

**Universidade de São Paulo
Escola Superior de Agricultura “Luiz de Queiroz”**

**Effects of phosphorus-solubilizing bacteria on plant development
and soil microbiology**

Mayara Martins e Martins

Thesis presented to obtain the degree of Doctor in
Science: Area: Soil and Plant Nutrition

**Piracicaba
2024**

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DEDICATION

**To my parents Flávia and Pedro,
For love, my creation and education**

**My sister, Letícia,
For being my best friend, trust me and always be there**

**My grandmother Jieva (Eva),
For being my example of a woman, love and humility**

**To my fiancé Fábio,
For your strength, patience and affection throughout all these years**

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I finish a stage with a mix of emotions, but certainly fulfilled by this mission accomplished.

Thank you very much!

EPIGRAPH

“Unless we change the way we think, we will not be able to solve the problems caused by the way we have become accustomed to seeing the world.”

Albert Einstein

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RESUMO

Efeitos de solubilizadores de fósforo no desenvolvimento das plantas e na microbiologia do solo

Os microrganismos solubilizadores de fosfato desempenham papel crucial no crescimento e desenvolvimento das plantas, influenciando diretamente a disponibilidade de fósforo (P) e, conseqüentemente, o desenvolvimento vegetal. Este estudo investiga como esses solubilizadores afetam não apenas a nutrição das plantas, mas também as mudanças na microbiologia do solo, avaliando sua contribuição para a saúde e fertilidade do solo. Ao explorar a interação entre solubilizadores de P, plantas e microbiota do solo, busca-se aprofundar o entendimento sobre práticas agrícolas sustentáveis, visando o uso eficiente de nutrientes e o estímulo ao desenvolvimento vegetal em ambientes específicos, por meio do uso de microrganismos solubilizadores de fosfato. O objetivo deste estudo foi avaliar o desempenho da associação entre fontes de fosfato e microrganismos potencialmente solubilizadores de fosfato, com foco na utilização nutricional das plantas e no impacto no solo. Após uma análise detalhada dos mecanismos e funções dos microrganismos solubilizadores de fosfato no contexto dos desafios globais relacionados ao P, realizou-se um experimento em casa de vegetação, abrangendo dois cenários: manejo de fertilização residual, baseado em práticas históricas de fertilização (com TSP ou RRP), e manejo de fertilização fresca, envolvendo a aplicação direta de fertilizante fresco (TSP ou RRP). Ambos os cenários foram sujeitos ao fator de inoculação (com ou sem inoculação do isolado ATCC 7061 classificado como *Bacillus pumilus*). O delineamento experimental foi em blocos completos ao acaso em esquema fatorial (2 x 2). O material vegetal foi coletado para análise dos teores foliares de N, P, K, Ca, Mg e S. As avaliações de fertilidade incluíram determinações dos teores de P, K, Ca e Mg, matéria orgânica (MO), pH, H + Al e capacidade de troca de cátions (V%). Para a análise da comunidade microbiana, foram realizadas determinações da fosfatase ácida e beta-glucosidase, além da quantificação microbiana de P. Embora o emprego de microrganismos solubilizadores de fosfato tenha potencial para melhorar a absorção de fertilizantes fosfatados pelas plantas, não foram observadas mudanças positivas nas frações de P do solo após a inoculação bacteriana, conforme esperado, indicando a necessidade de estudos adicionais para compreender essa interação. No entanto, foi observado um aumento na fração não lábil de P nos tratamentos com Fosfato de Rocha Recalcitrante (RRP) e Superfosfato Triplo (TSP), sugerindo atividade bacteriana nos processos de degradação da rocha em ambos os cenários frescos e residuais.

Palavras-chave: 1. Microrganismo 2. Fertilização 3. Solubilização.

ABSTRACT

Effects of phosphorus-solubilizing bacteria on plant development and soil microbiology

Phosphate-solubilizing microorganisms play a crucial role in the growth and development of plants, directly influencing the availability of phosphorus (P) and, consequently, plant development. This study investigates how these solubilizers affect not only plant nutrition but also changes in soil microbiology, assessing their contribution to soil health and fertility. By exploring the interaction between P solubilizers, plants, and soil microbiota, the aim is to deepen the understanding of sustainable agricultural practices, promoting efficient nutrient use and stimulating plant development in specific environments through the use of phosphate-solubilizing microorganisms. The objective of this study was to evaluate the performance of the association between phosphate sources and potentially phosphate-solubilizing microorganisms, focusing on plant nutritional uptake and soil impact. Following a detailed analysis of the mechanisms and functions of phosphate-solubilizing microorganisms in the context of global P challenges, an experiment was conducted in a greenhouse, covering two scenarios: residual fertilization management, based on historical fertilization practices (with TSP or RRP), and fresh fertilization management, involving the direct application of fresh fertilizer (TSP or RRP). Both scenarios were subjected to the inoculation factor (with or without inoculation of the ATCC 7061 isolate classified as *Bacillus pumilus*). The experimental design was a randomized complete block design in a factorial scheme (2 × 2). Plant material was collected for analysis of leaf contents of N, P, K, Ca, Mg, and S. Fertility assessments included determinations of P, K, Ca, and Mg levels, organic matter (OM), pH, H + Al, and cation exchange capacity (V%). For microbial community analysis, determinations of acid phosphatase and beta-glucosidase, as well as microbial P quantification, were performed. Although the use of phosphate-solubilizing microorganisms has the potential to enhance the absorption of phosphate fertilizers by plants, no positive changes in soil P fractions were observed after bacterial inoculation as expected, indicating the need for further studies to understand this interaction. However, an increase in the non-labile P fraction was observed in treatments with Recalcitrant Rock Phosphate (RRP) and Triple Superphosphate (TSP), suggesting bacterial activity in rock degradation processes in both fresh and residual scenarios.

Keywords: Microorganism 2. Fertilization 3. Solubilize.

1. INTRODUCTION

Phosphorus (P), although generally abundant in the soil, confronts limitations in its availability to plants due to its highly reactive nature. Deficiencies in this essential nutrient manifest in sluggish, stunted growth and diminished productivity. Phosphorus undergoes modulation by precipitation and adsorption mechanisms, binding and reacting on the surface of soil particles (cations) such as iron (Fe), aluminum (Al), and calcium (Ca), resulting in the formation of insoluble compounds inaccessible to plants (Wild, 1988).

Playing a fundamental role in photosynthesis, growth and reproduction, P is irreplaceable (Shen et al., 2011). Recognized as an essential nutrient in agricultural production, it is crucial for young plant tissues, root growth, physiological processes of flowering, seed and fruit formation (Poirier, Jaskolowski, & Clúa, 2022). Furthermore, it facilitates the absorption of other elements such as nitrogen (N) and potassium (K), increasing assimilation rates (Maathuis, 2009; Bhatla et al., 2018).

In tropical soils, P emerges as a limiting factor for the production of plant biomass due to intense weathering, resulting in high levels of iron (Fe) and aluminum (Al) oxides in the clay fraction (Osorio, 2011). These compounds selectively adsorb phosphorus, reducing its availability to plants (Tian, 2021).

To increase P availability in the soil, several management techniques have been proposed, including addition of phosphate fertilizers, use of phosphate solubilizing microorganisms (PSM) and adequate management of soil organic matter (SOM). Furthermore, choosing plant species adapted to tropical soil can optimize biomass production (Sharma et al., 2013 and Kalayu, 2019).

Limited soil P availability results from complex interactions involving soil chemical and physical characteristics, weathering intensity, and P removal by agricultural production (Fink et al., 2016; Penn and Camberato, 2019). The excessive addition of P sources to agricultural soils can precipitate environmental problems such as water eutrophication (Tilman et al., 2001), with increased algae production and deterioration in water quality (Gomes et al., 2014). Therefore, the conscious use of P in agriculture is crucial (Johan, 2021).

The use of phosphate solubilizing microorganisms (PSM) is a strategy studied to improve the availability of P in the soil, aiming to increase agricultural productivity. PSMs have advantages of environmental compatibility, potential cost savings (less fertilizers), and high biological efficiency (Timofeeva, Galyamova, and Sedykh, 2022).

However, obstacles persist, requiring solutions for the widespread adoption of this practice in agricultural management. Challenges include identifying effective microorganisms

for each soil and crop type, defining optimal strategies for applying microorganisms, and market considerations (costs and storage) for these PSM.

Thus, the use of PSM emerges as a promising strategy to improve the availability of P in the soil, aiming to enhance the productivity of agricultural crops, with positive implications for the environment and the agricultural economy. The objective of this study was to evaluate the performance of the association of phosphate sources with potentially phosphate-solubilizing microorganisms, aiming to assess the impact of this association on plant nutritional utilization and its performance in the soil.

Within this scope, the hypotheses of this study are:

I. Inoculation with potentially phosphate-solubilizing microorganisms along with phosphate fertilizer sources [triple superphosphate (TSP) or reactive natural phosphate (RNP)] promotes an increase in plant biomass and enhanced phosphorus (P) utilization by the plants.

II. Inoculation with potentially phosphate-solubilizing microorganisms in soils with residual P stocks from different P sources (TSP and RNP) promotes an increase in plant biomass and enhanced phosphorus (P) utilization by the plants.

III. The association of phosphate sources with phosphate-solubilizing microorganisms increases the availability of organic forms of P in the soil due to the production/exudation of phosphatase enzymes.

The present work is divided into two chapters: 1. Mechanisms and roles of phosphate-solubilizing microorganisms in the context of global phosphorus challenges; 2. Microorganismos potencialmente solubilizadores de fosfato associados.

References

- Bindraban, P. S., Dimkpa, C. O., & Pandey, R. (2020). Exploring phosphorus fertilizers and fertilization strategies for improved human and environmental health. *Biology and Fertility of Soils*, 56(3), 299-317.
- Fink, J.R.; Inda, A.; Tiecher, T.; Barrón, V. 2016. Iron oxides and organic matter on soil phosphorus availability. *Cienc. Agrotecnologia*, 40, 369–379.
- Gomes, E. A., Silva, U. D. C., Marriel, I. E., De Oliveira, C. A., e Lana, U. G. D. P. (2014). Rock phosphate solubilizing microorganisms isolated from maize rhizosphere soil. *Revista brasileira de Milho e Sorgo*, 13(1), 69-81.
- Johan, P. D., Ahmed, O. H., Omar, L., & Hasbullah, N. A. (2021). Phosphorus transformation in soils following co-application of charcoal and wood ash. *Agronomy*, 11(10), 2021.
- Kalay, G. (2019). Phosphate solubilizing microorganisms: promising approach as biofertilizers. *International Journal of Agronomy*, 2019, 1-7.
- Khan, F., Siddique, A. B., Shabala, S., Zhou, M., & Zhao, C. (2023). Phosphorus plays key roles in regulating plants' physiological responses to abiotic stresses. *Plants*, 12(15), 2861.
- Maathuis, F. J. (2009). Physiological functions of mineral macronutrients. *Current opinion in plant biology*, 12(3), 250-258.

- Osorio, N. W. (2011). Effectiveness of phosphate solubilizing microorganism in increasing plant phosphate uptake and growth in tropical soils. *Bacteria in agrobiologia: plant nutrient management*, 65-80.
- Penn, C. J., & Camberato, J. J. (2019). A critical review on soil chemical processes that control how soil pH affects phosphorus availability to plants. *Agriculture*, 9(6), 120.
- Poirier, Y., Jaskolowski, A., e Clúa, J. (2022). Phosphate acquisition and metabolism in plants. *Current Biology*, 32(12), R623-R629.
- Sharma, S. B., Sayyed, R. Z., Trivedi, M. H., Gobi, T. A. (2013). Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. *SpringerPlus*, 2, 1-14.
- Shen, J., Yuan, L., Zhang, J., Li, H., Bai, Z., Chen, X., Zhang, W e Zhang, F. (2011). Phosphorus dynamics: from soil to plant. *Plant physiology*, 156(3), 997-1005.
- Tian, J., Ge, F., Zhang, D., Deng, S., Liu, X. (2021). Roles of phosphate solubilizing microorganisms from managing soil phosphorus deficiency to mediating biogeochemical P cycle. *Biology*, 10(2), 158.
- Tilman D, Fargione J, Wolff B, D'Antonio C, Dobson A, Howarth R, Schindler D, Schlesinger WH, Simberloff D, Wackhamer D (2001). Forecasting agriculturally driven global environmental change. *Science*, 292: 281-284. 10.1126/science.1057544
- Timofeeva, A., Galyamova, M., Sedykh, S. (2022). Prospects for using phosphate-solubilizing microorganisms as natural fertilizers in agriculture. *Plants*, 11(16), 2119.
- Vishwakarma, K., Kumar, N., Shandilya, C., Mohapatra, S., Bhayana, S., & Varma, A. (2020). Revisiting plant–microbe interactions and microbial consortia application for enhancing sustainable agriculture: a review. *Frontiers in Microbiology*, 11, 560406.
- Wild, A. (1988). Plant nutrients in soil: phosphate. *Russell's soil conditions and plant growth*. Eleventh edition, 695-742.

2. MECHANISMS AND ROLES OF PHOSPHATE-SOLUBILIZING MICROORGANISMS IN THE CONTEXT OF GLOBAL PHOSPHORUS CHALLENGES

Abstract

Phosphorus (P) faces challenges in sustainable management amidst global population growth, highlighting the need for efficient utilization, especially in agriculture, facing global P challenges. Strategies involve land use, agricultural management and use of soil microorganisms, especially phosphorus-solubilizing microorganisms (PSMs), which aim to increase P efficiency, while considering environmental sustainability. Studies indicate that the use of phosphate-solubilizing microorganisms promotes system sustainability, combined with phosphate fertilizers with lower solubility, making P available to crops, promoting an increase in production. Despite several studies on this association, the insertion of one or more microorganisms can interfere with the soil's microbial community, and therefore their use must be analyzed so as not to generate competition with the community already existing in the soil. From what was presented, there are several insights into what the inclusion of PSM would be like in the current management of phosphate fertilizers, and the behavior of the association of microorganisms with P sources, which still require further studies.

Keyword: Phosphate. Legacy P. Soil.

2.1. Introduction

Phosphorus (P) is universally acknowledged as a vital nutrient for all known life forms, serving as a critical element in bridging the gap between the living and non-living components of the biosphere. A key challenge already evident in this context relates to the sustainable management of resources, with a specific emphasis on the use and efficient utilization of P (Withers et al., 2018). As projections suggest, the world's population is expected to reach 9.7 billion by 2050 and 10.9 billion by 2100, representing a significant increase from the 7.8 billion recorded in mid-2020 (Gu et al., 2021). Food production plays a critical role in supporting both human development and food security. An issue regarding phosphorus as a finite resource essentially revolves around a tripod: (i) mining and the international market, (ii) the constant need for supply to sustain agricultural productivity as an essential element, and (iii) the use and efficiency of this resource in situations involving highly weathered soils and/or landscape degradation process as soil erosion and runoff (Solangi et al., 2023). At the core of this tripod

lies agriculture, playing a vital role in addressing the Global Phosphorus Challenges, especially in highly weathered soils that are dependent on phosphate rock-derived fertilizers supply (Gatiboni et al., 2020a).

As inorganic fertilizers are introduced into the soil with the purpose of addressing nutrient deficiencies, P undergoes a series of sorption, precipitation, and complexation reactions in the soil environment (Hallama et al., 2019). These reactions result in the transformation of P into forms that are not readily accessible for plant uptake. Consequently, the worldwide use of P fertilizer on agricultural lands has seen a substantial rise, going from approximately 5 Tg P yr⁻¹ in 1961 to 18 Tg P yr⁻¹ as of 2013 (Lu and Tian, 2017). Projections indicate that this trend is expected to continue, with estimates suggesting it could reach 22–27 Tg P yr⁻¹ by 2050 (Mogollón et al., 2018).

Investigating the natural soil P reservoirs and the long-standing buildup of P over the past five decades, commonly referred to as "legacy P" (Pavinato et al., 2020) could enhance the efficiency of P utilization in most soils. The pathways being pursued to increase P efficiency rate and the utilization of the legacy P encompass land use and farm management, fertilization with alternative sources, and the implementation and management of soil microorganisms (Gatiboni et al., 2020b). This scenario has led to a growing interest in the biological processes that occur in the soil, especially regarding Phosphorus-Solubilizing Microorganisms (PSMs). This is due to the possibility of adopting an environmentally friendly approach that can achieve similar outcomes as synthetic fertilizers but without drawbacks.

The phenomenon of insoluble P solubilization by microorganisms was initially documented by Pikovskaya (1948). It is noteworthy that there exists a rich diversity of bacterial, archaeal, and fungal strains that exhibit the capacity for P solubilization. While they are indeed widespread, their presence and P-solubilizing potential can vary across various ecosystems, different phyla, species, and even among isolates (Divjot et al., 2021). Although the umbrella term "PSMs" encompasses various microorganisms with the remarkable ability to convert plant non-labile phosphorus compounds into soluble fractions, differences arise in both the mode of action and the specific locations where these microorganisms operate. A diverse range of microorganisms directly engage with mineral surfaces and organic matter complexes within the soil itself, while others, such as Arbuscular mycorrhizal fungi (AMF) and plant growth-promoting bacteria (PGPB), exert their influence primarily within the rhizosphere (Sharma et al., 2013).

The PSMs have the capacity to enhance P bioavailability by lowering soil pH, solubilizing inorganic P, mineralizing organic P and chelating P from aluminum (Al³⁺), calcium (Ca²⁺), and iron (Fe³⁺) sources (Etesami, 2020; Rawat et al., 2021). Also, they release various substances such as organic acids, like citric acid, oxalic acid, and succinic acid, as well as

enzymes like phosphatases and phytases, along with ion-chelating molecules like siderophore (Wang et al., 2023). Furthermore, PSMs enhance plant growth in various environments, solubilizing potassium, facilitating nitrogen fixation, producing growth regulators, and protecting against pathogens through antibiotic and antifungal excretion (Kalayu, 2019).

This review article elucidates the diverse array of species and mechanisms of action utilized by PSMs for soil phosphorus dissolution. Furthermore, it highlights their pivotal role in fostering plant growth and underscores their significance as a tool for optimizing soil P utilization efficiency and accessing the legacy P.

2.2. Phosphorus cycle and its bioavailability

Phosphorus, denoted by its atomic number 15, possesses an atomic mass of 30.97 and follows an electron configuration of $1s^2 2s^2 2p^6 3s^2 3p^3$. In the context of phosphorus chemistry, it's important to note the relatively minor energy distinction between the 3s and 3d orbitals. This minimal energy gap allows for the active participation of vacant d-orbitals in bonding processes, leading to the formation of hybridized orbitals. This accessibility to d-orbitals contributes to the substantial diversity in potential electron configurations around the nucleus, consequently accounting for the wide range of phosphorus compounds observed in soil chemistry (Jaisi and Blake, 2014). These periodic characteristics confer to the P cycle cyclical oxidation and reduction of P compounds, involving electron transfer reactions that span from -3 (PH_3 , phosphine gas) to +5 (PO_4^{3-} , phosphate) during various redox reactions. These reactions are not yet fully understood (Ohtake et al., 1996), but it is noteworthy that microorganisms play a significant role in this process. In any discussion of P dynamics, it's crucial to consider the complete P cycle because the immobilization, mineralization, and redistribution of P in the soil depend on physical-chemical properties, such as P sorption by colloidal surfaces, just as much as they depend on microbial, mycorrhizal, or plant uptake of P (Stewart and Tiessen, 1987).

The global biogeochemical cycling of P involves three primary components (Ruttenberg, 2001). First, it encompasses tectonic uplift and the exposure of phosphorus-bearing rocks to weathering forces. Second, the interactions of various factors (e.g., geologic material, climate, slope, organisms and time) and processes (translocation, transformation, addition-removal) inherent in soil formation lead primary minerals toward achieving thermodynamic equilibrium with stable pedogenic forms (Fink et al., 2016). Throughout these transformations, P is released from primary minerals into the soil solution, thereby becoming accessible for plant uptake. Simultaneously, elements such as calcium (Ca), magnesium (Mg), potassium (K), and sodium (Na), along with silicates and carbonates, are leached from the soil matrix. The

transformation of iron (Fe) and aluminum (Al) into oxides, hydroxides, or oxyhydroxides introduces novel functional groups capable of adsorbing P. Finally, P undergoes sedimentation, becoming associated with organic and mineral matter and eventually being buried within sediments. The P pathway can be described as sedimentary because P does not undergo rapid cycling comparable to carbon (C), nitrogen (N), and sulfur (S), which are transported not only within soil and water but also in the atmosphere.

Soil P exists in both inorganic (Pi) and organic (Po) forms, and the ratio of Pi to Po undergoes changes as soils develop (Cross and Schlesinger, 1995). Globally, the concentrations of total P in surface soils typically vary between 200 to 800 mg kg⁻¹ (Tiessen, 2008). When P is present in the soil, it triggers a series of processes and mechanisms (Figure 1). In alkaline soils or under conditions of over-liming, it can initiate the precipitation of phosphate with particular cations, ultimately leading to the formation of calcium phosphate through neo-formation (Hemwall, 1957). Also, phosphate may be adsorbed by functional groups found on iron or aluminum oxides, forming thermodynamically stable complexes (Gypser et al., 2018).

