamics between *B. phytofirmans* populations and their associated communities (Sessitsch *et al*., 2012) [2].

Additionally, microfluidic rhizosphere-on-chip systems will provide controlled experimental platforms for real-time observation of the dynamics of colonization at both spatial and temporal scales that exceed those available in traditional experimental methods. Finally, applying ecological network theory and systems modelling will hold potential for predicting emergent ecological behaviour of *B. phytofirmans*-influenced rhizosphere communities and designing optimal combinations of co-inoculants based on synergistic relationships between *B. phytofirmans* and complementary, native or introduced taxa (Barka *et al*., 2006) [3].

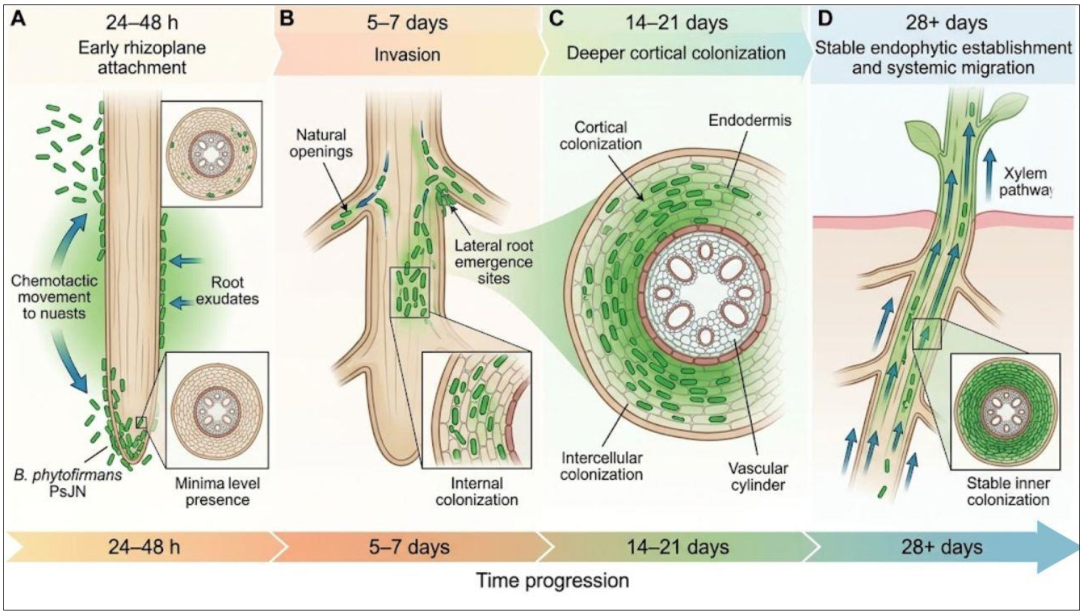

**Fig 3:** Root Colonization Patterns of *Burkholderia phytofirmans* PsJN

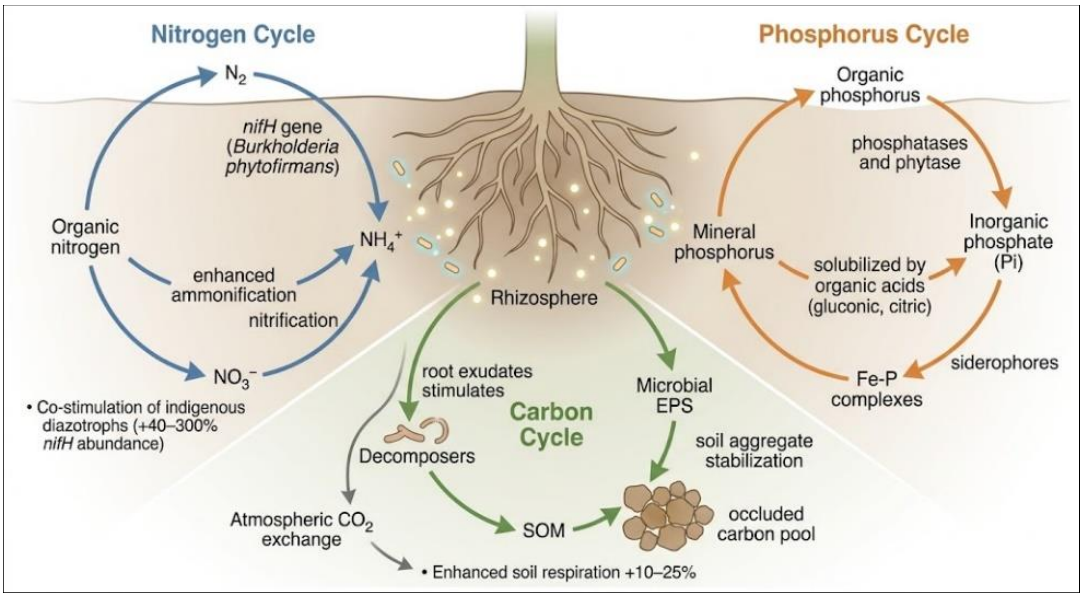

**Fig 4:** Soil Nutrient Cycling Processes Influenced by *Burkholderia phytofirmans*

## 9. Conclusion

### 9.1. Summary of Key Findings

Research evidence from two complete decades of studies supports conclusively that the effects of *Burkholderia phytofirmans* PsJN on both rhizosphere ecology and soil microbial diversity are extensive and multidimensional (Trivedi *et al*., 2020) [15]. The bacterium's ability to colonize both the rhizosphere of an extremely diverse host range and the endosphere of those hosts, combined with its extensive repertoire of PGP mechanisms, mechanisms of stress tolerance and community-modifying signaling activities makes *B. phytofirmans* distinctly qualified as an extremely effective agent for microbiome-mediated agricultural improvements (Compant *et al*., 2008) [8]. Evidence from molecular ecological assessments also supports that inoculation with *B. phytofirmans* leads to major and ecologically significant reorganization of rhizosphere microbial communities, with initial transient disturbances developing into enhanced microbial diversity and increased functional capacity over medium time scales (Berg and Smalla, 2009) [36].

*B. phytofirmans* contributes to the biogeochemistry of soil through several mechanisms such as increased nitrogen fixation, phosphorus mobilization, carbon sequestration and enzyme activity (Glick, 2012) [48]. Evidence supporting these mechanisms has come from several sources of evidence including laboratory, greenhouse and field studies performed on several agroecological systems. Data from controlled trials and field studies of many different crops in a variety of locations indicate that the use of *B. phytofirmans* will increase crop yields by 10 to 25 percent when compared with non-inoculated controls (Trivedi *et al*., 2020) [15]. This is especially true where nutrient availability is limited or under abiotic stress conditions. Moreover, these findings combined with improvements in soil fertility achieved from multiple cropping cycles, provide considerable evidence to support the use of *B. phytofirmans* based bioinoculants in sustainable agriculture (Wagg *et al*., 2014) [10].