Alternatively, it can transform into biologically active organic compounds that persist as organic P within the soil (Tiessen et al., 1984). The decomposition rates of litter are governed by soil organisms, environmental conditions, and the chemical composition of the litter. The conversions between Pi and organic Po forms are regulated by various factors influencing mineralization and immobilization processes, such as microbial activity, moisture levels, and the physico-chemical and mineralogical properties of the soil (Holland and Turekian, 2003).

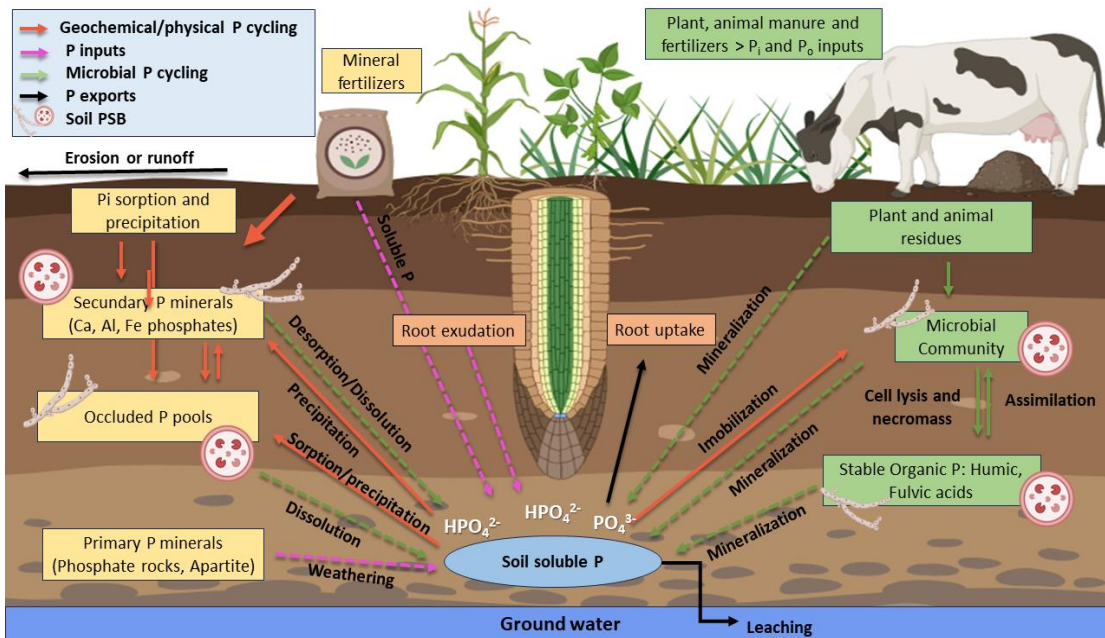


Figure 1. Schematic diagram of soil phosphorus (P) biochemical cycle. The Orange arrows indicate geochemical or geophysical reactions on P cycle. Pink arrows indicate P inputs by fertilizers, SOM degradation or by natural primary minerals weathering. Green arrows indicate phosphate solubilizing microorganisms (PSM) inducing P reactions and P cycle. Black arrows indicate P exports on soil by erosion, runoff or by root absorption.

It is essential to acknowledge that while the correlation between C:P in SOM may be weaker in comparison to N or S, a substantial portion of the P_o utilized by soil microorganisms originates from the soil organic matter (SOM) (McGill and Cole, 1981). Crucially, P_i is consistently cycled through microbial biomass (Tamburini et al., 2012). The SOM consists of humic acids (HA), fulvic acids (FA), and humin. Among these components, HA, along with fulvic acids, make up to 70% of SOM (Cotrufo et al., 2015). Furthermore, various fractions of SOM, such as dissolved organic matter (DOM) plays a significant role in the P cycle are primarily responsible for cation binding capacity in most soils (Bravin et al., 2012). This range of organic compounds, which arises from the decomposition of organic matter in the soil, has the dual effect of reducing adsorption and promoting the desorption process of previously sorbed P up to a certain energy threshold.

The influence of SOM on soil phosphorus availability involves three main processes: (1) competitive adsorption between SOM and phosphate on soil colloid surfaces, (2) metal complexation, and (3) the mineralization of P_o and P_i (Figure 1). Essentially, SOM and SOC compete for adsorption sites with phosphate (Haynes and Zhou, 2018). Additionally, when SOM is previously attached to colloids, it generates a repelling surface effect. (Groeneveld et al., 2020). Although the binding between phosphate and Fe/Al oxyhydroxides is not charge-dependent (Wang et al., 2019), the increase in the negative surface area of colloids due to

SOM reduces the chances of contact between P and soil minerals surface. Additionally, both SOM and SOC establish binding bridges between P and the colloid surface, offering the potential for phosphate complexation and subsequent release into the soil solution (Debicka et al., 2023).

Microorganisms play an essential role in Earth's P cycle. They serve as both a source and a sink for P, influencing its distribution and availability in the ecosystem. These microorganisms are integral to the cycle, directly participating in the formation and stabilization of SOM. Just as the formation of SOM relies closely on the action of microorganisms, its effect directly influences the cycling of P compounds as nucleotides, phosphides, phosphoproteins, phosphosaccharides (ATP), nucleic acids (DNA, RNA), co-enzymes, humic and fulvic acids, and phosphatides actively participate in the P cycle (Tamburini et al., 2012), and consequently the availability of Pi. This highlights that the enduring organic carbon in soil may not primarily originate from plant litter or its remnants, but instead from carbon that has undergone initial microbial biomass processing (Benner, 2011; Grandy et al., 2009).

The fast pool consists of constant Po from the soil solution, which becomes immobilized in microbial biomass and replenishes the slow pool upon cell death (Tian et al., 2021). Soil-soluble orthophosphate ions can be incorporated into microbial cells to enhance biomass growth. Studies have shown that a significant portion of the P released from organic P by phosphate-solubilizing microbes (PSM) is assimilated into bacterial cells as cellular P (Rawat et al., 2021). Simultaneously, these soil microbes can promptly release Po into the slow pool through processes like cell lysis, death, and predation by soil fauna (Prabhu et al., 2019). The most common sources of slow Po include plant debris, deceased organisms, microbes, and non-living Po fertilizers (e.g., dry straw and animal manure) (Alvarez et al., 2021). These sources directly contribute to replenishing soil orthophosphate levels through geochemical or biological decomposition, ultimately benefiting plant-available P supply, and improving soil quality (Figure 1). The activities of phosphatase enzymes in both PSM and soils primarily regulate these biogeochemical processes (Tao et al., 2008).

2.3. Legacy P

The concept of "Legacy Phosphorus" can be interpreted differently from various perspectives. This diversity in interpretation can create difficulties in effectively addressing the real challenges associated with maintaining crop production, reducing losses, and responsibly managing P resources. Two basic questions arise from the term legacy P: (1) What strategies can be employed to minimize the loss of phosphorus from agriculture into surface waters,

thereby mitigating the risk of eutrophication? Additionally, (2) how can we effectively utilize the phosphorus stored in soil to enhance food production?

There are four primary areas of debate concerning how to define, measure, and assess legacy phosphorus (Figure 2) in soils (Condrón, 2022). The first revolves around the concept of mass balance, essentially referring to the difference between the natural P content in the soil and the current total P content. However, this concept has limitations. Even in long-term experiments with meticulous monitoring of input and output data, accurately quantifying P losses resulting from erosion, runoff, or wind dispersion can be challenging. Consequently, when using the mass balance concept, estimates may only be feasible through modeling. The second definition is rooted in agronomic concepts (Figure 2), considering only the phosphorus accumulated in the soil above agronomic recommendation limits. However, this concept presents challenges because agronomic recommendations vary among different crop species. Additionally, the critical phosphorus level varies with factors such as soil type, crop type, and climate, as noted by Syers et al., (2008), and depends on the models used to represent the relationship between soil P extractors and yield (Mallarino and Blackmer, 1992) which are often presented as wide ranges and methods (e.g. Olsen P, Melich, Resin, etc). The third ("environmental definition") sets a boundary for legacy P above the risk threshold for losses. Like the previous agronomic threshold concept, this definition also poses challenges and quantification difficulties. Nonetheless, it underscores a critical connection between environmental losses and agronomic recommendations. The fourth definition relates to the utilization of the pool of natural P that isn't accessible to plants, commonly referred to as "natural legacy P". The utilization of these natural reserves provides an opportunity to either maintain or enhance crop productivity, reduce the reliance on mineral fertilizers (thereby improving the efficiency of previous applications), and mitigate environmental risks. In this context, advancements in genetics, optimization of soil management practices, and the utilization of PSMs stand at the forefront of managing soil P in its "legacy P" context.

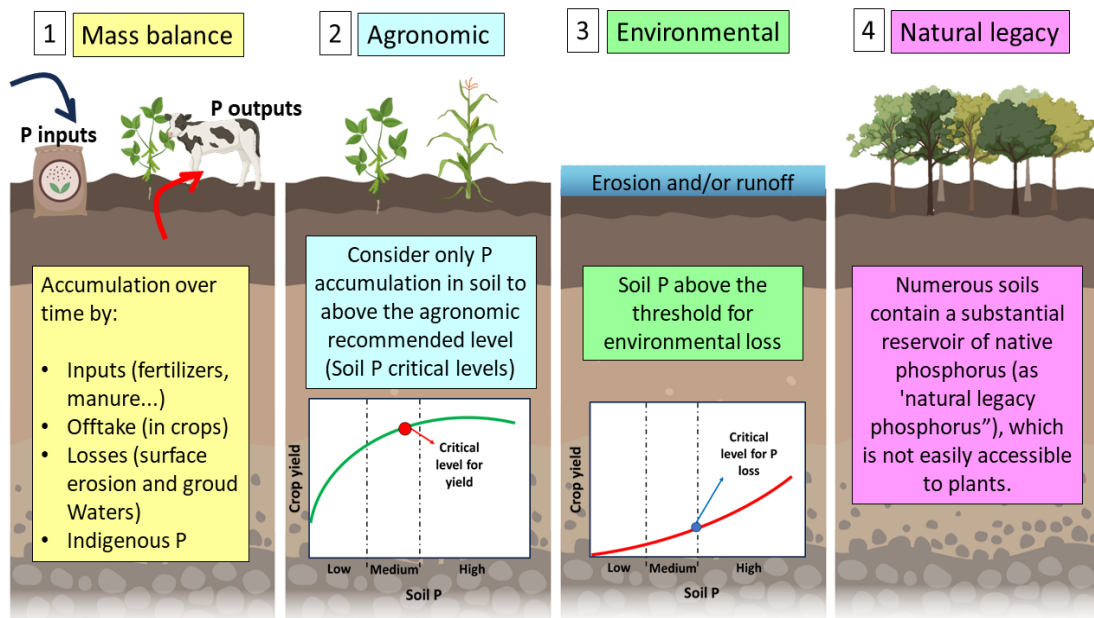


Figure 2. Conceptualization of different definitions of legacy P.

The interactions between P soil minerals are controlled by a variety of physiochemical factors, including pH, P loadings, and mineral phases (Wang et al., 2019). To predict and assess the fate and bioavailability of P, it is essential to have a comprehensive understanding of P speciation on mineral surfaces. This understanding is still lacking, particularly when it comes to phosphate sorption on aluminum oxides, which are commonly found in acidic soils.

Phosphate primarily forms inner-sphere complexes on the surfaces of iron (Fe) and aluminum (Al) oxides through a ligand exchange reaction, as documented in studies from references (Stumm, 1995). The assertion that phosphate forms bidentate-binuclear (BB) complexes on ferrihydrite surfaces is substantiated by the P–Fe interatomic distance (measuring 3.23 Å) of these complexes, as determined through differential atomic pair distribution function (d-PDF) analysis (Han and Ro, 2019).

To quantify, qualify, and define boundaries between soil P fractions, sequential extraction has historically been employed, and their relative lability is determined based on the chemical species extracted (Condon and Newman, 2011). These P fractionations of soil P are mainly divided in inorganic (Pi) and organic (Po) predicated on the principle that extractants with increasing acidity and alkalinity successively remove P with decreasing lability or bioavailability (Hedley et al., 1982). Multiple adaptations of the Hedley fractionation method are available, but they generally adhere to a similar extraction sequence. Then, soil P fractions can generally be categorized as (a) soil solution Pi (normally $<10 \mu\text{mol L}^{-1}$) extracted by water, (b) soluble Pi, extracted by resin bag or strip (in HCO_3^- form) in water, (c) labile Pi and Po, extracted by 0.5 M NaHCO_3 (pH 8.5), (d) moderately labile Pi and Po, with 0.1 M NaOH, primary mineral P,

extracted with 1.0 M HCl and (e) residual P (occluded P), by digestion with H₂SO₄ and H₂O₂ (Hedley et al., 1982).

The desorption of P by PSMs often coincides with a decrease in pH. This phenomenon is thought to be a consequence of increased solubility of Fe and Al through potential complexation with low molecular weight organic acids (Hoberg et al., 2005; Osorio and Habte, 2013). The extent to which phosphate remains dissolved in solution is determined by the equilibrium between precipitation and dissolution, as well as the processes of adsorption and desorption onto compounds with varying charges (Lindsay, 1979). The PSMs act by solubilizing P bound to minerals, achieving this through the dissolution of P-bearing minerals and the desorption of phosphate from mineral surfaces. These processes are primarily driven by the release of (i) low-molecular-weight organic acids (LMWOAs), (ii) protons (H⁺), and (iii) siderophores and exopolysaccharides (Rawat et al., 2021).

Organic acids and anions have the capacity to displace P_i from adsorption sites through ligand exchange mechanisms that are instigated by microbial activities and the temporary obstruction of P_i adsorption sites (Menezes-Blackburn et al., 2016). The effectiveness of organic acids in promoting the desorption of P_i relies on their dissociation characteristics and the presence of carboxylic groups, which introduce unique negative charges that assist in this process (Marschner, 2008).

Pastore et al., (2020) discovered that microbial communities within acidic beech forest soils were notably more effective at releasing phosphate from hydroxyapatite than from goethite. This enhanced phosphate release from hydroxyapatite was primarily attributed to two key processes: acidification and the production of LMWOAs. Biswas et al., (2022) verified that phosphate-solubilizing bacteria (PSB) can enhance the solubility of P_i derived from low-grade rock phosphate (LGRP) and consequently augment P accessibility for crops. Moreover, the use of PSB-inoculated LGRP can ensure a sustained and consistent supply of available P in the soil over an extended period. This durability stems from the fact that PSB, being living organisms, can persistently generate organic acids and facilitate the solubilization of P from LGRP for an extended duration.

2.4. Mechanisms of Phosphorus Solubilization by PSMs

The PSMs perform a critical function in converting insoluble phosphorus into accessible forms that plants can readily absorb. Soil contains a substantial population of these PSMs, and their capacity for phosphorus solubilization can be assessed through both qualitative and quantitative methods (Mehta and Nautiyal, 2001). A wide array of biological groups of

microorganisms, encompassing bacteria, fungi, actinomycetes, and algae, possess the ability to solubilize and mineralize P (Table 1). These microorganisms include Actinobacteria, Ascomycota, Bacteroidetes, Basidiomycota, Euryarchaeota, Firmicutes, Mucoromycota, and Proteobacteria (Devi et al., 2022; Nidhin and Chattopadhyay, 2022; Yadav et al., 2019). According to Kour et al., (2021), bacteria have several representatives of genere as *Azospirillum*, *Bacillus*, *Erwinia*, *Enterobacter*, *Nitrosomonas*, *Pseudomonas*, *Pantoea*, *Rhizobium*, *Serratia*, and *Xanthomonas*. Basically, among the non-mycorrhizal fungi encompass *Alternaria*, *Arthrobotrys*, *Aspergillus*, *Fusarium*, *Helminthosporium*, *Penicillium*, *Trichoderma* (Cosme et al., 2018; Sisti et al., 2019). Among actinobacteria, the genera *Actinomyces*, *Gardnerella*, *Frankia*, *Mycobacterium*, *Norcardiopsis*, *Streptomyces*, *Thermobifida* (Barka et al., 2016) and for cyanobacteria, *Calothrix braunii*, *Anabaena variabilis*, *Scytonema* sp. and *Westiellopsis prolifica* (Yang et al., 2017).

The process of solubilizing and transforming less readily available forms into forms that are readily accessible to plants through PSMs entails a variety of mechanisms and processes (Figure 3). Basically, the primary mechanisms employed by PSMs encompass (1) the release of extracellular enzymes (biochemical mineralization), (2) the liberation of phosphorus during substrate degradation (biological mineralization), and (3) the secretion of mineral-dissolving complexes or compounds (such as siderophores, protons, hydroxyl ions, and organic acids) (Divjot et al., 2021; Li et al., 2023; Sharma et al., 2013).

Table 1. Organic acids produced during the solubilization process of microorganisms that solubilize P

Organisms	Organic acids	Mechanism	Reference
<i>Halolamina sp.</i>	Gluconic acid, citric acid		Yadav A et al. (2015)
<i>Azospirillum brasilense</i>	Gluconic acid	pH reduction/solubilization	Rodríguez et al. (2004)
<i>Azospirillum lipoferum</i>	Gluconic acid	pH reduction/solubilization	Rodríguez et al. (2004)
<i>Serratia marcescens</i>	Citric acid, gluconic acid, succinic acid, lactic acid, unknown acid	pH reduction	Chen et al. (2006)
<i>Arthrobacter sp.</i>	Citric acid, lactic acid	pH reduction	Chen et al. (2006)
<i>Bacillus megaterium</i>	Citric acid, lactic acid, unknown acid		Chen et al. (2006)
<i>Leclercia adecarboxylata</i>	Formic acid, gluconic acid, malonic acid, acetic acid, citric acid, succinic acid	pH reduction/solubilization	Teng et al. (2019b)
<i>Penicillium oxalicum</i> , <i>Aspergillus niger</i>	Oxalic acid, formic acid, tartaric acid, malic acid, citric acid, acetic acid		Li et al. (2016)
<i>Trichoderma flavus</i> , <i>Trichoderma helicus</i> , <i>Penicillium purpurogenum</i> , <i>Penicillium janthinellum</i>	Glucuronic acid, gluconic acid, succinic acid, butyric acid, valeric acid, citric acid, fumaric acid, propionic acid, acetic acid	pH reduction/solubilization	Scervino et al. (2010)
<i>Bacillus cereus</i> , <i>Bacillus subtilis</i> , <i>Paenibacillus sp.</i>	Oxalic acid, malic acid, formic acid, acetic acid, tartaric acid, gluconic acid	pH reduction/solubilization	Chawngthu et al. (2020)
<i>Pseudomonas corrugata</i>	Gluconic acid, citric acid		Trivedi and Sa (2008)
<i>Aspergillus aculeatus</i> , <i>Penicillium daleae</i> , <i>Aspergillus versicolor</i>	Phytase, phosphatases		Adhikari and Pandey (2019)

2.5. Extracellular Enzyme Release (Biochemical Mineralization)

Within the context of soil microbiology, microorganisms are prolific producers of extracellular enzymes. The non-specific acid phosphatases (NSAPs), also known as phosphomonoesterases, represent a category of enzymes either associated with the lipoprotein membranes of microorganisms or released into the extracellular environment (Duff et al., 1994). Acid (EC 3.1.3.2) and alkaline (EC 3.1.3.1) phosphatases are classified as a

NSAP with potential of hydrolysis simple phosphate monoesters to acquire orthophosphate (Eivazi and Tabatabai, 1977). Soil phosphatases catalyze the hydrolysis of soil P_o into its inorganic forms, HPO_4^- and $H_2PO_4^-$, prior to its absorption and uptake by plant roots from the soil solution. Chemically, this reaction can be described as follows by substrate, enzymatic action, hydrolysis reaction and products (Gandhi and Chandra, 2012; Manzoor et al., 2022). The substrate for phosphatase is typically a phosphate ester, such as organic phosphate found in molecules like adenosine triphosphate (ATP) or inorganic phosphate, such as calcium phosphate ($Ca_3(PO_4)_2$) present in soil minerals (Fransson and Jones, 2007). The enzyme action occurs upon the substrate. It has an active site that promotes the breaking of the bond between the phosphate and the functional group to which it is attached. During the reaction catalyzed by phosphatase, a hydrolysis reaction takes place. Then, the water molecule (H_2O) is added to the substrate, resulting in the cleavage of the phosphate bond and the separation of products.

Following their release from plant roots as exudates or microbial secretions, extracellular phosphatases engage in various types of associations. These associations encompass (1) the formation of enzyme-substrate complexes, (2) adsorption onto clay minerals, (3) complexation with soil organic matter surfaces through entrapment, absorption, or co-polymerization, and/or (4) existence in freely diffusible forms within the soil solution (Burns et al., 2013).

2.5.1 Phytases

Phytases, a distinct subset of phosphatases, are specialized in breaking down phytate and generating lower phosphorylated inositol compounds. They are broadly distributed throughout nature, originating primarily from plants, microbes (including bacteria and fungi), and specific animal tissues (Singh and Satyanarayana, 2011). There are two types of phytases: 3-phytases (EC 3.1.3.8) and 6-phytases (EC 3.1.3.26) (Webb, 1992). This classification is based on which phosphate group the enzyme cleaves first. Microbes primarily produce 3-phytases, whereas 6-phytases are typically found in plant enzymes (Singh and Satyanarayana, 2011). The mechanism of action for all phytases involves the enzymatic hydrolysis of bonds between inositol and phosphoric acid residues (Rizwanuddin et al., 2023).

Phytases have the capability to hydrolyze phytic acid by targeting the phosphomonoester bonds within the compound, resulting in two subgroups: myo-inositol hexaphosphate or phytate in its salt form (Kour et al., 2020). This enzymatic process not only releases P but also makes other associated nutrients, such as zinc, accessible (Sadaf et al., 2022). In a study conducted by Estrada-Bonilla et al., (2021), the utilization of compost and PSB was investigated to improve soil P availability. Within the tested groups, Group 2, where *Bacillus*

sp. BACBR04, *Bacillus* sp. BACBR06, and *Rhizobium* sp. RIZBR01 were co-inoculated, exhibited a notable increase in the abundance of phytate-degrading enzymes. This increase in enzyme activity was correlated with the enhancement of labile Po in the soil when PSB were co-inoculated.

2.6. Inorganic phosphate-solubilization mechanisms

2.6.1 Organic acids

Among the various strategies employed by microorganisms, the release of low molecular weight organic acids (LMWOAs) has long been recognized and widely accepted as a key mechanism for P-solubilization. Several studies have identified and quantified these organic acids, providing a clearer understanding of their role in the solubilization process (Chen et al., 2016, 2006; Sharma et al., 2011).

PSMs have been discovered to produce various acids in liquid media. These acids include acetic and formic (monocarboxylic acids), lactic, gluconic, and glycolic (monocarboxylic hydroxy acids), 2-keto gluconic (monocarboxylic keto acid), oxalic and succinic (dicarboxylic acids), malic (dicarboxylic hydroxy acids), and citric (tricarboxylic hydroxy acids) (Prabhu et al., 2019).

Fermentation, the breakdown of organic carbon compounds through respiration, and direct oxidation are fundamental metabolic processes utilized by PSMs to synthesize organic acids, ultimately causing acidification in their immediate vicinity (Sindhu et al., 2014). The organic acids act by chelating cations bound to P through their hydroxyl and carboxyl groups. Acids such as 2-keto-gluconic acid, humic acid, and fulvic acid are recognized for their strong ability to chelate cations. They are highly effective at dissolving P_i compounds that are bound to calcium, iron, and aluminum (Fouad et al., 2023). Additionally, it plays a role in lowering the pH within the rhizosphere by influencing the exchange of gases (O_2/CO_2) and the balance of protons and bicarbonate. This collective action results in the release of bound P (Khan et al., 2014).

2.6.2 Inorganic acid production

Another mechanism through which microorganisms facilitate the solubilization of mineral phosphates is by generating inorganic acids, including sulfuric (Pradhan and Sukla, 2006), nitric (Khan et al., 2009), and carbonic acids (Kalayu, 2019). Inorganic acids are generated by nitrifying and sulfur-oxidizing bacteria while oxidizing nitrogen-containing or inorganic sulfur

compounds. These inorganic acids produced then interact with insoluble phosphate compounds, transforming them into soluble forms (Sand, 1997).

Acidophilic and sulfur-oxidizing bacteria generate H₂S as a metabolic byproduct during the microbial decomposition of organic matter, sulfate reduction, and various biochemical reactions. This H₂S then reacts with ferric phosphate, forming ferrous sulfate and releasing the bound phosphorus (Florentino et al., 2016). Jazaeri et al., (2016) verified that the inoculation of *Thiobacillus* bacteria with elemental sulfur had a significant effect on the availability of Olsen P and P uptake by plants.

2.6.3 Proton release from assimilation and respiration

Phosphate solubilization has also been documented to happen even without the production of acids (Roos and Luckner, 1984). The acidification of the medium through the excretion of H⁺ ions and the production of respiratory H₂CO₃ is documented as an alternative mechanism for solubilizing inorganic phosphate (Arvieu et al., 2003).

Within the microbial cell, NH₄⁺ undergoes conversion into ammonia (NH₃), leading to the release of excess protons (H⁺) into the cytoplasm (Ortiz-Medina et al., 2019). This results in the acidification of the immediate environment around the microbial cell, which in turn assists in the breakdown of insoluble phosphates (Yadav, 2022).

2.6.4 Siderophores

Siderophores are compounds with high-affinity ferric-ion-specific ligand that are excreted by microorganisms and plants in response to iron stress in the environment (Scavino and Pedraza, 2013). Siderophores primarily serve to chelate Fe(III) under different environmental conditions, thus making this element accessible to plants and microorganisms (Soares, 2022).

PSMs have also been demonstrated to have the capability to produce siderophores (Emami-Karvani and Chitsaz-Esfahani, 2021; Jiang et al., 2018; Saha et al., 2016). Siderophores improve P availability for plants (Figure 3) through processes such as ligand exchange and chelation of elements that form complexes with P (Řezanka et al., 2019).

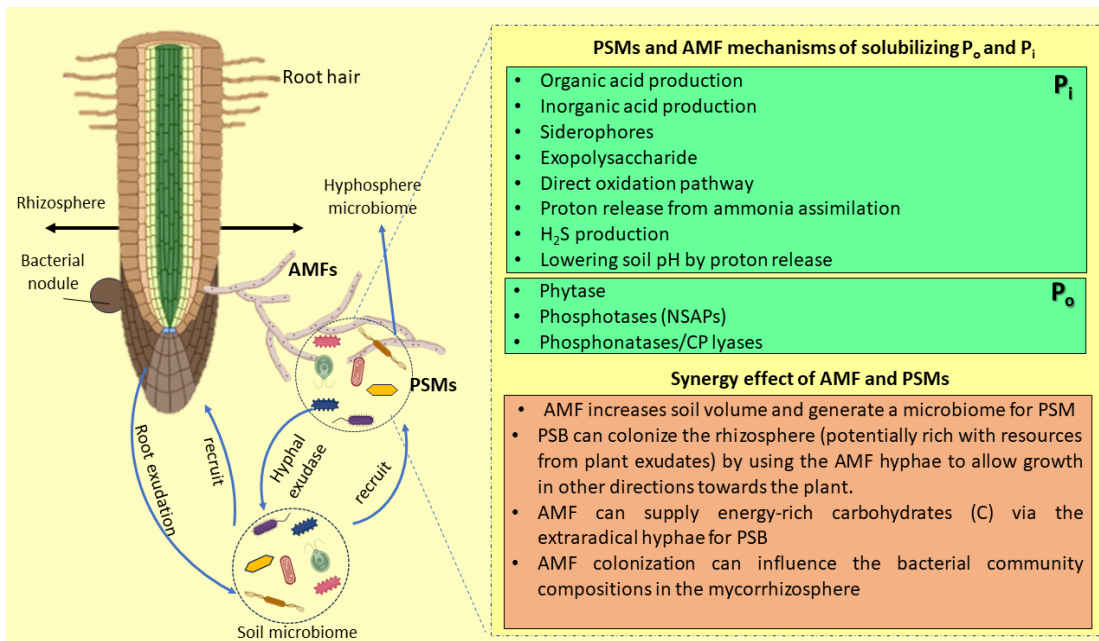


Figure 3. Schematic representation of how AMFs and PSMs act in solubilizing phosphate and its interaction.

2.7. Final considerations and perspectives

The phosphate solubilizing microorganisms have the capacity to enhance the process of dissolving phosphate fertilizers, promoting the sustainability of the system. Some studies show that these microorganisms, combined with phosphate fertilizers with lower solubility, make P available to crops, in addition to promoting, in general, increases in production.

Despite several studies on this association, the insertion of one or more microorganisms can interfere with the soil's microbial community, and therefore their use must be analyzed so as not to generate competition with the community already existing in the soil.

From what was presented, insights emerge about what the inclusion of phosphate-solubilizing microorganisms would be like in the current management of phosphate fertilizers, that is, the behavior of the association of microorganisms with P sources and what the behavior of these microorganisms would be like in soils with a history of phosphate fertilizers.

References

- Alvarez, A.L., Weyers, S.L., Goemann, H.M., Peyton, B.M., Gardner, R.D., 2021. Microalgae, soil and plants: A critical review of microalgae as renewable resources for agriculture. *Algal Res.* 54, 102200.
- Arvieu, J.-C., Leprince, F., Plassard, C., 2003. Release of oxalate and protons by ectomycorrhizal fungi in response to P-deficiency and calcium carbonate in nutrient solution. *Ann. For. Sci.* 60, 815–821.

- Barka, E.A., Vatsa, P., Sanchez, L., Gaveau-Vaillant, N., Jacquard, C., Klenk, H.-P., Clément, C., Ouhdouch, Y., van Wezel, G.P., 2016. Taxonomy, physiology, and natural products of Actinobacteria. *Microbiol. Mol. Biol. Rev.* 80, 1–43.
- Benner, R., 2011. Biosequestration of carbon by heterotrophic microorganisms. *Nat. Rev. Microbiol.* 9, 75. <https://doi.org/10.1038/nrmicro2386-c3>
- Biswas, S.S., Biswas, D.R., Ghosh, A., Sarkar, A., Das, A., Roy, T., 2022. Phosphate solubilizing bacteria inoculated low-grade rock phosphate can supplement P fertilizer to grow wheat in sub-tropical inceptisol. *Rhizosphere* 23, 100556. <https://doi.org/https://doi.org/10.1016/j.rhisph.2022.100556>
- Bravin, M.N., Garnier, C., Lenoble, V., Gérard, F., Dudal, Y., Hinsinger, P., 2012. Root-induced changes in pH and dissolved organic matter binding capacity affect copper dynamic speciation in the rhizosphere. *Geochim. Cosmochim. Acta* 84, 256–268.
- Burns, R.G., DeForest, J.L., Marxsen, J., Sinsabaugh, R.L., Stromberger, M.E., Wallenstein, M.D., Weintraub, M.N., Zoppini, A., 2013. Soil enzymes in a changing environment: Current knowledge and future directions. *Soil Biol. Biochem.* 58, 216–234. <https://doi.org/https://doi.org/10.1016/j.soilbio.2012.11.009>
- Chen, W., Yang, F., Zhang, L., Wang, J., 2016. Organic acid secretion and phosphate solubilizing efficiency of *Pseudomonas* sp. PSB12: effects of phosphorus forms and carbon sources. *Geomicrobiol. J.* 33, 870–877.
- Chen, Y.P., Rekha, P.D., Arun, A.B., Shen, F.T., Lai, W.-A., Young, C.C., 2006. Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. *Appl. soil Ecol.* 34, 33–41.
- Condrón, L., 2022. Legacy Phosphorus in agricultural soils: Maintaining crop yields and minimising losses to water. *Eur. Sustain. Phosphorus Platf.* 1–21.
- Condrón, L.M., Newman, S., 2011. Revisiting the fundamentals of phosphorus fractionation of sediments and soils. *J. Soils Sediments* 11, 830–840. <https://doi.org/10.1007/s11368-011-0363-2>
- Cosme, M., Fernández, I., Van der Heijden, M.G.A., Pieterse, C.M.J., 2018. Non-mycorrhizal plants: the exceptions that prove the rule. *Trends Plant Sci.* 23, 577–587.
- Cotrufo, M.F., Soong, J.L., Horton, A.J., Campbell, E.E., Haddix, M.L., Wall, D.H., Parton, W.J., 2015. Formation of soil organic matter via biochemical and physical pathways of litter mass loss. *Nat. Geosci.* 8, 776–779. <https://doi.org/10.1038/ngeo2520>
- Cross, A.F., Schlesinger, W.H., 1995. A literature review and evaluation of the Hedley fractionation: Applications to the biogeochemical cycle of soil phosphorus in natural ecosystems. *Geoderma* 64, 197–214. [https://doi.org/10.1016/0016-7061\(94\)00023-4](https://doi.org/10.1016/0016-7061(94)00023-4)
- de Almeida, R.F., Naves, E.R., da Mota, R.P., 2015. Soil quality: Enzymatic activity of soil β -glucosidase. *Glob. J. Agric. Res. Rev.* 3, 146–450.
- Debicka, M., Morshedizad, M., Leinweber, P., 2023. The Effects of Dissolved Organic Matter Derived from Agricultural Waste Materials on Phosphorus Sorption in Sandy Soils.
- Devi, R., Kaur, T., Kour, D., Yadav, A., Yadav, A.N., Suman, A., Ahluwalia, A.S., Saxena, A.K., 2022. Minerals solubilizing and mobilizing microbiomes: A sustainable approach for managing minerals' deficiency in agricultural soil. *J. Appl. Microbiol.* 133, 1245–1272.
- Divjot, K., Rana, K.L., Tanvir, K., Yadav, N., Yadav, A.N., Kumar, M., Kumar, V., Dhaliwal, H.S., Saxena, A.K., 2021. Biodiversity, current developments and potential biotechnological applications of phosphorus-solubilizing and-mobilizing microbes: A review. *Pedosphere* 31, 43–75.
- Duff, S.M.G., Sarath, G., Plaxton, W.C., 1994. The role of acid phosphatases in plant phosphorus metabolism. *Physiol. Plant.* 90, 791–800.
- Eivazi, F., Tabatabai, M.A., 1977. Phosphatases in soils. *Soil Biol. Biochem.* 9, 167–172.
- Emami-Karvani, Z., Chitsaz-Esfahani, Z., 2021. Phosphorus solubilization: mechanisms, recent advancement and future challenge. *Soil Microbiomes Sustain. Agric. Funct. Annot.* 85–131.
- Estrada-Bonilla, G.A., Durrer, A., Cardoso, E.J.B.N., 2021. Use of compost and phosphate-solubilizing bacteria affect sugarcane mineral nutrition, phosphorus availability, and the

- soil bacterial community. *Appl. Soil Ecol.* 157, 103760. <https://doi.org/https://doi.org/10.1016/j.apsoil.2020.103760>
- Etesami, H., 2020. Enhanced phosphorus fertilizer use efficiency with microorganisms. *Nutr. Dyn. Sustain. Crop Prod.* 215–245.
- Fink, J.R., Inda, A.V., Tiecher, T., Barrón, V., 2016. Iron oxides and organic matter on soil phosphorus availability. *Ciência e Agrotecnologia* 40, 369–379. <https://doi.org/10.1590/1413-70542016404023016>
- Fouad, F.A., Youssef, D.G., Shahat, F.M., Abd El-Ghany, M.N., 2023. Role of Microorganisms in Biodegradation of Pollutants, in: *Handbook of Biodegradable Materials*. Springer, pp. 221–260.
- Fransson, A.M., Jones, D.L., 2007. Phosphatase activity does not limit the microbial use of low molecular weight organic-P substrates in soil. *Soil Biol. Biochem.* 39, 1213–1217.
- Gandhi, N.U., Chandra, S.B., 2012. A comparative analysis of three classes of bacterial non-specific acid phosphatases and archaeal phosphoesterases: evolutionary perspective. *Acta Inform. Medica* 20, 167.
- Gatiboni, L., Brunetto, G., Pavinato, P.S., George, T.S., 2020a. Legacy phosphorus in agriculture: role of past management and perspectives for the future. *Front. Earth Sci.*
- Gatiboni, L., Brunetto, G., Pavinato, P.S., George, T.S., 2020b. Editorial: Legacy Phosphorus in Agriculture: Role of Past Management and Perspectives for the Future. *Front. Earth Sci.* 8, 1–2. <https://doi.org/10.3389/feart.2020.619935>
- Grandy, A.S., Strickland, M.S., Lauber, C.L., Bradford, M.A., Fierer, N., 2009. The influence of microbial communities, management, and soil texture on soil organic matter chemistry. *Geoderma* 150, 278–286. <https://doi.org/https://doi.org/10.1016/j.geoderma.2009.02.007>
- Groeneveld, M., Catalán, N., Attermeyer, K., Hawkes, J., Einarsdóttir, K., Kothawala, D., Bergquist, J., Tranvik, L., 2020. Selective adsorption of terrestrial dissolved organic matter to inorganic surfaces along a boreal inland water continuum. *J. Geophys. Res. Biogeosciences* 125, e2019JG005236.
- Gu, D., Andreev, K., & Dupre, M. E. (2021). Major trends in population growth around the world. *China CDC weekly*, 3(28), 604.
- Guelfi, D., Nunes, A.P.P., Sarkis, L.F., Oliveira, D.P., 2022. Innovative Phosphate Fertilizer Technologies to Improve Phosphorus Use Efficiency in Agriculture. *Sustainability* 14. <https://doi.org/10.3390/su142114266>
- Gypser, S., Hirsch, F., Schleicher, A.M., Freese, D., 2018. Impact of crystalline and amorphous iron- and aluminum hydroxides on mechanisms of phosphate adsorption and desorption. *J. Environ. Sci.* 70, 175–189. <https://doi.org/https://doi.org/10.1016/j.jes.2017.12.001>
- Hallama, M., Pekrun, C., Lambers, H., Kandeler, E., 2019. Hidden miners – the roles of cover crops and soil microorganisms in phosphorus cycling through agroecosystems. *Plant Soil* 434, 7–45. <https://doi.org/10.1007/s11104-018-3810-7>
- Han, J., Ro, H.-M., 2019. Characterizing Preferential Adsorption of Phosphate on Binary Sorbents of Goethite and Maghaemite using in situ ATR-FTIR and 2D Correlation Spectroscopy. *Sci. Rep.* 9, 6130. <https://doi.org/10.1038/s41598-019-42575-2>
- Han, Y., Liu, W., Chang, N., Sun, L., Bello, A., Deng, L., Zhao, L., Egbeagu, U.U., Wang, B., Zhao, Y., 2023. Exploration of β -glucosidase-producing microorganisms community structure and key communities driving cellulose degradation during composting of pure corn straw by multi-interaction analysis. *J. Environ. Manage.* 325, 116694.
- Haynes, R.J., Zhou, Y.F., 2018. Competitive and noncompetitive adsorption of silicate and phosphate by two acid Si-deficient soils and their effects on P and Si extractability. *Soil Sci. Plant Nutr.* 64, 535–541. <https://doi.org/10.1080/00380768.2018.1473008>
- Hedley, M. J., Stewart, J.W.B., Chauhan, B.S., 1982. Changes in inorganic and organic soil phosphorus fractions induced by cultivation practices and by laboratory incubations 1. *Soil Sci. Soc. Am. J.* 46, 970–976.
- Hedley, M. J., Stewart, J.W.B., Chauhan, B.S., 1982. Changes in inorganic and organic soil phosphorus fractions induced by cultivation practices and by laboratory incubations. *Soil Sci. Soc. Am. J.* 46, 970–976.