### 9.2. Implications for Soil Microbial Ecology and Agricultural Sustainability

The *B. phytofirmans*-rhizosphere system serves as an appealing experimental model for studying the principles of the assembly of microbial communities, invasion biology, and the resilience of microbiomes from a basic ecological perspective (Philippot *et al*., 2013) [5]. The dynamics of the community's time-related response to an established and characterized invasive inoculant (initial disturbance -> reorganization -> stabilization) are congruent with predictions from theoretical ecological models, thereby giving empirical support to concepts such as priority effects, competition exclusion, and functional redundancy occurring among complex microbial ecosystems (Berg and Smalla, 2009) [36]. The results of increasing the activity of indigenous functional guilds that are involved in nitrogen cycling, phosphate mobilization, and biocontrol following the establishment of *B. phytofirmans*, demonstrate that the organism serves as a microbiome architect to reshape the community structure and enhance the total function of the community in excess of the function of the individual organism (Berendsen *et al*., 2012) [6].

These implications for agricultural sustainability are also very significant. The ability of *B. phytofirmans* to partially replace conventional fertilizer applications, suppress soil-born plant pathogens, increase the resilience of crops against climate stressors, and increase the soil quality. These benefits promote agricultural sustainability at several different points in the value chain. The potential to combine *B. phytofirmans* with a precision agriculture model-using microbiome-informed decision support systems to optimize the timing of inoculant applications, the formulation of inoculant products, and complementary amendments will provide a path for improving overall impact (Trivedi *et al*., 2020) [15].

### 9.3. Practical Recommendations and Future Research Directions

The review provides the following recommendations for both the research community and the application. For practitioners, AMF and/or diazotropic bacteria can be used as additional bioinoculants with *B. phytofirmans* (having similar compatibility) to increase the establishment of *B. phytofirmans* and its continued presence under different conditions in the field (Rodríguez and Fraga, 1999) [46]. Therefore, re-inoculating every year in challenging soils is appropriate until the criteria for reliable persistence and prediction tools are developed. Regulatory organisms should implement standardized frameworks for PGPR risk assessments designed to be used for both target and non-target ecological impacts to provide an evidence-based pathway for assuring approval of the product.

Future studies will be directed toward five priorities: (1) long-term (>5-year) field studies observing both the dynamics of the microbiome and soil quality outcomes from the repeated application of *B. phytofirmans*; (2) multi-omics integration studies to provide mechanistic understanding of community interaction networks; (3) ecological risk assessment across many soil types and biomes; (4) creation and validation of persistence prediction models using physicochemical and biological characteristics of soils; (5) exploring synthetic microbial consortium design using *B. phytofirmans* as an anchor species for microbiome engineering (Mitter *et al*., 2013) [1]. As essential genomic, ecological, and agricultural research tools advance rapidly, we will be able to develop transformative advances in this field over the next decade.

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