- <https://doi.org/10.2136/sssaj1982.03615995004600050017x>
- Hemwall, J.B., 1957. The fixation of phosphorus by soils. *Adv. Agron.* 9, 95–112.
- Hoberg, E., Marschner, P., Lieberei, R., 2005. Organic acid exudation and pH changes by *Gordonia* sp. and *Pseudomonas fluorescens* grown with P adsorbed to goethite. *Microbiol. Res.* 160, 177–187. <https://doi.org/https://doi.org/10.1016/j.micres.2005.01.003>
- Holland, H.D., Turekian, K.K., 2003. *Treatise on geochemistry: 8. Biogeochemistry.*
- Hu, X., Gu, H., Liu, J., Wei, D., Zhu, P., Cui, X., Zhou, B., Chen, X., Jin, J., Liu, X., Wang, G., 2023. Metagenomic strategies uncover the soil bioavailable phosphorus improved by organic fertilization in Mollisols. *Agric. Ecosyst. Environ.* 349, 108462. <https://doi.org/https://doi.org/10.1016/j.agee.2023.108462>
- Jaisi, D.P., Blake, R.E., 2014. Advances in using oxygen isotope ratios of phosphate to understand phosphorus cycling in the environment. *Adv. Agron.* 125, 1–53.
- Jazaeri, M., Akhgar, A., Sarcheshmehpour, M., Mohammadi, A.H., 2016. Bioresource efficacy of phosphate rock, sulfur, and *Thiobacillus inoculum* in improving soil phosphorus availability. *Commun. Soil Sci. Plant Anal.* 47, 1441–1450.
- Jiang, H., Qi, P., Wang, T., Wang, M., Chen, M., Chen, N., Pan, L., Chi, X., 2018. Isolation and characterization of halotolerant phosphate-solubilizing microorganisms from saline soils. *3 Biotech* 8, 1–8.
- Kalayu, G., 2019. Phosphate solubilizing microorganisms: promising approach as biofertilizers. *Int. J. Agron.* 2019, 1–7.
- Khan, M.S., Zaidi, A., Ahmad, E., 2014. Mechanism of phosphate solubilization and physiological functions of phosphate-solubilizing microorganisms. *Phosphate solubilizing Microorg. Princ. Appl. microphos Technol.* 31–62.
- Khan, M.S., Zaidi, A., Wani, P.A., 2009. Role of phosphate solubilizing microorganisms in sustainable agriculture-a review. *Sustain. Agric.* 551–570.
- Kour, D., Kaur, T., Yadav, N., Rastegari, A.A., Singh, B., Kumar, V., Yadav, A.N., 2020. Phytases from microbes in phosphorus acquisition for plant growth promotion and soil health, in: *New and Future Developments in Microbial Biotechnology and Bioengineering.* Elsevier, pp. 157–176.
- Kour, D., Rana, K.L., Kaur, T., Yadav, N., Yadav, A.N., Kumar, M., Kumar, V., Dhaliwal, H.S., Saxena, A.K., 2021. Biodiversity, current developments and potential biotechnological applications of phosphorus-solubilizing and -mobilizing microbes: A review. *Pedosphere* 31, 43–75. [https://doi.org/10.1016/S1002-0160\(20\)60057-1](https://doi.org/10.1016/S1002-0160(20)60057-1)
- Li, H.P., Han, Q.Q., Liu, Q.M., Gan, Y.N., Rensing, C., Rivera, W.L., Zhao, Q., Zhang, J.L., 2023. Roles of phosphate-solubilizing bacteria in mediating soil legacy phosphorus availability. *Microbiol. Res.* 272, 127375. <https://doi.org/10.1016/j.micres.2023.127375>
- Lindsay, W.L., 1979. *Chemical equilibria in soils.* John Wiley and Sons Ltd.
- Lu, C., Tian, H., 2017. Global nitrogen and phosphorus fertilizer use for agriculture production in the past half century: shifted hot spots and nutrient imbalance. *Earth Syst. Sci. Data* 9, 181–192. <https://doi.org/10.5194/essd-9-181-2017>
- Mallarino, A.P., Blackmer, A.M., 1992. Comparison of methods for determining critical concentrations of soil test phosphorus for corn. *Agron. J.* 84, 850–856.
- Manzoor, A., Dippold, M.A., Loepmann, S., Blagodatskaya, E., 2022. Two-phase conceptual framework of phosphatase activity and phosphorus bioavailability. *Front. Plant Sci.* 13, 935829.
- Mehta, S., Nautiyal, C.S., 2001. An Efficient Method for Qualitative Screening of Phosphate-Solubilizing Bacteria. *Curr. Microbiol.* 43, 51–56. <https://doi.org/10.1007/s002840010259>
- Menezes-Blackburn, D., Paredes, C., Zhang, H., Giles, C.D., Darch, T., Stutter, M., George, T.S., Shand, C., Lumsdon, D., Cooper, P., 2016. Organic acids regulation of chemical–microbial phosphorus transformations in soils. *Environ. Sci. Technol.* 50, 11521–11531.
- Mogollón, J. M., A. H. W. Beusen, H. J. M. van Grinsven, H. Westhoek, and A. F. Bouwman. (2018). Future agricultural phosphorus demand according to the shared socioeconomic pathways. *Global Environmental Change* 50:149-163.

- Nidhin, I.K., Chattopadhyay, I., 2022. 2 Emerging technologies in environmental microbiology. *Environ. Microbiol. Emerg. Technol.* 33.
- Ohtake, H., Wu, H., Imazu, K., Anbe, Y., Kato, J., Kuroda, A., 1996. Bacterial phosphonate degradation, phosphite oxidation and polyphosphate accumulation. *Resour. Conserv. Recycl.* 18, 125–134.
- Ortiz-Medina, J.F., Grunden, A.M., Hyman, M.R., Call, D.F., 2019. Nitrogen gas fixation and conversion to ammonium using microbial electrolysis cells. *ACS Sustain. Chem. Eng.* 7, 3511–3519.
- Osorio, N.W., Habte, M., 2013. Phosphate desorption from the surface of soil mineral particles by a phosphate-solubilizing fungus. *Biol. Fertil. Soils* 49, 481–486. <https://doi.org/10.1007/s00374-012-0763-5>
- Pastore, G., Kaiser, K., Kernchen, S., Spohn, M., 2020. Microbial release of apatite- and goethite-bound phosphate in acidic forest soils. *Geoderma* 370, 114360. <https://doi.org/https://doi.org/10.1016/j.geoderma.2020.114360>
- Pavinato, P.S., Cherubin, M.R., Soltangheisi, A., Rocha, G.C., Chadwick, D.R., Jones, D.L., 2020. Revealing soil legacy phosphorus to promote sustainable agriculture in Brazil. *Sci. Rep.* 1–11. <https://doi.org/10.1038/s41598-020-72302-1>
- Pikovskaya R.I. Mobilization of phosphorus in soil in connection with vital activity of some microbial species (1948). *Microbiology.* 17:362–370.
- Poirier, Y., Jaskolowski, A., e Clúa, J. (2022). Phosphate acquisition and metabolism in plants. *Current Biology*, 32(12), R623-R629.
- Prabhu, N., Borkar, S., Garg, S., 2019. Chapter 11 - Phosphate solubilization by microorganisms: Overview, mechanisms, applications and advances, in: Meena, S.N., Naik, M.M. (Eds.), *Advances in Biological Science Research*. Academic Press, pp. 161–176. <https://doi.org/https://doi.org/10.1016/B978-0-12-817497-5.00011-2>
- Pradhan, N., Sukla, L.B., 2006. Solubilization of inorganic phosphates by fungi isolated from agriculture soil. *African J. Biotechnol.* 5.
- Rawat, P., Das, S., Shankhdhar, D., Shankhdhar, S.C., 2021. Phosphate-solubilizing microorganisms: mechanism and their role in phosphate solubilization and uptake. *J. Soil Sci. Plant Nutr.* 21, 49–68.
- Řezanka, T., Palyzová, A., Faltýsková, H., Sigler, K., 2019. Siderophores: amazing metabolites of microorganisms. *Stud. Nat. Prod. Chem.* 60, 157–188.
- Rizwanuddin, S., Kumar, Vijay, Singh, P., Naik, B., Mishra, S., Chauhan, M., Saris, P.E.J., Verma, A., Kumar, Vivek, 2023. Insight into phytase-producing microorganisms for phytate solubilization and soil sustainability. *Front. Microbiol.* 14, 1127249.
- Roos, W., Luckner, M., 1984. Relationships between proton extrusion and fluxes of ammonium ions and organic acids in *Penicillium cyclopium*. *Microbiology* 130, 1007–1014.
- Ruttenberg, K.C., 2001. Phosphorus cycle. *Encycl. Ocean Sci.* 4, 401–412.
- Sadaf, N., Haider, M.Z., Iqbal, N., Abualreesh, M.H., Alatawi, A., 2022. Harnessing the Phytase production potential of soil-borne fungi from wastewater irrigated fields based on eco-cultural optimization under shake flask method. *Agriculture* 12, 103.
- Saha, M., Sarkar, S., Sarkar, B., Sharma, B.K., Bhattacharjee, S., Tribedi, P., 2016. Microbial siderophores and their potential applications: a review. *Environ. Sci. Pollut. Res.* 23, 3984–3999.
- Sand, W., 1997. Microbial mechanisms of deterioration of inorganic substrates—a general mechanistic overview. *Int. Biodeterior. Biodegradation* 40, 183–190.
- Scavino, A.F., Pedraza, R.O., 2013. The role of siderophores in plant growth-promoting bacteria, in: *Bacteria in Agrobiolgy: Crop Productivity*. Springer, pp. 265–285.
- Sharma, S., Kumar, V., Tripathi, R.B., 2011. Isolation of phosphate solubilizing microorganism (PSMs) from soil. *J. Microbiol. Biotechnol. Res.* 1, 90–95.
- Sharma, S.B., Sayyed, R.Z., Trivedi, M.H., Gobi, T.A., 2013. Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. *Springerplus* 2, 1–14.
- Sindhu, S.S., Phour, M., Choudhary, S.R., Chaudhary, D., 2014. Phosphorus cycling:

- prospects of using rhizosphere microorganisms for improving phosphorus nutrition of plants. *Geomicrobiol. Biogeochem.* 199–237.
- Singh, B., Satyanarayana, T., 2011. Microbial phytases in phosphorus acquisition and plant growth promotion. *Physiol. Mol. Biol. Plants* 17, 93–103.
- Sisti, L.S., Flores-Borges, D.N.A., Andrade, S.A.L. de, Koehler, S., Bonatelli, M.L., Mayer, J.L.S., 2019. The role of non-mycorrhizal fungi in germination of the mycoheterotrophic orchid *pogoniopsis schenckii* cogn. *Front. Plant Sci.* 10, 1589.
- Soares, E. V., 2022. Perspective on the biotechnological production of bacterial siderophores and their use. *Appl. Microbiol. Biotechnol.* 106, 3985–4004.
- Solangi, F., Zhu, X., Khan, S., Rais, N., Majeed, A., Sabir, M.A., Iqbal, R., Ali, S., Hafeez, A., Ali, B., 2023. The Global Dilemma of Soil Legacy Phosphorus and Its Improvement Strategies under Recent Changes in Agro-Ecosystem Sustainability. *ACS omega* 8, 23271–23282.
- Stewart, J.W.B., Tiessen, H., 1987. Dynamics of soil organic phosphorus. *Biogeochemistry* 4, 41–60. <https://doi.org/10.1007/BF02187361>
- Stumm, W., 1995. The inner-sphere surface complex: A key to understanding surface reactivity. ACS Publications.
- Syers, J.K., Johnston, A.E., Curtin, D., 2008. Efficiency of soil and fertilizer phosphorus use. *FAO Fertil. plant Nutr. Bull.* 18, 5–50.
- Tamburini, F., Pfahler, V., Bünemann, E.K., Guelland, K., Bernasconi, S.M., Frossard, E., 2012. Oxygen isotopes unravel the role of microorganisms in phosphate cycling in soils. *Environ. Sci. Technol.* 46, 5956–5962.
- TAO, G.-C., TIAN, S.-J., CAI, M.-Y., XIE, G.-H., 2008. Phosphate-Solubilizing and -Mineralizing Abilities of Bacteria Isolated from Soils. *Pedosphere* 18, 515–523. [https://doi.org/https://doi.org/10.1016/S1002-0160\(08\)60042-9](https://doi.org/https://doi.org/10.1016/S1002-0160(08)60042-9)
- Tian, J., Ge, F., Zhang, D., Deng, S., Liu, X., 2021. Roles of phosphate solubilizing microorganisms from managing soil phosphorus deficiency to mediating biogeochemical P cycle. *Biology (Basel)*. 10, 158.
- Tiessen, H., 2008. Phosphorus in the global environment 1–7. https://doi.org/10.1007/978-1-4020-8435-5_1
- Tiessen, H., Stewart, J.W.B., Cole, C. V., 1984. Pathways of Phosphorus Transformations in Soils of Differing Pedogenesis. *Soil Sci. Soc. Am. J.* 48, 853–858. <https://doi.org/10.2136/sssaj1984.03615995004800040031x>
- Wang, C., Pan, G., Lu, X., Qi, W., 2023. Phosphorus solubilizing microorganisms: potential promoters of agricultural and environmental engineering. *Front. Bioeng. Biotechnol.* 11, 1–5. <https://doi.org/10.3389/fbioe.2023.1181078>
- Wang, X., Phillips, B.L., Boily, J.-F., Hu, Y., Hu, Z., Yang, P., Feng, X., Xu, W., Zhu, M., 2019. Phosphate sorption speciation and precipitation mechanisms on amorphous aluminum hydroxide. *Soil Syst.* 3, 20.
- Webb, E.C., 1992. Enzyme nomenclature 1992. Recommendations of the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology on the Nomenclature and Classification of Enzymes. Academic Press.
- Wild, A. (1988). Plant nutrients in soil: phosphate. *Russell's soil conditions and plant growth*. Eleventh edition, 695-742.
- Withers, P.J.A., Rodrigues, M., Soltangheisi, A., De Carvalho, T.S., Guilherme, L.R.G., Benites, V.D.M., Gatiboni, L.C., De Sousa, D.M.G., Nunes, R.D.S., Rosolem, C.A., Andreote, F.D., Oliveira, A. De, Coutinho, E.L.M., Pavinato, P.S., 2018. Transitions to sustainable management of phosphorus in Brazilian agriculture. *Sci. Rep.* 8, 1–13. <https://doi.org/10.1038/s41598-018-20887-z>
- Yadav, A.N., 2022. Phosphate-solubilizing microorganisms for agricultural sustainability. *J. Appl. Biol. Biotechnol.* 10, 1–6.
- Yadav, A.N., Yadav, N., Sachan, S.G., Saxena, A.K., 2019. Biodiversity of psychrotrophic microbes and their biotechnological applications. *J. Appl. Biol. Biotechnol.* 7, 99–108.
- Yang, L., Liu, Y., Cao, X., Zhou, Z., Wang, S., Xiao, J., Song, C., Zhou, Y., 2017. Community

composition specificity and potential role of phosphorus solubilizing bacteria attached on the different bloom-forming cyanobacteria. *Microbiol. Res.* 205, 59–65.

3 POTENTIALLY PHOSPHATE-SOLUBILIZING MICROORGANISMS ASSOCIATED WITH DIFFERENT PHOSPHATE SOURCES

Abstract

Phosphorus is essential for plants, but its scarcity in soil requires fertilizers. Growing demand and concerns about finite resources underscore the need for efficient P use. Thus, employing phosphate-solubilizing microorganisms associated with phosphate fertilization may enhance P use efficiency. This study aimed to evaluate the performance of phosphate source-microorganism associations on plant nutritional absorption and development. Effects on P fractions and microbial communities were analyzed, including leaf nutrient analysis, soil chemical analysis, P fractionation (labile P, moderately labile P, non-labile P), 16s rRNA, beta-glucosidase, acid phosphatase, ITS, P biomass, and PhoD. The experiment, conducted at Luiz de Queiroz College of Agriculture (ESALQ-USP) in Piracicaba, São Paulo, Brazil, encompassed two scenarios: residual fertilization management and fresh fertilization management, both subjected to inoculation (with and without inoculation of isolate *Bacillus pumilus*). Employing phosphate-solubilizing microorganisms holds promise for enhancing plant uptake of phosphate fertilizers. However, no positive changes in soil P fractions were observed post-bacterial inoculation as anticipated, necessitating further studies to assess this interaction. Nevertheless, an increase in the non-labile P fraction was observed in the reactive rock phosphate (RRP) and triple superphosphate (TSP) treatments, indicating bacterial activity in rock degradation processes in both fresh and residual scenarios.

Keywords: Fertilizing. Phosphorus. RRP. TSP. *Bacillus*.

3.1 Introduction

Phosphorus (P) is an essential element for plants and consequently a limiting factor for agricultural production. Its unavailability is a characteristic feature of tropical soils due to the characteristics derived from intense weathering and acidity in these soils (Alovisi, 2020). P can exist in inorganic (Pi) and organic (Po) forms, and its transformation into soluble forms is often mediated by soil microbiota (Rheinheimer et al., 2020).

Due to the limited availability of P in the soil for plants, the use of phosphate fertilizers is necessary to meet these nutritional requirements. Concerns about this finite resource, coupled with the increasing demand and pressure for high agricultural productivity, draw attention to alternatives for the efficient use of P (Alori, Glick, and Babalola, 2017). Thus, an alternative approach involves the use of phosphorus-solubilizing microorganisms (Osorio, 2011; Guelfi et al., 2022).

The primary mechanisms employed by microorganisms to make P available are related to the production of organic and inorganic acids and enzymatic exudation, transforming phosphate present in the environment into readily available assimilable forms (Kalayu, 2019). Furthermore, the production of acids and the concomitant reduction in soil pH can favor increased P fixation by increasing positive charges in the exchange complex on the mineral surface of soil particles (Shibata et al., 2003).

Microorganisms temporarily break down part of the P in their cells (microbial biomass P), releasing some of it through mineralization. Microbial P can act as a reserve for organic P, preventing the adsorption process, immobilizing P temporarily, and avoiding adsorption to inorganic soil colloids. Part of the released P associates with active and passive fractions of soil organic matter (SOM), subject to storage and future release. These organic forms are also slowly mineralized, releasing phosphate ions that can be absorbed by roots, thus repeating the cycle (Selvi et al., 2017). The rate of phosphate release from microbial biomass depends on the amount of available P in the soil (Spohn and Widdig, 2017), C availability (Malik, 2012), soil texture (Chen and He, 2002), and microbial community composition (Dinh et al., 2017).

Studies conducted by Vieira et al. (2015) and Abreu et al. (2017) demonstrated that bacteria found in the maize rhizosphere can solubilize P. Among P-solubilizing bacteria, *Arthrobacter* and *Streptomyces* have been proven effective in promoting maize plant growth (Araújo et al., 2023).

Maize (*Zea mays*) cultivation is widely practiced in Brazil, being a major economic segment of Brazilian agribusiness and the second most cultivated and exported grain (after soybeans). It is extensively used in animal feed production (mainly for the meat production chain) and exported for biofuel production (Almeida, 2019; Abimilho, 2022).

To achieve the desired productivity, the use of phosphate fertilizers is necessary, and according to data from the 2021/22 harvest, with a production of 113,133.6 thousand tons, there was an average demand of 4.6 kg of P ton⁻¹ of grain (Conab, 2022). Based on these data, to connect high cereal productivity, high P demand by plants, and food security, alternatives and management tools are needed to increase the efficiency of P use, considering potential and production needs.

Considering the above listed issues, the use of P-solubilizing bacteria associated with natural phosphate fertilization increases the efficiency of P use compared to soluble phosphate application. Thus, the objective of this study was to evaluate the performance of the association between phosphate sources and potentially phosphate-solubilizing microorganisms to assess the effects of this association and the nutritional uptake and development of plants. In addition, we analyzed the treatments effects upon P fractions and upon microbial communities (represented by leaf nutrient analysis, soil chemical analysis, P fractionation (labile P,

moderate labile P, non-labile P), 16s.rRNA, Beta-glucosidase, acid phosphatase, ITS, P biomass and PhoD).

3.2 Materials and methods

3.2.1 Characteristics and experimental design

The experiment was conducted in a greenhouse located at the Luiz de Queiroz College of Agriculture (ESALQ-USP) in Piracicaba, São Paulo, Brazil (22°42'S, 47°38'W), from March 2021 to April 2021. The soil used for this experiment was collected from the experimental area of the Cerrados Agricultural Research Center (Embrapa Cerrados) in Planaltina, DF (latitude 15°36'06"S and longitude 47°42'26"W), at an altitude of 1,014 m, and with a Cwa climate (according to the Köppen classification). The soil is classified as Dystrophic Red Latosol according to the Brazilian Soil Classification System (Embrapa, 2013), characterized by good permeability, high clay content (64% clay), and low nutrient levels.

This soil has a history of long-term experiments (already concluded) conducted over 25 years in a no-till system (SSD), with alternating crops of soybeans and corn in the summer and legumes (mucuna/guandu/crotalaria) in the winter. Over these decades, P was annually applied as topdressing, fertilized with reactive natural phosphate (RRP) or triple superphosphate (TSP), at a rate of 80 kg ha⁻¹ of P₂O₅ per year. The RRP contained 28.2% total P₂O₅ (44% soluble in a 2% citric acid solution), and the TSP contained 47.6% total P₂O₅ (92% soluble in a 2% citric acid solution). Consequently, the soil retains residues from the fertilizers and, thus, a P stock. Table 1 presents the soil analysis before the installation of the experiment.

Table 1. Soil analysis before installing the experiment in a greenhouse.

	pH CaCl ₂	P ----- mg dm ⁻³	K ----- mg dm ⁻³	S ----- mg dm ⁻³	Ca ----- mg dm ⁻³	Mg ----- mg dm ⁻³	Al ----- cmol _c dm ⁻³	H+Al ----- cmol _c dm ⁻³	SB ----- cmol _c dm ⁻³	CTC ----- cmol _c dm ⁻³
Soil managed with TSP	4,4	1,9	79	9	1,6	0,6	<1	6,8	2	8,8
Soil managed with RRP	4,4	3,7	85	14	1,7	0,4	<1	6,4	2	8,4

The soil was amended throughout its volume with calcium carbonate and magnesium oxide, in addition to the application of potassium chloride and ammonium nitrate, following the recommendations for maize cultivation. It was incubated for seven days with moisture above

70% of field capacity (FC) to restore nutrient balance in the solution and, simultaneously, natural microorganisms. The test crop for this trial was maize (*Zea mays*, cultivar AL ALVARE).

The greenhouse experiment consisted of two scenarios (Figure 1). The residual fertilization management scenario involved the historical fertilization practices (managed with TSP or RRP), while the fresh fertilization management scenario involved the fresh application of fertilizer (TSP or RRP). Both scenarios were subjected to the inoculation factor (with and without inoculation of the isolate ATCC 7061 classified as *Bacillus pumilus*) (Compaction, 2012).

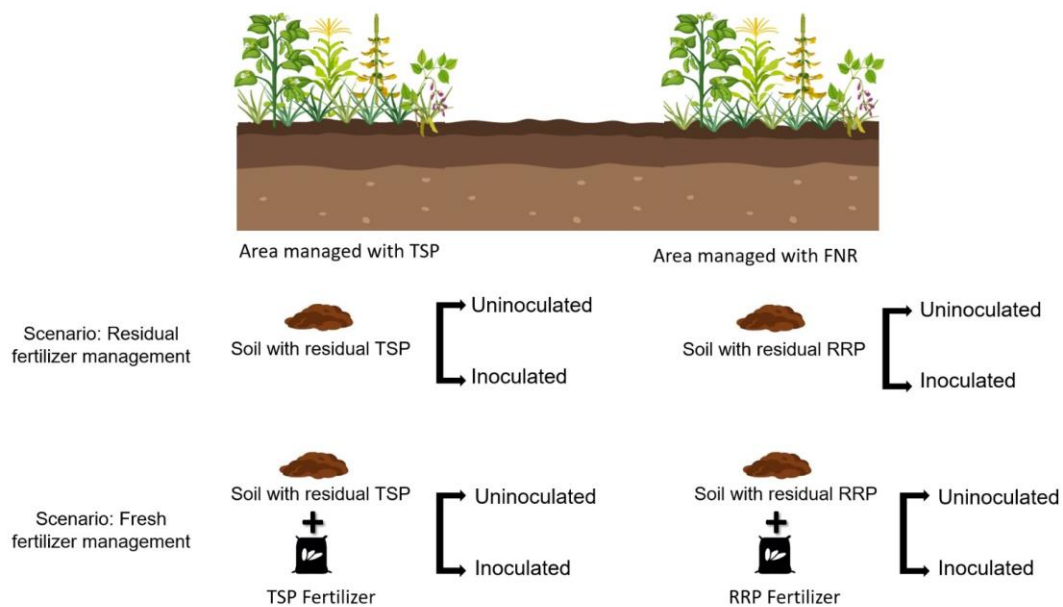


Figure 1. Design of the scenarios studied during the experiment.

In each scenario, the experimental design followed a randomized complete block design in a factorial scheme (2×2). The first factor was P management (triple superphosphate – TSP or reactive natural phosphate - RRP), and the second factor was inoculation (with or without inoculation). For each scenario, treatments were implemented in four blocks, resulting in 16 experimental units for each scenario ($2 \text{ P sources} \times 2 \text{ inoculation modes} \times 4 \text{ blocks}$), totaling 32 experimental units. The experimental units consisted of 4 L capacity pots filled with 3 kg of air-dried soil, which had already been corrected. After soil incubation, the treatments were applied.

In the residual fertilization management scenario, after soil correction, it only received the inoculation factor. Meanwhile, in the fresh fertilization management scenario, a fresh dose of fertilizer was applied (according to the initial soil management, TSP, or RRP). To ensure uniform application throughout the pot volume, phosphate fertilizers were ground and sifted

through a 0.250 mm mesh (60 mesh), provided to the soil in a powder/granular form, and thoroughly mixed (using a concrete mixer). The soil managed for years with TSP received a dose of 100 mg kg⁻¹ P (TSP source), and the soil managed with RRP received a dose of 100 mg kg⁻¹ P (RRP source).

The microorganism utilized in this experiment, a potentially phosphorus-solubilizing bacterium (*Bacillus pumilus*), is part of the isolates collection at the Soil Microbiology Laboratory of ESALQ/USP. It was cultivated in a 10% TSB liquid medium (Trypticase Soy Broth) supplemented with 10 g L⁻¹ of dextrose, 5 g L⁻¹ of yeast extract, and 5 mg of tryptophan (1000 µg mL⁻¹), maintained at 28 °C, in the dark, and under constant agitation for 24 hours. After this period, the suspension was centrifuged for 5 min at 6500g at 10 °C to obtain the cells pellet. The cell mass was adjusted to a concentration of 10⁹ CFU/mL, measured by spectrophotometry (OD550 = 0.5). This inoculum was subsequently used to inoculate corn seeds at the time of sowing in the pots.

Corn seeds were disinfected using the following procedure: immersion in 70% ethanol for 3 min, followed by submersion in hypochlorite for 7 min, immersed again in 70% ethanol for 1 min, and finally rinsed several times with autoclaved distilled water. After disinfection, seeds that would receive inoculation were separated from those that would not; thus, one part of the seeds was immersed in the *B. pumilus* suspension, and the other part was immersed in water, both for 2 hours.

Five corn seeds were sown per pot at a depth of 3 cm. Ten days after planting, when the seedlings were well-established, thinning was performed, leaving only two seedlings per pot. During the experiment, the pots were manually irrigated with deionized water to maintain 70% of field capacity humidity. The seedlings received Hoagland & Arnon's (1950) nutrient solution without the addition of P sources, according to their needs. The temperature and relative humidity were monitored during the experiment, ranging from 26-32 °C and 50-80%, respectively.

3.2.2 Analyzes performed

The experiment lasted for forty-five days after sowing. At the conclusion of the experiment, soil and plant samples were collected for analysis. All analyses were conducted in the Soil Analysis and Soil Microbiology Laboratories at the Luiz de Queiroz College of Agriculture (ESALQ/USP).

The aboveground plant material was collected and subjected to drying in a forced air circulation oven at 65 °C for approximately 72 hours for subsequent analysis of dry mass production and plant nutritional content. Following weighing, the material underwent leaf

grinding using a Wiley mill equipped with a 1 mm sieve. From these samples, foliar contents of N, P, K, Ca, Mg, and S were determined according to the methodology described by Malavolta, Vitti, and Oliveira (1997). Root material was collected, washed with deionized water, and subjected to drying in a forced air circulation oven at 65 °C for about 72 hours, followed by obtaining the root dry mass.

For fertility assessments, the soils were collected and air-dried. The contents of P, K, Ca, and Mg were determined, extracted with anionic and cationic exchange resins. Organic matter (OM) was determined by colorimetry using potassium dichromate; pH in CaCl₂ (1:2.5 soil:solution ratio); H + Al (correlated to the SMP index); and cation exchange capacity (V%) was mathematically determined according to Raij et al. (2001).

The P fractionation, proposed by Hedley et al. (1982) with modifications by Gatiboni et al. (2007), is a widely used methodology in studies of P dynamics in agricultural systems. The quantified P fractions were grouped based on the predicted P lability by the extractors. Labile P consists of PiRTA, combined with PBIC (Pi and Po); moderately labile P consists of PHID-0.1 (Pi and Po) added to PiHCl, and non-labile P comprises the sum of PHID-0.5 (Pi and Po) and RESIDUAL.

For microbial community analyses, soil samples were collected and stored in thermal boxes with ice. For the determination of acid phosphatase and beta-glucosidase enzymes and microbial P quantification, the samples were stored for two days in a cold chamber at 4 °C. The soil collected for DNA extraction and subsequent microbial community quantification by quantitative real-time PCR (qPCR) and the evaluation of the microbial community structure by Terminal Restriction Fragment Length Polymorphism (T-RFLP) was stored in Eppendorf tubes and kept refrigerated at -20 °C.

Soil microbial activity was assessed through the enzymes β -glucosidase and acid phosphatase following the method proposed by Tabatabai (1994). Briefly, 1.0 g of fresh soil was added to 10 mL test tubes, where 2 mL of MUB buffer solution (pH 6 for β -glucosidase and pH 5 for acid phosphatase, respectively) was added. The solutions containing soil were stirred and incubated (37 °C, 1 h) with 0.05 M p-nitrophenyl buffer solution (p-nitrophenyl- β -D-glucopyranoside for β -glucosidase and p-nitrophenyl phosphate for acid phosphatase, respectively). The enzymatic activity was determined based on the colorimetric determination (400 nm) of p-nitrophenol released. The P content in the microbial biomass was determined using the method proposed by Hedley and Stewart (1982), which involves extracting the available Pi with resin before the fumigation process and extraction with NaHCO₃ (Brookes et al., 1981), and adjusting the soil adsorption capacity (Morel et al., 1996).

The total DNA from soil samples was extracted using the Power Soil DNA Isolation Kit (Qiagen, Hilden, Germany) based on 0.25 g, following the manufacturer's instructions. Briefly,

the soil sample was homogenized, and the microbial cells were lysed using mechanical and chemical methods. After that, the total genomic DNA was captured on a silica membrane in a spin column format, washed, and eluted from the membrane to obtain the extract. Additionally, the DNA concentration was measured using the Qubit fluorometer (Invitrogen, Carlsbad, USA) according to the manufacturer's protocol. Finally, the DNA quality was checked through 1% sodium boric acid agarose gel electrophoresis analysis (Brody and Kern 2004).

Terminal Restriction Fragment Length Polymorphism (T-RFLP) analysis was used to determine variations in the bacterial community structure in all samples. The 16S rRNA gene was amplified with primers 8-FM (5' - AGA CTT TGA TCM TGG CTC AG - 3') labeled with 6-carboxyfluorescein (6-FAM) and 926r (5' - CCG TCA ATT CCT TTR AGT TT - 3') (Schütte et al., 2009). The thermal cycling conditions were 95°C for 4 min (initial denaturation), followed by 30 cycles of 95°C for 30 s, 57°C for 30 s, and 72°C for 45 s, with a final extension step at 72°C for 10 min. The final volume of the reactions was 50 µL, containing 6 µL of MgCl₂ (final concentration of MgCl₂ 3 mM), 0.2 µL (1 U) of Taq polymerase, 5 µL of Mg-free buffer (Sinapse Inc, São Paulo, Brazil), 4 µL of deoxynucleotide triphosphate (dNTP) mix (0.2 mM of each nitrogenous base) (Invitrogen Corporation, Carlsbad, CA, USA), 0.1 µL of each primer (0.01 mM), and 1 µL of DNA template, and 33.6 µL of ultrapure water. The amplification products were digested with HhaI endonuclease (5 U) (Invitrogen Corporation, Carlsbad, CA, USA), precipitated with 125 mM EDTA and 3 M sodium acetate. Then, the amplification products were resuspended using Hi-Di™ formamide (Applied Biosystems, Life Technologies) with GeneScan™ 1200 LIZ® size standard (Applied Biosystems, Life Technologies) and analyzed on an ABI Prism 3500 automatic sequencer (Applied Biosystems, Life Technologies).

The T-RFLP profiles were analyzed using GeneMapper® 4.1 (Applied Biosystems, Life Technologies) and then subjected to two-dimensional non-metric multidimensional scaling (NMDS) using Primer 5. Differences between treatments were assessed through analysis of similarity (ANOSIM) using the Paleontological Statistics freeware package (PASTv.3 - Hammer et al. (Hammer; Harper; Rayan, 2001)).

The absolute abundance of bacterial communities (represented by the 16S rRNA marker gene), total fungi (ITS), and phosphate-solubilizing bacteria (phoD) was quantified through real-time quantitative PCR (qPCR) analysis using the StepOnePlus™ system (Applied Biosystems, Foster City, CA, USA). Each gene was amplified in 20 µL final volume reactions with 10 µL SYBR Green/ROX qPCR Master Mix (2x) (Abgene, Epsom, United Kingdom), 0.5 µL of each primer (10 µM), 0.3 µL bovine serum albumin (BSA, 10 mg mL⁻¹), and 1 µL DNA (approximately 10 ng). Primers and reaction conditions are presented in Table 2.

Reactions were performed in duplicate, and two negative controls were added in all quantifications to monitor possible contaminations. Standard curves were obtained using serial dilutions (10^1 to 10^8) of model DNA amplicons with known copy numbers. All amplification reactions showed efficiency values between 92% and 110%, with R2 values of standard curves always exceeding 0.99. The results were analyzed using StepOnePlus™ Real-Time version 2.2.2 software (Applied Biosystems, Foster City, CA, USA).

Table 2. Primers and cycling conditions used to amplify target genes.

qPCR primers	Primer sequence (5'-3')	Thermal cycling conditions
Total bacteria (16S rRNA)		
341f ¹	CCT-ACG-GGA-GGC-AGC-AG	95°C 10 min, 1 cycle; 94°C 30 s, 55°C 30 s, 72°C 30 s, 35 cycles
518r ¹	ATT-ACC-GCG-GCT-GCT-GG	
Total fungi (ITS1)		
ITS1F ² 5.8S ³	CTT-GGT-CAT-TTA-GAG-GAA-GTA-A CGC- TGC-GTT-CTT-CAT-CG	94°C 15 min, 1 cycle; 94°C 1 min, 53°C 30 s, 72°C 1 min, 40 cycles
Gene <i>phoD</i>		
ALPS-F730 ⁴	CAG-TGG-GAC-GACCAC-GAG-GT GAG-GCC-GAT- CGG-CAT-GTC-G	95 °C, 10 min
ALPS-R1101 ⁴		95 °C, 40 cycles de 30 s 57 °C, 1 min 72 °C, 30 s

¹Muyzer et al. (1993); ²Gardes and Bruns (1993); ³Fierer et al. (2005); ⁴Sakurai et al. (2008)

3.2.3 Analyze statistical data

The data were analyzed in a randomized block design with a factorial scheme (2x2), where the first factor was the P source (TSP and FNR), and the second factor was the inoculation (with and without inoculation) in the two studied scenarios (residual fertilization management and fresh fertilization management). The data were subjected to normality testing using the Shapiro-Wilk test, followed by variance homoscedasticity testing using the Bartlett test. Once the criteria (normality and homoscedasticity) for analysis of variance (ANOVA) were met, and a significant F value was obtained, a multiple comparison test (Tukey's test at 5% and 10%) was performed using the ExpDes statistical package in the R® program (R CORE TEAM, 2017).

The analysis of P fractions (Hedley) was conducted in a double factorial scheme for each scenario. Additionally, a post-hoc statistical test was applied for mean comparisons using the Tukey test at a 5% probability.

For the T-RFLP data analysis, we calculated the relative abundance of peak areas for terminal restriction fragments (T-RFs). The relative abundance was defined as the percentage of each T-RF in the sum of all peak areas in the T-RFLP profile. Differences between T-RFLP profiles were confirmed through Analysis of Dissimilarity (ANOSIM) and visualized using Principal Coordinates Analysis (PCoA) with the statistical package Vegan in the R® program (R CORE TEAM, 2017).

The qPCR data were analyzed through ANOVA and mean tests. In the distance-based redundancy analysis (dbRDA), T-RFLP profiles served as response variables, while explanatory variables included enzymatic activities, gene abundance, and chemical data obtained by Lopes (2017). Differences were confirmed through the ADONIS test using the statistical package Vegan in the R® program (R CORE TEAM, 2017)."

3.3 Results

3.3.1 Root and aboveground biomass

In the fresh fertilization scenario (Figure 1A), a difference was observed between the P sources, such as TSP, resulting in an increase in shoot biomass to 14.5 g when uninoculated and 16.5 g when inoculated. As for root biomass, the TSP treatment led to an increase of 16 g when uninoculated and 21.3 g when inoculated.

In the residual scenario (Figure 1B), there was an effect on the fertilizer source, with RRP showing lower values for root biomass (15 g) when compared to TSP in both uninoculated and inoculated cases. No differences were observed for shoot biomass between the fertilizer sources and inoculation.

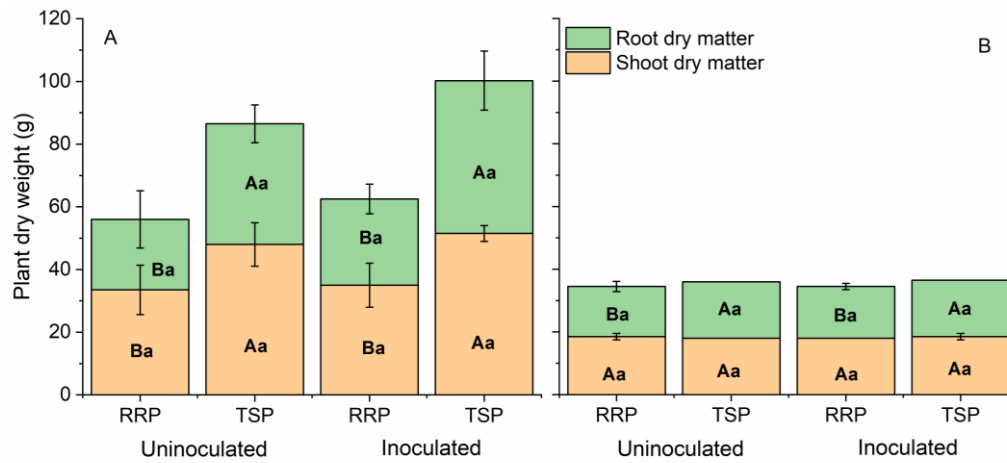


Figure 1: Aboveground and root biomass of corn plants in scenarios (A) Fresh fertilization management and (B) Residual fertilization management. Lowercase letters compare difference between inoculation factors within the same P source, whilst uppercase letters compare difference between P sources within the same inoculation level by the Tukey test at 5% ($p \leq 0.05$). TSP: triple superphosphate, RRP: reactive rock phosphate

3.3.2 Nutrients uptake accumulation

In both scenarios, there was no effect of inoculation on the nutrients accumulated in the plants. For residual fertilization management, concerning the P sources, it is noted that TSP was superior to RRP only in the S content (a significant difference of $4.6 \text{ g S plant}^{-1}$).

As for fresh fertilization management, overall, the soluble source (TSP) was superior to the reactive source (RRP) in all analyzed parameters. Regarding the accumulated N content, TSP ($1314 \text{ g N plant}^{-1}$) was superior to RRP ($982 \text{ g N plant}^{-1}$), showing a difference of $331 \text{ g N plant}^{-1}$. The same pattern was observed for the accumulated S content, with a difference of $25 \text{ g S plant}^{-1}$ from TSP to RRP (Figure 2A and F). Concerning the accumulated P content in the plant, TSP ($88 \text{ g P plant}^{-1}$) was superior to RRP ($57 \text{ g P plant}^{-1}$), with an approximate difference of $30 \text{ g P plant}^{-1}$ (Figure 2B).

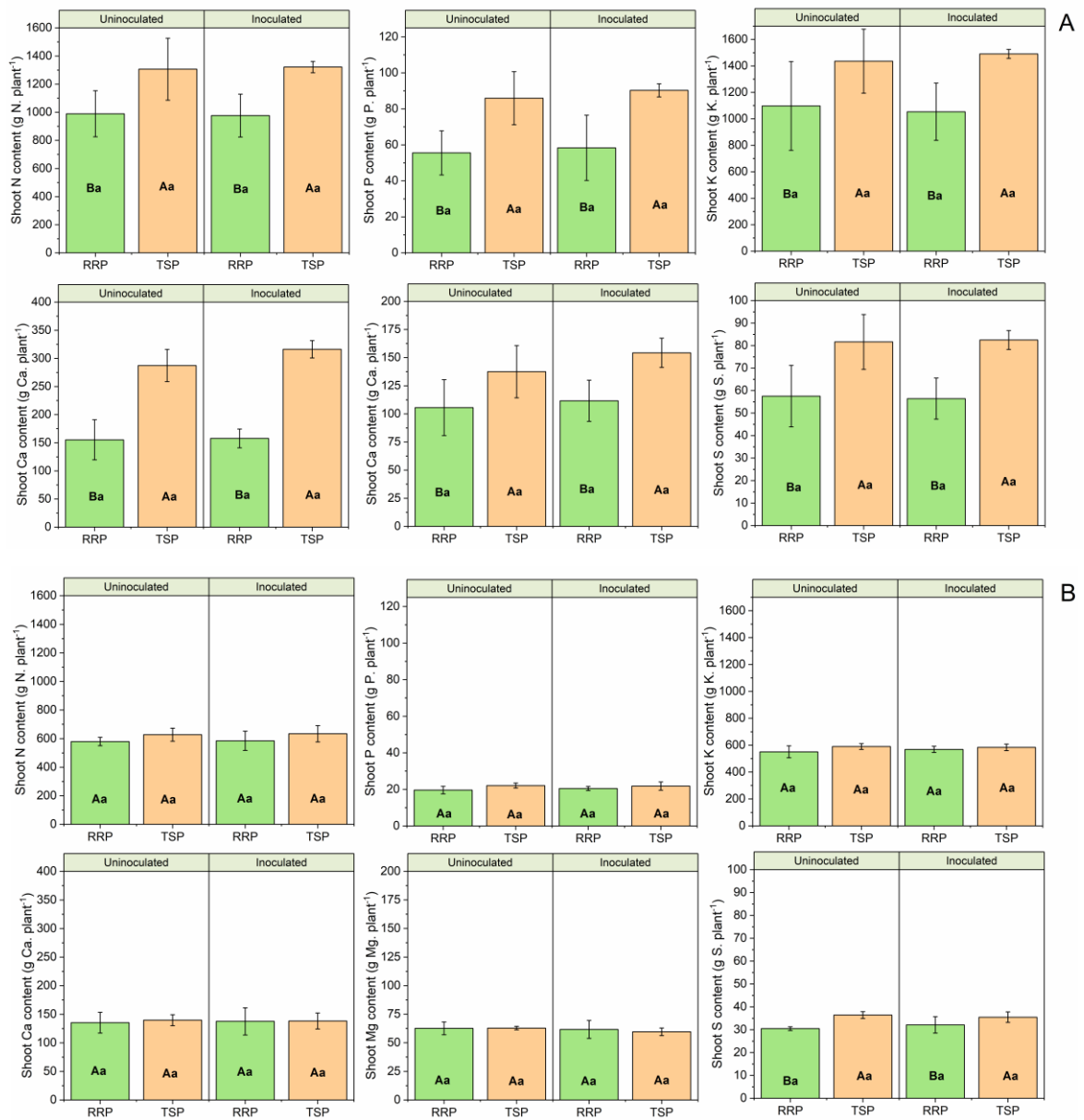


Figure 2. Nutrients uptake accumulated in (A) Fresh and (B) residual scenario. Lowercase letters compare difference between inoculation factors within the same P source, whilst uppercase letters compare difference between P sources within the same inoculation level by the Tukey test at 5% ($p \leq 0.05$). TSP: triple superphosphate, RRP: reactive rock phosphate.

3.3.3 Soil chemical attributes

In both scenarios studied, there was no effect of inoculation on soil chemical attributes. In the residual fertilization management, for the parameters pH, SOM, Ca, Mg, SB, and V%, RRP was superior to TSP. Under the conditions evaluated during the trial, no differences were observed for the parameters P, K, H+Al, and CEC.

On the other hand, for fresh fertilization management, there was a significant effect of P sources, with a similar pattern for both P and Mg. RRP was superior to TSP, exhibiting higher quantities of P and Mg in the soil, nearly double the amount of these nutrients in the soil. For the other analyzed attributes, there was no significant effect of P sources.

Table 3. Effect of P sources (RRP: reactive natural phosphate and TSP: triple superphosphate) and bacterial inoculation on soil chemical attributes in the two scenarios studied.

P Sources Bacterial Inoculation	Residual fertilizer scenario				Fresh fertilizer scenario			
	RRP		TSP		RRP		TSP	
	Inoculated	Uninoculated	Inoculated	Uninoculated	Inoculated	Uninoculated	Inoculated	Uninoculated
pH (CaCl ₂)	4.8 ± 0.2 Aa	4.9 ± 0.2 Aa	4.5 ± 0.1 Ba	4.4 ± 0.1 Ba	4.8 ± 0.2	4.8 ± 0.1	4.7 ± 0.3	4.6 ± 0.2
SOM (g kg ⁻¹)	42.7 ± 1.9 Aa	42.6 ± 2.2 Aa	37.1 ± 1.3 Ba	35.8 ± 0.9 Ba	40.4 ± 1.9	39.6 ± 1.7	41.6 ± 1.8	39.2 ± 0.9
P (mg kg ⁻¹)	22.7 ± 6.1	36.1 ± 40.6	13.8 ± 1.3	12.1 ± 0.8	61.8 ± 32.5 Aa	64.3 ± 31.8 Aa	36.68 ± 1.21 Ba	33.85 ± 6.45 Ba
K (mmol _c kg ⁻¹)	15.2 ± 3.7	16.2 ± 6.4	18.3 ± 0.7	15.2 ± 0.7	8.6 ± 6.4	10.0 ± 5.5	6.1 ± 3.6	7.1 ± 6.6
Ca (mmol _c kg ⁻¹)	38.8 ± 4.04 Aa	40.47 ± 1.94 Aa	32.8 ± 0.9 Ba	30.8 ± 2.4 Ba	30.0 ± 3.6	29.1 ± 3.3	33.08 ± 0.70	30.3 ± 5.8
Mg (mmol _c kg ⁻¹)	7.05 ± 1.28 Aa	7.5 ± 0.9 Aa	6.4 ± 0.1 Ba	5.9 ± 0.7 Ba	9.2 ± 1.9 Aa	8.7 ± 1.8 Aa	4.33 ± 2.61 Ba	4.7 ± 0.8 Ba
H+Al (mmol _c kg ⁻¹)	33.8 ± 4.8	31.3 ± 5.1	40.40 ± 6.52	38.1 ± 8.4	38.1 ± 6.1	35.4 ± 5.3	35.7 ± 5.9	39.1 ± 4.5
SB (mmol _c kg ⁻¹)	61.1 ± 8.3 Aa	64.1 ± 5.9 Aa	57.65 ± 0.68 Ba	51.8 ± 2.8 Ba	47.8 ± 10.9	47.8 ± 8.2	43.5 ± 5.2	42.1 ± 13.1
CEC (mmol _c kg ⁻¹)	94.8 ± 11.2	95.4 ± 8.6	72.2 ± 41.1	89.87 ± 10.70	85.8 ± 11.4	83.2 ± 13.2	79.4 ± 6.7	81.2 ± 8.6
V (%)	64.3 ± 2.9 Aa	67.3 ± 3.6 Aa	59.3 ± 3.8 Ba	58.0 ± 4.0 Ba	55.3 ± 7.2	57.5 ± 1.7	56.0 ± 6.2	51.0 ± 9.6

Means followed by the standard deviation. Lowercase letters compare difference between inoculation factors within the same P source, whilst uppercase letters compare difference between P sources within the same inoculation level by the Tukey test at 5% ($p \leq 0.05$). Absence of letters indicates no statistical difference. pH: active acidity, SOM: soil organic matter, P: phosphorus content, K: potassium content, Ca: calcium content, Mg: magnesium content, S: sulfur, H+Al: potential acidity, SB: sum of bases (Ca, Mg and K), CEC: cation exchange capacity, V: base saturation

3.3.4 Biological attributes

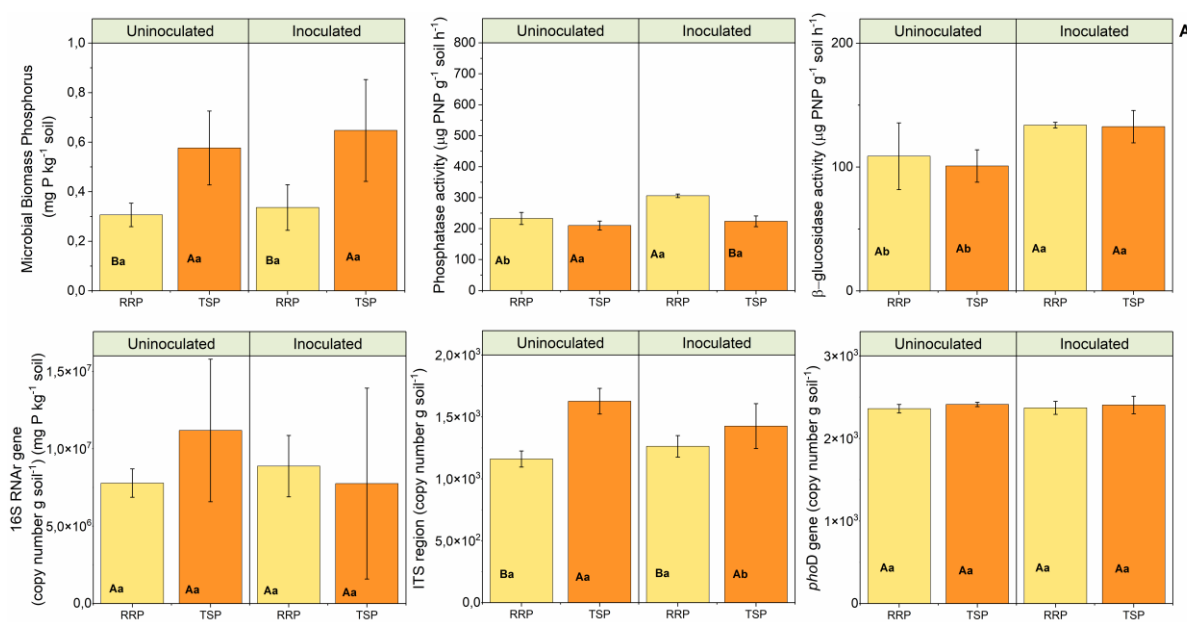
For residual fertilization management, there was no effect of inoculation within the biological parameters analyzed in this study, with only the effect of P sources observed. The acid phosphatase activity in RRP (565 $\mu\text{g PNP g}^{-1} \text{ soil h}^{-1}$) was higher than in TSP (411 PNP

$\text{g}^{-1} \text{ soil h}^{-1}$), representing a difference of $154 \text{ PNP g}^{-1} \text{ soil h}^{-1}$. The total abundance of the bacterial community (16S rRNA gene) showed the same behavior, with RRP being superior to TSP, with a difference of $1.52\text{E}+00 \text{ copy number g soil}^{-1}$. This indicates that the less soluble source promoted a greater abundance of the 16S rRNA gene in the soil.

For fresh fertilization management, there was a source effect on the parameters P microbial biomass, acid phosphatase, and ITS region. Microbial biomass in TSP was higher than in RRP (a difference of $0.30 \text{ mg P kg soil}^{-1}$). There was an inoculation effect for the RRP source; inoculated RRP was superior to non-inoculated RRP for acid phosphatase activity. In this sense, the less soluble source, when inoculated (inoculated RRP), was superior to the absence of inoculation, showing a statistical difference of $73.19 \text{ } \mu\text{g PNP g}^{-1} \text{ soil h}^{-1}$. When comparing P sources, RRP was superior to TSP (a difference of $52.7 \text{ } \mu\text{g PNP g}^{-1} \text{ soil h}^{-1}$).

The beta-glucosidase activity did not show a significant difference for P sources; it only differed in response to inoculation. Inoculated RRP ($133.84 \text{ } \mu\text{g PNP g}^{-1} \text{ soil h}^{-1}$) exhibited higher activity than non-inoculated RRP ($108.74 \text{ } \mu\text{g PNP g}^{-1} \text{ soil h}^{-1}$). Similarly, inoculated TSP ($132.63 \text{ } \mu\text{g PNP g}^{-1} \text{ soil h}^{-1}$) showed higher activity compared to non-inoculated TSP ($100.86 \text{ } \mu\text{g PNP g}^{-1} \text{ soil h}^{-1}$). In essence, inoculation led to increased beta-glucosidase activity, indicating a boost in the native microbial population in the soil.

The abundance of fungi (ITS gene) was significant concerning P sources, with TSP ($1.43\text{E}+03$) surpassing RRP ($1.21\text{E}+03$). The inoculation effect was observed in the soluble source, resulting in a higher number of gene copies in the absence of inoculation. Under these conditions, inoculation decreased the quantity of fungi in the soil.



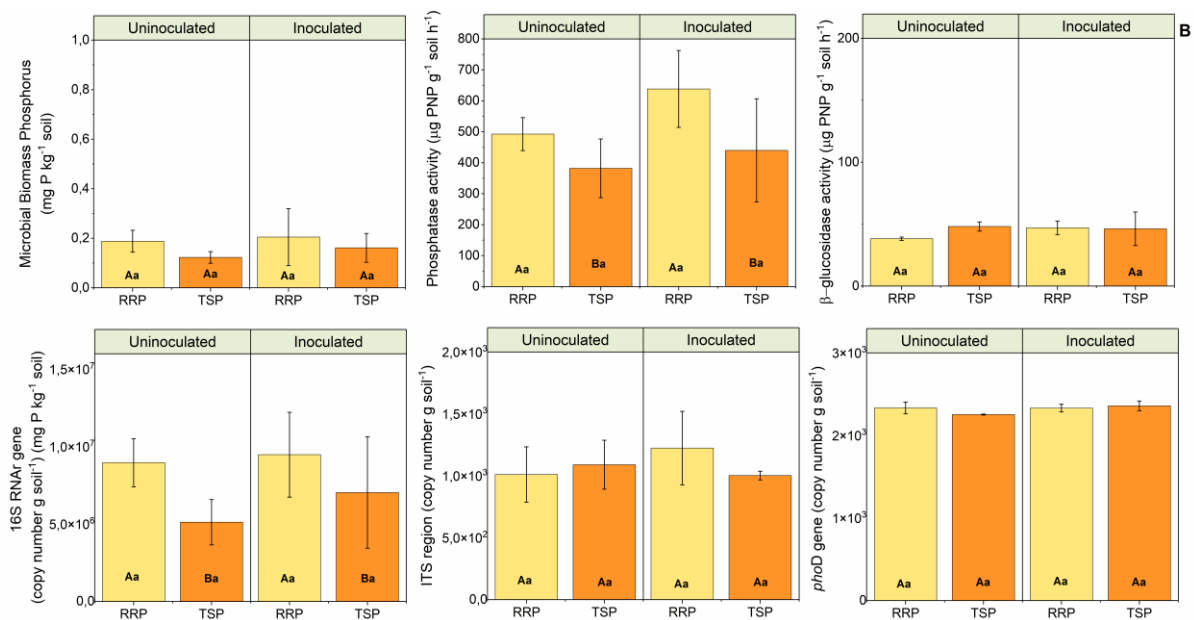


Figure 3. Biological soil attributes (Microbial P, Phosphatase, Beta, 16S, ITS, PhoD). A- fresh scenario and B – residual scenario. Lower case letters indicate differences between inoculation factors within the same P source, while uppercase letters indicate differences between P sources within the same inoculation level, determined by the Tukey test at 5% significance level ($p \leq 0.05$). TSP: triple superphosphate, RRP: reactive rock phosphate.

3.3.5 Soil phosphorus fractions

In the fresh scenario (Figure 4A), treatments uninoculated and with the RRP source exhibited lower P levels in the labile and moderately labile fractions (38,8 and 122.3 mg kg⁻¹, respectively). On the other hand, an increase in the labile P fraction on RRP inoculated was observed. Furthermore, an increase was noted in the non-labile P and total P fractions (304.6 and 584.9 mg kg⁻¹) when comparing the inoculated treatment and RRP source treatments with the others. For the residual scenario (Figure 4B), the inoculation leads a lower P labile fractions (38.4 mg kg⁻¹) in RRP when compared with the other treatments. There were observed an increase in the non-labile P fraction in RRP inoculated treatment (1243.5 mg kg⁻¹). The moderately labile and total P fraction showed no differences between the treatments.

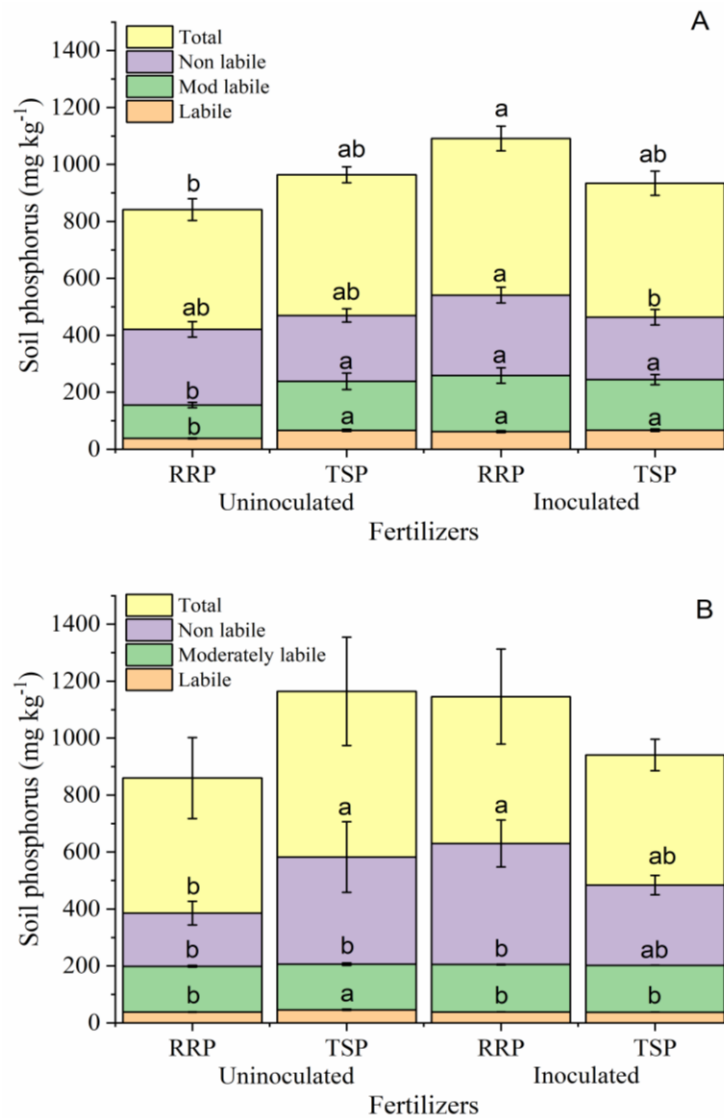


Figure 4. Phosphorus fractions in: A) fresh scenario and B) residual scenario. Lowercase letters indicate differences between inoculation factors within the same P source, while uppercase letters indicate differences between P sources within the same inoculation level, determined by the Tukey test at a 5% significance level ($p \leq 0.05$). TSP: triple superphosphate, RRP: reactive rock phosphate.

3.3.6 Principal component analysis

The P sources and inoculation influenced the soil microbial community structure. The multivariate representation of the soil bacterial community was depicted through PCoA analysis (Figure 5), explaining 49% of the data variation (considering the sum of axes 1 and 2). Additionally, the Adonis test (based on Bray-Curtis distance and 999 permutations) indicated a significant interaction between P sources and inoculation ($P=0.03$, Annex 1). The

pairwise Adonis test revealed differences in the bacterial community between the RRP treatment and the non-inoculated treatment compared to the other treatments (Annex 2).

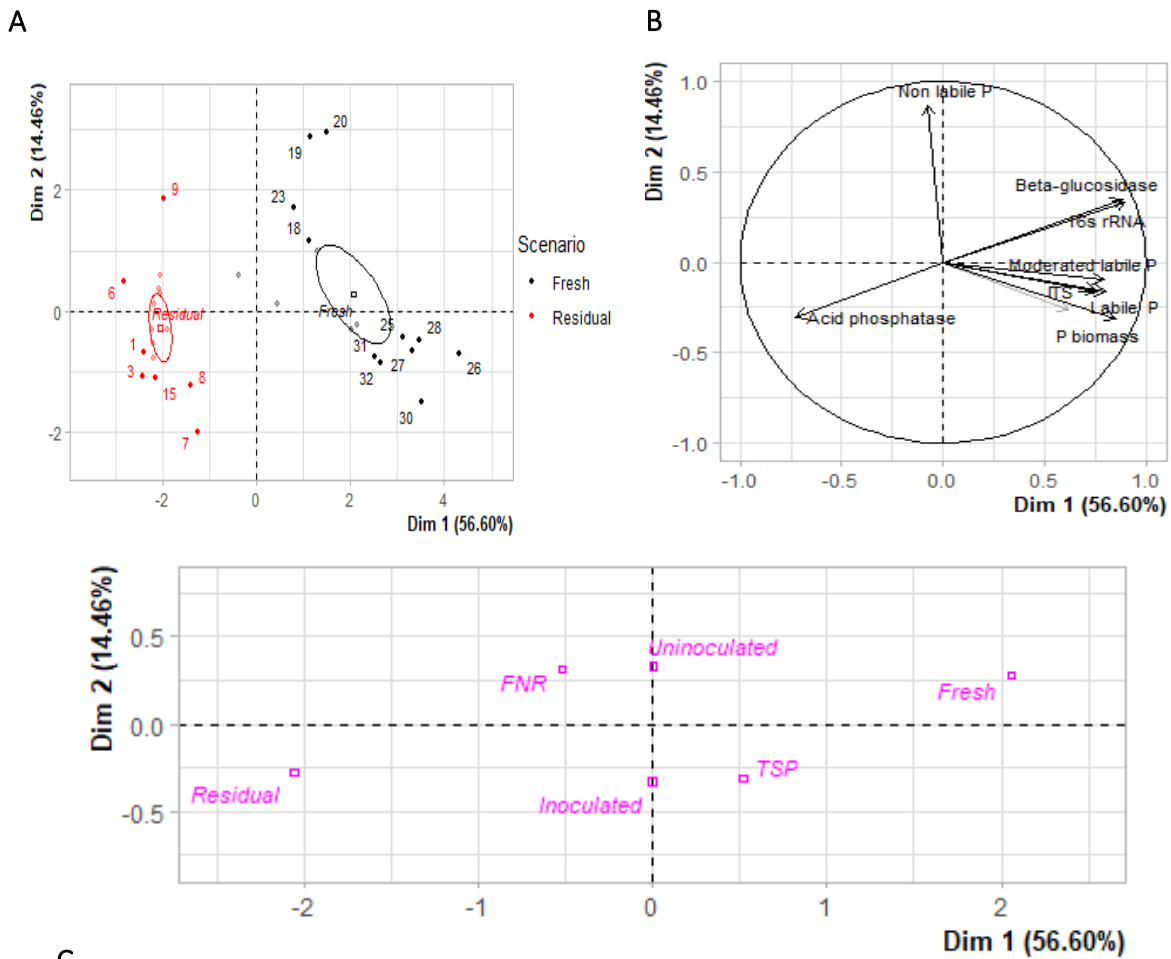


Figure 5. Principal Coordinate Analysis (PCoA) of the structure of the soil bacteria community subjected to the application of Triple Superphosphate (TSP) and Natural Reactive Phosphate (RRP), with and without *Bacillus pumilus* inoculation. A - Individuals factor map (PCA) The labeled individuals are those with the higher contribution to the plane construction B - Variables factor map (PCA) The labeled variables are those the best shown on the plane and C - Qualitative factor map (PCA).

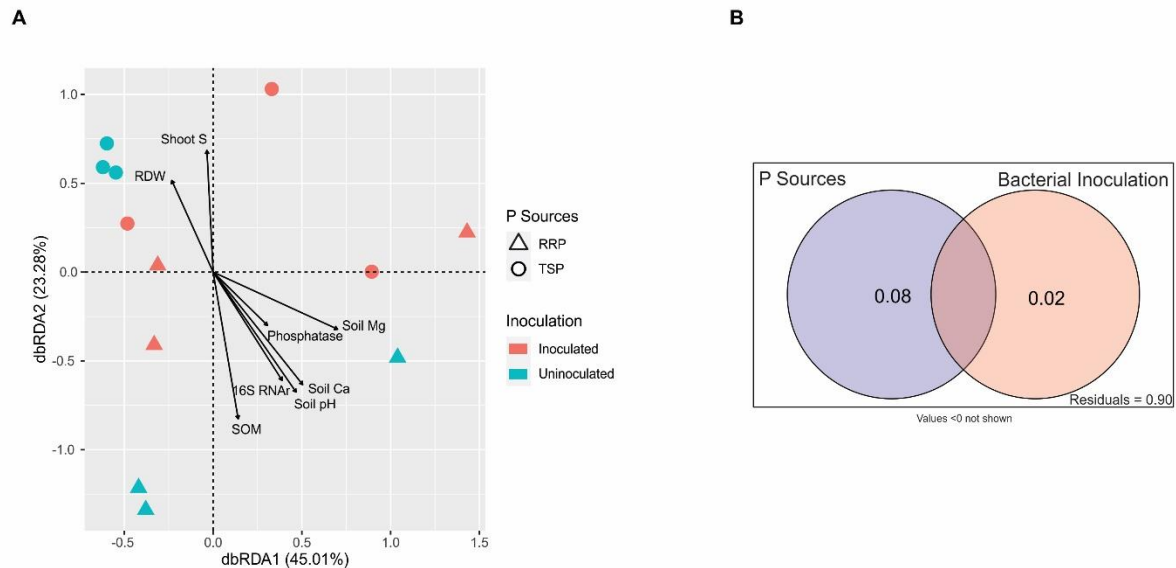
Dimension 1 reveals a distinct separation between two groups of samples, positioning fresh to the right of the graph with a significantly positive coordinate on the axis. In contrast, the residual treatment is situated on the left side, characterized by a strongly negative coordinate on the axis. The group characterized by positive coordinates shares a distinct profile marked by elevated values in key variables such as Labile P, P biomass, ITS, Moderated labile P, 16s rRNA, PhoD, and Beta-glucosidase, arranged in descending order of intensity. In contrast, these samples display lower values for the variable Acid.phosphatase. Conversely, in the opposing group, whose members have negative coordinates, we observe a contrasting

pattern. These samples present elevated values for the Acid.phosphatase variable, indicating a response opposite to that of the first group. Additionally, they exhibit lower values for variables such as 16s.rRNA, Beta-glucosidase, Moderated labile P, Labile P, ITS, P biomass, and PhoD, arranged in ascending order of intensity. It's worth noting that the variables Fresh and Residual display a high correlation with Dimension 1 (with respective correlations of 0.98, 0.98). Hence, these variables may effectively capture the essence of Dimension 1.

The Distance-based Redundancy Analysis (dbRDA) was conducted to assess the interaction of the community with the chemical and biological attributes of the soil, as depicted in Figure 6 (Annexes 2 and 3). The parameters under study are, for the most part, associated with the TST source, while β -glucosidase, Soil P AER, and Soil Mg are linked to the RRP source, specifically in the absence of inoculation (Figure 6A). The first and second axes combined accounted for nearly 60% of the total variance in the soil bacterial community.

Upon partitioning the variance, it is observed that inoculation explains only 2% (0.02), whereas the P source explains 13% (0.13). The remaining 85% is explained by factors other than the source and inoculation, potentially including environmental factors, soil, and plant-related aspects (Figure 6B).

Scenario A - Scenario Residual fertilizer



Scenario B - Fresh fertilizer scenario

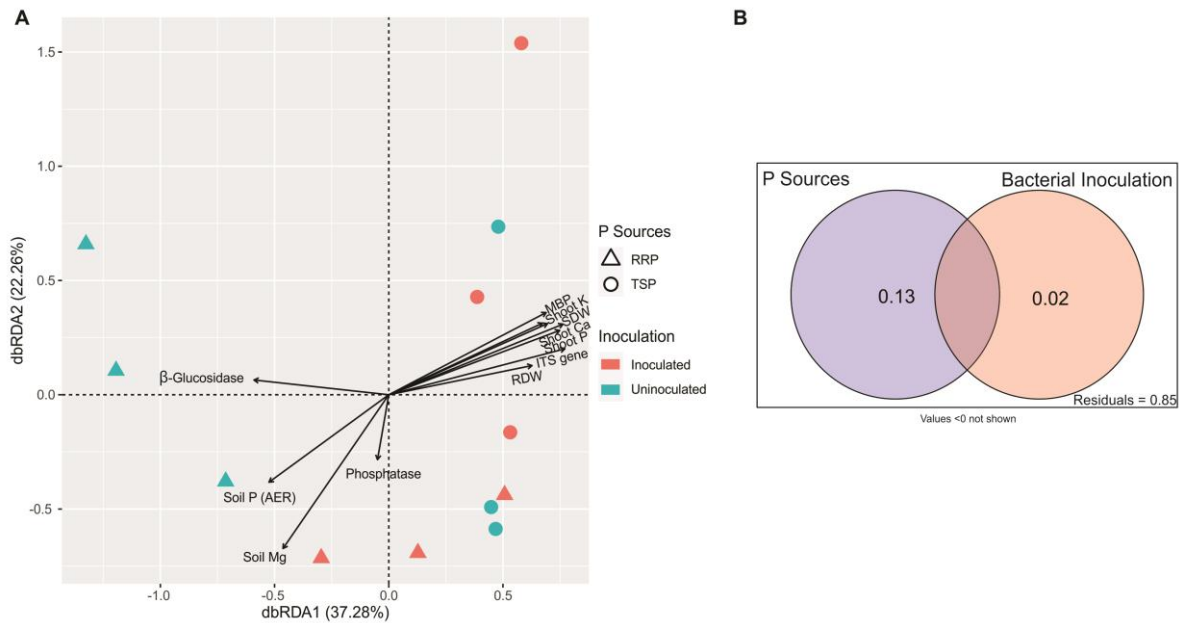


Figure 6. A) Distance-based Redundancy Analysis (dbRDA) evaluating the interaction between community structure and the chemical and biological attributes in the soil that exhibited differences in univariate analysis. B) RDA partitioning to understand the extent to which each factor explains the dynamics of community structure.

3.4 Discussion

3.4.1 Shoot and root biomass

The observed difference between shoot and root biomass was primarily noted in the fresh scenario. This occurs because, instead of the phosphorus solubilization potential of the bacteria, the fertilizer source can alter P availability persistently.

A more gradual release of phosphorus from reactive phosphate rocks is associated with the gradual process of solubilization. Solubilization is the phenomenon whereby phosphorus compounds, usually in the form of phosphate, present in the rock, undergo transformation into water-soluble forms, thereby becoming available for plants (Illmer and Schinner, 1995).

Phosphate rocks, such as apatite, are primarily composed of minerals containing phosphorus in the form of phosphate. Phosphate in these rocks is often present in the form of hydroxyapatite, an insoluble substance in water (Rajan and Martin, 2021). The presence of insoluble minerals contributes to the naturally low solubility of phosphate rocks (Amarasinghe et al., 2022).

On the other hand, acidulated phosphate fertilizers undergo an acidulation process during their manufacture. Acids such as sulfuric acid or phosphoric acid are added to natural phosphates, resulting in acid-base reactions. These chemical reactions transform the insoluble phosphates present in the rock into more soluble forms, such as monoacid phosphate (H_2PO_4^-) or diacid phosphate (HPO_4^{2-}), depending on specific process conditions (Guelfi et al., 2022).

The shoot and root biomass (Figure 1A and 1B) demonstrate that providing a soluble P source, such as TSP, enhances P availability to plant roots, which, in turn, can lead to an increase in total biomass and, consequently, crop productivity (Meyer et al., 2018). Furthermore, inoculation with microorganisms may enhance nutrient availability, leading to an increase in the total biomass of corn plants (Bradáčová et al., 2020). However, the efficiency of this conversion depends on the bacterial species used, soil conditions such as pH, humidity, temperature, the microorganisms life cycle, and P (Silva et al., 2023).

Converting insoluble phosphorus into soluble forms, Phosphorus-Solubilizing Microorganisms (PSMs) function as biofertilizers (Zhu et al., 2012). The inorganic solubilization of phosphorus by PSMs primarily results from the production of organic acids. This production not only reduces the pH but also enhances the chelation of cations bound to P, competes with P for adsorption sites in the soil, and forms soluble complexes with metal ions associated with insoluble phosphorus, such as calcium, aluminum, and iron (Sharma et al., 2013).

Although this large array of molecules capable of increasing the solubilization potential of P, the efficiency of solubilization by microorganisms depends on their ability to adapt and establish themselves in the environment after application (Elhaissofi et al., 2022). Besides that, the PSB may be more evident in subsequent crops (residual effects of microbial activity), as inoculated bacteria can cause modifications in the community that may increase the demand for P in the system, leading to a consequent increase in enzymatic activity and a potential increase in P availability to plants (Cheng et al., 2023). Therefore, the tendency for an increase in total biomass with the use of RRP and inoculation can be justified by the improvement in nutrient availability in the soil (Eida et al., 2017).

In the case of the residual scenario, a decreased development in shoot and root was observed. This can be attributed to the maximum adsorption capacity that soils may possess. Generally, soils with a high degree of weathering tend to exhibit a high adsorption capacity for phosphorus (Jørgensen et al., 2015). This is due to the presence of specific clays, such as Fe/Al oxyhydroxides, which demonstrate a high affinity for orthophosphate in these soils (Gérard, 2016). Consequently, the residual scenario becomes a more responsive setting for assessing the enhancement of phosphorus efficiency by microorganisms, given the low labile phosphorus in the soil. However, no response was observed in the inoculated treatments for shoot and root biomass. The efficiency of PSM in mobilizing phosphorus bound in the solid

phase of the soil is significantly influenced by the chemical and physical nature of phosphorus in the soil (Jones and Oburger, 2011). The effectiveness, encompassing gross release and/or solubilization, of PSM is closely regulated by the characteristics of the phosphorus present. For example, phosphate rock consists of various phosphorus minerals with diverse solubility profiles. While PSM are typically chosen for their ability to dissolve phosphate rock in laboratory settings, it's crucial to recognize that phosphate rock may not accurately represent the phosphorus forms commonly found in diverse soils (Baveye, 2015). This difference is likely a primary factor contributing to the varied responses observed with PSM in situ.

3.4.2 Nutrient uptake

The P and Mg levels accumulated in the aboveground part were inversely proportional to the levels present in the soil (Figure 2). In the aboveground part, the TSP source was superior to the RRP, while in the soil, the RRP was superior to the TSP. The TSP source showed greater efficiency in promoting aboveground growth compared to the RRP source, while in the soil, the RRP source proved to be more effective than TSP.

A possible explanation for higher P concentrations in the soil with the RRP source is related to its low solubility in the soil, meaning it is not readily available for plant absorption, thus showing a residual effect in the soil. Souza et al. (2020), observed that maize grain yields were affected by the initial application of rock phosphates, sustaining an influence for up to four years, especially in plots where an annual addition of soluble phosphate fertilizer was not applied.

3.4.3 Biological attributes

Soil microbial biomass is sensitive to environmental changes, influenced by factors such as climate, land use, root density and diversity, soil physical and chemical attributes, and the availability of organic substrate for microbial growth (Vezzani et al., 2018).

The availability of P in the soil plays a crucial role in increasing microbial biomass, directly influencing the development and activity of the microorganisms present (Mehnaz et al., 2019). Under favorable conditions, microorganisms can sense the adequate presence of P, triggering biological responses, including an increase in microbial biomass (Prommer et al., 2020).

Despite PSB being able to access a portion of the unavailable or occluded P in the soil environment, the initial content of this nutrient in the soil can affect the synthesis of essential molecules such as nucleic acids (DNA and RNA), adenosine triphosphate (ATP), and phospholipids in cell membranes (Hemkemeyer et al., 2021). Additionally, in the case of RRP or under conditions of low availability, such as the residual scenario, there is greater competition between soil microorganisms and the present plants.

On the other hand, the increase in microbial biomass (Figure 2 and Table 3) with the use of TSP as the fertilizer source in the fresh scenario can be explained by the high availability of phosphorus from this fertilizer source.

Compared to plants, microorganisms seem to harbor more diverse metabolic capabilities to enhance the bioavailability of various recalcitrant forms of P in soils (Hu et al., 2023). The enzymatic activity of acid phosphatase is an indicator of soil management practices and is inversely proportional to the available P content (Nannipieri et al., 2011). Soil microorganisms acted on the less soluble P source (RRP) to make phosphate available in the soil (Silva et al., 2023). In contrast, the soluble source (TSP) did not respond to the inoculation (Figure 2). Nevertheless, the inoculation's effect on P availability in the soil through acid phosphatase activity is evident, as observed in fresh fertilizer management.

The enzymatic activity of β -glucosidase is related to the action of soil microorganisms for carbon consumption (de Almeida et al., 2015). The β -glucosidase activity (Figure 2) showed no difference between the fertilizer's sources, however, there was an increase in that activity when inoculated. Besides that, there was a decrease in the β -glucosidase activity in the residual scenario when compared with the fresh scenario. High levels of soil carbon can inhibit β -glucosidase activity (Sheng et al., 2022). β -glucosidase is an enzyme involved in the breakdown of complex organic compounds, including cellulose, in the soil. However, when soil carbon levels are elevated, it can lead to a phenomenon known as substrate inhibition, where the abundance of available carbon compounds inhibits the activity of certain enzymes, including β -glucosidase (Han et al., 2023). This is because the microorganisms in the soil may prioritize the utilization of more easily accessible carbon sources over the more complex ones, leading to a decrease in the activity of enzymes like β -glucosidase (Tian et al., 2020).

Soil microbial communities can adjust their metabolism and composition based on nutrient content in the soil (Yao et al., 2018). Under the conditions observed in this study, there was no effect of P sources and inoculation on the abundance of the 16S rRNA gene. One possible explanation for these results may be related to the establishment time of the communities (low), and there is a possibility that the inoculation of *B. pumilus* did not interfere with the native soil community (Sengupta, Gunri, Biswas, 2017). However, this cannot be

confirmed, as an analysis of the bacterial community before and after inoculation was not conducted.

Fungal abundance requires time for establishment and altering the microbiological community. Studies on forest soils show better responses due to the time needed for community establishment (Merloti et al., 2019). The soluble source (TSP) outperformed the less soluble source (RRP). A likely explanation for the association of lower abundance of the ITS gene (Figure 2) in the inoculated TSP could be that the inoculation stimulated certain groups while suppressing other microbial groups in the soil.

3.4.4 Phosphorus fractions

Since P is a highly reactive element in the soil when in solution, the type of fertilizer source applied can influence the labile nature of this nutrient over time (Lizcano-Toledo et al., 2021). Fertilizers such as rock phosphates (RP), which have low solubilization, tend to solubilize small amounts of P over time, even with an increased potential for solubilization by PSM (Timofeeva et al., 2022).

Studies suggest that combining PSBs with phosphate fertilizers has the potential to enhance the agronomic efficiency of these fertilizers in soils (Bargaz et al., 2018). For instance, the growth of maize showed significant enhancement when inoculated with PSBs in conjunction with various types of phosphate fertilizers, including livestock manure, bird droppings, single superphosphate, and rock phosphates (Adnan et al., 2020). However, such synergistic effects between the fertilizer source and PSB were not observed in the present study.

In the fresh scenario (Figure 4A), a decrease in the labile and moderately labile P fractions was observed in the RRP source when inoculated. On the other hand, there was also an observed increase in the non-labile fractions of total P in this treatment. Both the decrease in labile and moderately labile P and the increase in non-labile P are due to the dynamic balance between P in solution and the adsorption reactions and specific (high-affinity) bonds between P and mineral surfaces (Violante et al., 2002). Even with the solubilizing mechanisms of PSB, all P made available by RRP solubilization can be spontaneously adsorbed upon entering the soil solution. In other words, the solubilizing effect of PSB is also limited by the soil's maximum adsorption capacity and competition for P nutrition by plants (Bargaz et al., 2021). More studies are needed to assess how the adsorption capacity of soils limits or competes with the solubilizing capacity of soil microorganisms.

3.4.5 Multivariate analysis

The PCoA demonstrates a distinct separation between the fresh and residual scenarios (Figure 5). The significantly positive coordinates associated with the fresh scenario, involving variables such as labile P, P biomass, 16s rRNA, and β -glucosidase, contrast with low values for acid phosphatase. This highlights that in the fresh scenario, the impact of PSM inoculation on increasing P efficiency from mineral sources is less pronounced. On the other hand, individuals from the negative group of PCoA, such as non-labile P, exhibit high values for acid phosphatase, indicating a close association between the activity of this enzyme and P availability in the environment (Margalef et al., 2017). However, the activity of acid phosphatase in natural soils differs when PSM are exposed to controlled environments and closed systems (Tian et al., 2021).

The PCoA reduces the dimensionality of bacterial community data (Figure 6). A segregation of positions is noted according to the treatment applied. The non-inoculated RRP is isolated from the other treatments (left), while the remaining treatments are positioned to the right of the matrix. Probably, the inoculation in the less soluble source (RRP) altered the microbial community, as this treatment behaved similarly to the TSP source (in the presence or absence of inoculation), thus highlighting the importance of inoculation.

3.5 Conclusion

The utilization of PSM is a promising avenue to increase the use and efficiency of phosphatic fertilizers by plants. The use of reactive rocks holds significant value considering the global scenario of phosphatic fertilizers and the challenges of sustainable phosphorus utilization as a finite resource. However, no positive changes in soil P fractions were observed after bacterial inoculation, as anticipated. Nevertheless, an increase in the non-labile P fraction was observed in the treatment with Recalcitrant Rock Phosphate (RRP) as well as Triple Superphosphate (TSP), indicating bacterial activity in the rock degradation process in both the fresh and residual scenarios. This suggests that the bacterial solubilizing potential could not surpass the soil's P adsorption capacity. Further studies are required to understand how soil adsorption capacity affects the solubilization potential of phosphorus-solubilizing bacteria.

References

Adnan, M., Fahad, S., Zamin, M., Shah, S., Mian, I.A., Danish, S., Zafar-ul-Hye, M., Battaglia,

- M.L., Naz, R.M.M., Saeed, B., 2020. Coupling phosphate-solubilizing bacteria with phosphorus supplements improve maize phosphorus acquisition and growth under lime induced salinity stress. *Plants* 9, 900.
- Alvarez, A.L., Weyers, S.L., Goemann, H.M., Peyton, B.M., Gardner, R.D., 2021. Microalgae, soil and plants: A critical review of microalgae as renewable resources for agriculture. *Algal Res.* 54, 102200.
- Amarasinghe, T., Madhusa, C., Munaweera, I., Kottegoda, N., 2022. Review on mechanisms of phosphate solubilization in rock phosphate fertilizer. *Commun. Soil Sci. Plant Anal.* 53, 944–960.
- Arvieu, J.-C., Leprince, F., Plassard, C., 2003. Release of oxalate and protons by ectomycorrhizal fungi in response to P-deficiency and calcium carbonate in nutrient solution. *Ann. For. Sci.* 60, 815–821.
- Bargaz, A., Elhaissofi, W., Khourchi, S., Benmrid, B., Borden, K.A., Rchiad, Z., 2021. Benefits of phosphate solubilizing bacteria on belowground crop performance for improved crop acquisition of phosphorus. *Microbiol. Res.* 252, 126842.
- Bargaz, A., Lyamlouli, K., Chtouki, M., Zeroual, Y., Dhiba, D., 2018. Soil microbial resources for improving fertilizers efficiency in an integrated plant nutrient management system. *Front. Microbiol.* 9, 1606.
- Barka, E.A., Vatsa, P., Sanchez, L., Gaveau-Vaillant, N., Jacquard, C., Klenk, H.-P., Clément, C., Ouhdouch, Y., van Wezel, G.P., 2016. Taxonomy, physiology, and natural products of Actinobacteria. *Microbiol. Mol. Biol. Rev.* 80, 1–43.
- Baveye, P.C., 2015. Looming scarcity of phosphate rock and intensification of soil phosphorus research. *Rev. Bras. Ciência do Solo* 39, 637–642.
- Benner, R., 2011. Biosequestration of carbon by heterotrophic microorganisms. *Nat. Rev. Microbiol.* 9, 75. <https://doi.org/10.1038/nrmicro2386-c3>
- Biswas, S.S., Biswas, D.R., Ghosh, A., Sarkar, A., Das, A., Roy, T., 2022. Phosphate solubilizing bacteria inoculated low-grade rock phosphate can supplement P fertilizer to grow wheat in sub-tropical inceptisol. *Rhizosphere* 23, 100556. <https://doi.org/https://doi.org/10.1016/j.rhisph.2022.100556>
- Bravin, M.N., Garnier, C., Lenoble, V., Gérard, F., Dudal, Y., Hinsinger, P., 2012. Root-induced changes in pH and dissolved organic matter binding capacity affect copper dynamic speciation in the rhizosphere. *Geochim. Cosmochim. Acta* 84, 256–268.
- Burns, R.G., DeForest, J.L., Marxsen, J., Sinsabaugh, R.L., Stromberger, M.E., Wallenstein, M.D., Weintraub, M.N., Zoppini, A., 2013. Soil enzymes in a changing environment: Current knowledge and future directions. *Soil Biol. Biochem.* 58, 216–234. <https://doi.org/https://doi.org/10.1016/j.soilbio.2012.11.009>
- Chen, W., Yang, F., Zhang, L., Wang, J., 2016. Organic acid secretion and phosphate solubilizing efficiency of *Pseudomonas* sp. PSB12: effects of phosphorus forms and carbon sources. *Geomicrobiol. J.* 33, 870–877.
- Chen, Y.P., Rekha, P.D., Arun, A.B., Shen, F.T., Lai, W.-A., Young, C.C., 2006. Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. *Appl. soil Ecol.* 34, 33–41.
- Cheng, Y., Narayanan, M., Shi, X., Chen, X., Li, Z., Ma, Y., 2023. Phosphate-solubilizing bacteria: Their agroecological function and optimistic application for enhancing agro-productivity. *Sci. Total Environ.* 166468.
- Condon, L., 2022. Legacy Phosphorus in agricultural soils: Maintaining crop yields and minimising losses to water. *Eur. Sustain. Phosphorus Platf.* 1–21.
- Condon, L.M., Newman, S., 2011. Revisiting the fundamentals of phosphorus fractionation of sediments and soils. *J. Soils Sediments* 11, 830–840. <https://doi.org/10.1007/s11368-011-0363-2>
- Cosme, M., Fernández, I., Van der Heijden, M.G.A., Pieterse, C.M.J., 2018. Non-mycorrhizal plants: the exceptions that prove the rule. *Trends Plant Sci.* 23, 577–587.
- Cotrufo, M.F., Soong, J.L., Horton, A.J., Campbell, E.E., Haddix, M.L., Wall, D.H., Parton, W.J., 2015. Formation of soil organic matter via biochemical and physical pathways of litter

- mass loss. *Nat. Geosci.* 8, 776–779. <https://doi.org/10.1038/ngeo2520>
- Cross, A.F., Schlesinger, W.H., 1995. A literature review and evaluation of the Hedley fractionation: Applications to the biogeochemical cycle of soil phosphorus in natural ecosystems. *Geoderma* 64, 197–214. [https://doi.org/10.1016/0016-7061\(94\)00023-4](https://doi.org/10.1016/0016-7061(94)00023-4)
- de Almeida, R.F., Naves, E.R., da Mota, R.P., 2015. Soil quality: Enzymatic activity of soil β -glucosidase. *Glob. J. Agric. Res. Rev.* 3, 146–450.
- Debicka, M., Morshedizad, M., Leinweber, P., 2023. The Effects of Dissolved Organic Matter Derived from Agricultural Waste Materials on Phosphorus Sorption in Sandy Soils.
- Devi, R., Kaur, T., Kour, D., Yadav, A., Yadav, A.N., Suman, A., Ahluwalia, A.S., Saxena, A.K., 2022. Minerals solubilizing and mobilizing microbiomes: A sustainable approach for managing minerals' deficiency in agricultural soil. *J. Appl. Microbiol.* 133, 1245–1272.
- Divjot, K., Rana, K.L., Tanvir, K., Yadav, N., Yadav, A.N., Kumar, M., Kumar, V., Dhaliwal, H.S., Saxena, A.K., 2021. Biodiversity, current developments and potential biotechnological applications of phosphorus-solubilizing and-mobilizing microbes: A review. *Pedosphere* 31, 43–75.
- Duff, S.M.G., Sarath, G., Plaxton, W.C., 1994. The role of acid phosphatases in plant phosphorus metabolism. *Physiol. Plant.* 90, 791–800.
- Eida, A.A., Hirt, H., Saad, M.M., 2017. Challenges faced in field application of phosphate-solubilizing bacteria. *Rhizotrophs plant growth Promot. to bioremediation* 125–143.
- Eivazi, F., Tabatabai, M.A., 1977. Phosphatases in soils. *Soil Biol. Biochem.* 9, 167–172.
- Elhaisoufi, W., Ghoulam, C., Barakat, A., Zeroual, Y., Bargaz, A., 2022. Phosphate bacterial solubilization: A key rhizosphere driving force enabling higher P use efficiency and crop productivity. *J. Adv. Res.* 38, 13–28.
- Emami-Karvani, Z., Chitsaz-Esfahani, Z., 2021. Phosphorus solubilization: mechanisms, recent advancement and future challenge. *Soil Microbiomes Sustain. Agric. Funct. Annot.* 85–131.
- Estrada-Bonilla, G.A., Durrer, A., Cardoso, E.J.B.N., 2021. Use of compost and phosphate-solubilizing bacteria affect sugarcane mineral nutrition, phosphorus availability, and the soil bacterial community. *Appl. Soil Ecol.* 157, 103760. <https://doi.org/https://doi.org/10.1016/j.apsoil.2020.103760>
- Etesami, H., 2020. Enhanced phosphorus fertilizer use efficiency with microorganisms. *Nutr. Dyn. Sustain. Crop Prod.* 215–245.
- Fierer, N., Jackson, J.A., Vilgalys, R., Jackson, R.B., 2005. Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. *Appl. Environ. Microbiol.* 71, 4117–4120. <https://doi.org/10.1128/AEM.71.7.4117-4120.2005/ASSET/0915DD64-3705-4111-AD00-9ADCEF62A3CD/ASSETS/GRAPHIC/ZAM0070556110002.JPEG>
- Fink, J.R., Inda, A.V., Tiecher, T., Barrón, V., 2016. Iron oxides and organic matter on soil phosphorus availability. *Ciência e Agrotecnologia* 40, 369–379. <https://doi.org/10.1590/1413-70542016404023016>
- Fouad, F.A., Youssef, D.G., Shahat, F.M., Abd El-Ghany, M.N., 2023. Role of Microorganisms in Biodegradation of Pollutants, in: *Handbook of Biodegradable Materials*. Springer, pp. 221–260.
- Fransson, A.M., Jones, D.L., 2007. Phosphatase activity does not limit the microbial use of low molecular weight organic-P substrates in soil. *Soil Biol. Biochem.* 39, 1213–1217.
- Gandhi, N.U., Chandra, S.B., 2012. A comparative analysis of three classes of bacterial non-specific acid phosphatases and archaeal phosphoesterases: evolutionary perspective. *Acta Inform. Medica* 20, 167.
- GARDES, M., BRUNS, T.D., 1993. ITS primers with enhanced specificity for basidiomycetes - application to the identification of mycorrhizae and rusts. *Mol. Ecol.* 2, 113–118. <https://doi.org/10.1111/j.1365-294X.1993.tb00005.x>
- Gatiboni, L., Brunetto, G., Pavinato, P.S., George, T.S., 2020a. Legacy phosphorus in agriculture: role of past management and perspectives for the future. *Front. Earth Sci.*
- Gatiboni, L., Brunetto, G., Pavinato, P.S., George, T.S., 2020b. Editorial: Legacy Phosphorus

- in Agriculture: Role of Past Management and Perspectives for the Future. *Front. Earth Sci.* 8, 1–2. <https://doi.org/10.3389/feart.2020.619935>
- Gérard, F., 2016. Clay minerals, iron/aluminum oxides, and their contribution to phosphate sorption in soils—A myth revisited. *Geoderma* 262, 213–226.
- Grandy, A.S., Strickland, M.S., Lauber, C.L., Bradford, M.A., Fierer, N., 2009. The influence of microbial communities, management, and soil texture on soil organic matter chemistry. *Geoderma* 150, 278–286. <https://doi.org/https://doi.org/10.1016/j.geoderma.2009.02.007>
- Groeneveld, M., Catalán, N., Attermeyer, K., Hawkes, J., Einarsdóttir, K., Kothawala, D., Bergquist, J., Tranvik, L., 2020. Selective adsorption of terrestrial dissolved organic matter to inorganic surfaces along a boreal inland water continuum. *J. Geophys. Res. Biogeosciences* 125, e2019JG005236.
- Guelfi, D., Nunes, A.P.P., Sarkis, L.F., Oliveira, D.P., 2022. Innovative Phosphate Fertilizer Technologies to Improve Phosphorus Use Efficiency in Agriculture. *Sustainability* 14. <https://doi.org/10.3390/su142114266>
- Gypser, S., Hirsch, F., Schleicher, A.M., Freese, D., 2018. Impact of crystalline and amorphous iron- and aluminum hydroxides on mechanisms of phosphate adsorption and desorption. *J. Environ. Sci.* 70, 175–189. <https://doi.org/https://doi.org/10.1016/j.jes.2017.12.001>
- Hallama, M., Pekrun, C., Lambers, H., Kandeler, E., 2019. Hidden miners – the roles of cover crops and soil microorganisms in phosphorus cycling through agroecosystems. *Plant Soil* 434, 7–45. <https://doi.org/10.1007/s11104-018-3810-7>
- Han, J., Ro, H.-M., 2019. Characterizing Preferential Adsorption of Phosphate on Binary Sorbents of Goethite and Maghaemite using in situ ATR-FTIR and 2D Correlation Spectroscopy. *Sci. Rep.* 9, 6130. <https://doi.org/10.1038/s41598-019-42575-2>
- Han, Y., Liu, W., Chang, N., Sun, L., Bello, A., Deng, L., Zhao, L., Egbeagu, U.U., Wang, B., Zhao, Y., 2023. Exploration of β -glucosidase-producing microorganisms community structure and key communities driving cellulose degradation during composting of pure corn straw by multi-interaction analysis. *J. Environ. Manage.* 325, 116694.
- Haynes, R.J., Zhou, Y.F., 2018. Competitive and noncompetitive adsorption of silicate and phosphate by two acid Si-deficient soils and their effects on P and Si extractability. *Soil Sci. Plant Nutr.* 64, 535–541. <https://doi.org/10.1080/00380768.2018.1473008>
- Hedley, M. J., Stewart, J.W.B., Chauhan, B.S., 1982. Changes in inorganic and organic soil phosphorus fractions induced by cultivation practices and by laboratory incubations. *Soil Sci. Soc. Am. J.* 46, 970–976. <https://doi.org/10.2136/sssaj1982.03615995004600050017x>
- Hedley, M Ji, Stewart, J.W.B., Chauhan, Bs., 1982. Changes in inorganic and organic soil phosphorus fractions induced by cultivation practices and by laboratory incubations 1. *Soil Sci. Soc. Am. J.* 46, 970–976.
- Hemkemeyer, M., Schwalb, S.A., Heinze, S., Joergensen, R.G., Wichern, F., 2021. Functions of elements in soil microorganisms. *Microbiol. Res.* 252, 126832. <https://doi.org/https://doi.org/10.1016/j.micres.2021.126832>
- Hemwall, J.B., 1957. The fixation of phosphorus by soils. *Adv. Agron.* 9, 95–112.
- Hoberg, E., Marschner, P., Lieberei, R., 2005. Organic acid exudation and pH changes by *Gordonia* sp. and *Pseudomonas fluorescens* grown with P adsorbed to goethite. *Microbiol. Res.* 160, 177–187. <https://doi.org/https://doi.org/10.1016/j.micres.2005.01.003>
- Holland, H.D., Turekian, K.K., 2003. *Treatise on geochemistry: 8. Biogeochemistry.*
- Hu, X., Gu, H., Liu, J., Wei, D., Zhu, P., Cui, X., Zhou, B., Chen, X., Jin, J., Liu, X., Wang, G., 2023. Metagenomic strategies uncover the soil bioavailable phosphorus improved by organic fertilization in Mollisols. *Agric. Ecosyst. Environ.* 349, 108462. <https://doi.org/https://doi.org/10.1016/j.agee.2023.108462>
- Jaisi, D.P., Blake, R.E., 2014. Advances in using oxygen isotope ratios of phosphate to understand phosphorus cycling in the environment. *Adv. Agron.* 125, 1–53.
- Jazaeri, M., Akhgar, A., Sarcheshmehpour, M., Mohammadi, A.H., 2016. Bioresource efficacy of phosphate rock, sulfur, and *Thiobacillus inoculum* in improving soil phosphorus

- availability. *Commun. Soil Sci. Plant Anal.* 47, 1441–1450.
- Jiang, H., Qi, P., Wang, T., Wang, M., Chen, M., Chen, N., Pan, L., Chi, X., 2018. Isolation and characterization of halotolerant phosphate-solubilizing microorganisms from saline soils. *3 Biotech* 8, 1–8.
- Jones, D.L., Oburger, E., 2011. Solubilization of Phosphorus by Soil Microorganisms, in: Bünemann, E., Oberson, A., Frossard, E. (Eds.), *Phosphorus in Action: Biological Processes in Soil Phosphorus Cycling*. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 169–198. https://doi.org/10.1007/978-3-642-15271-9_7
- Jørgensen, C., Turner, B.L., Reitzel, K., 2015. Identification of inositol hexakisphosphate binding sites in soils by selective extraction and solution ^{31}P NMR spectroscopy. *Geoderma* 257–258, 22–28. <https://doi.org/10.1016/j.geoderma.2015.03.021>
- Kalayu, G., 2019. Phosphate solubilizing microorganisms: promising approach as biofertilizers. *Int. J. Agron.* 2019, 1–7.
- Khan, M.S., Zaidi, A., Ahmad, E., 2014. Mechanism of phosphate solubilization and physiological functions of phosphate-solubilizing microorganisms. *Phosphate solubilizing Microorg. Princ. Appl. microphos Technol.* 31–62.
- Khan, M.S., Zaidi, A., Wani, P.A., 2009. Role of phosphate solubilizing microorganisms in sustainable agriculture-a review. *Sustain. Agric.* 551–570.
- Kour, D., Kaur, T., Yadav, N., Rastegari, A.A., Singh, B., Kumar, V., Yadav, A.N., 2020. Phytases from microbes in phosphorus acquisition for plant growth promotion and soil health, in: *New and Future Developments in Microbial Biotechnology and Bioengineering*. Elsevier, pp. 157–176.
- KOUR, D., RANA, K.L., KAUR, T., YADAV, N., YADAV, A.N., KUMAR, M., KUMAR, V., DHALIWAL, H.S., SAXENA, A.K., 2021. Biodiversity, current developments and potential biotechnological applications of phosphorus-solubilizing and -mobilizing microbes: A review. *Pedosphere* 31, 43–75. [https://doi.org/10.1016/S1002-0160\(20\)60057-1](https://doi.org/10.1016/S1002-0160(20)60057-1)
- Li, H.P., Han, Q.Q., Liu, Q.M., Gan, Y.N., Rensing, C., Rivera, W.L., Zhao, Q., Zhang, J.L., 2023. Roles of phosphate-solubilizing bacteria in mediating soil legacy phosphorus availability. *Microbiol. Res.* 272, 127375. <https://doi.org/10.1016/j.micres.2023.127375>
- Lindsay, W.L., 1979. *Chemical equilibria in soils*. John Wiley and Sons Ltd.
- Lizcano-Toledo, R., Reyes-Martín, M.P., Celi, L., Fernández-Ondoño, E., 2021. Phosphorus dynamics in the soil–plant–environment relationship in cropping systems: A review. *Appl. Sci.* 11, 11133.
- Lu, C., Tian, H., 2017. Global nitrogen and phosphorus fertilizer use for agriculture production in the past half century: shifted hot spots and nutrient imbalance. *Earth Syst. Sci. Data* 9, 181–192. <https://doi.org/10.5194/essd-9-181-2017>
- Mallarino, A.P., Blackmer, A.M., 1992. Comparison of methods for determining critical concentrations of soil test phosphorus for corn. *Agron. J.* 84, 850–856.
- Manzoor, A., Dippold, M.A., Loepmann, S., Blagodatskaya, E., 2022. Two-phase conceptual framework of phosphatase activity and phosphorus bioavailability. *Front. Plant Sci.* 13, 935829.
- Margalef, O., Sardans, J., Fernández-Martínez, M., Molowny-Horas, R., Janssens, I.A., Ciais, P., Goll, D., Richter, A., Obersteiner, M., Asensio, D., 2017. Global patterns of phosphatase activity in natural soils. *Sci. Rep.* 7, 1337.
- Mehnaz, K.R., Corneo, P.E., Keitel, C., Dijkstra, F.A., 2019. Carbon and phosphorus addition effects on microbial carbon use efficiency, soil organic matter priming, gross nitrogen mineralization and nitrous oxide emission from soil. *Soil Biol. Biochem.* 134, 175–186.
- Mehta, S., Nautiyal, C.S., 2001. An Efficient Method for Qualitative Screening of Phosphate-Solubilizing Bacteria. *Curr. Microbiol.* 43, 51–56. <https://doi.org/10.1007/s002840010259>
- Menezes-Blackburn, D., Paredes, C., Zhang, H., Giles, C.D., Darch, T., Stutter, M., George, T.S., Shand, C., Lumsdon, D., Cooper, P., 2016. Organic acids regulation of chemical–microbial phosphorus transformations in soils. *Environ. Sci. Technol.* 50, 11521–11531.
- Muyzer, G., de Waal, E.C., Uitterlinden, A.G., 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-

- amplified genes coding for 16S rRNA. *Appl. Environ. Microbiol.* 59, 695–700.
- Nidhin, I.K., Chattopadhyay, I., 2022. 2 Emerging technologies in environmental microbiology. *Environ. Microbiol. Emerg. Technol.* 33.
- Ohtake, H., Wu, H., Imazu, K., Anbe, Y., Kato, J., Kuroda, A., 1996. Bacterial phosphonate degradation, phosphite oxidation and polyphosphate accumulation. *Resour. Conserv. Recycl.* 18, 125–134.
- Ortiz-Medina, J.F., Grunden, A.M., Hyman, M.R., Call, D.F., 2019. Nitrogen gas fixation and conversion to ammonium using microbial electrolysis cells. *ACS Sustain. Chem. Eng.* 7, 3511–3519.
- Osorio, N.W., Habte, M., 2013. Phosphate desorption from the surface of soil mineral particles by a phosphate-solubilizing fungus. *Biol. Fertil. Soils* 49, 481–486. <https://doi.org/10.1007/s00374-012-0763-5>
- Pastore, G., Kaiser, K., Kernchen, S., Spohn, M., 2020. Microbial release of apatite- and goethite-bound phosphate in acidic forest soils. *Geoderma* 370, 114360. <https://doi.org/https://doi.org/10.1016/j.geoderma.2020.114360>
- Pavinato, P.S., Cherubin, M.R., Soltangheisi, A., Rocha, G.C., Chadwick, D.R., Jones, D.L., 2020. Revealing soil legacy phosphorus to promote sustainable agriculture in Brazil. *Sci. Rep.* 1–11. <https://doi.org/10.1038/s41598-020-72302-1>
- Prabhu, N., Borkar, S., Garg, S., 2019. Chapter 11 - Phosphate solubilization by microorganisms: Overview, mechanisms, applications and advances, in: Meena, S.N., Naik, M.M. (Eds.), *Advances in Biological Science Research*. Academic Press, pp. 161–176. <https://doi.org/https://doi.org/10.1016/B978-0-12-817497-5.00011-2>
- Pradhan, N., Sukla, L.B., 2006. Solubilization of inorganic phosphates by fungi isolated from agriculture soil. *African J. Biotechnol.* 5.
- Prommer, J., Walker, T.W.N., Wanek, W., Braun, J., Zezula, D., Hu, Y., Hofhansl, F., Richter, A., 2020. Increased microbial growth, biomass, and turnover drive soil organic carbon accumulation at higher plant diversity. *Glob. Chang. Biol.* 26, 669–681.
- Rawat, P., Das, S., Shankhdhar, D., Shankhdhar, S.C., 2021. Phosphate-solubilizing microorganisms: mechanism and their role in phosphate solubilization and uptake. *J. Soil Sci. Plant Nutr.* 21, 49–68.
- Řezanka, T., Palyzová, A., Faltýsková, H., Sigler, K., 2019. Siderophores: amazing metabolites of microorganisms. *Stud. Nat. Prod. Chem.* 60, 157–188.
- Rizwanuddin, S., Kumar, Vijay, Singh, P., Naik, B., Mishra, S., Chauhan, M., Saris, P.E.J., Verma, A., Kumar, Vivek, 2023. Insight into phytase-producing microorganisms for phytate solubilization and soil sustainability. *Front. Microbiol.* 14, 1127249.
- Roos, W., Luckner, M., 1984. Relationships between proton extrusion and fluxes of ammonium ions and organic acids in *Penicillium cyclopium*. *Microbiology* 130, 1007–1014.
- Ruttenberg, K.C., 2001. Phosphorus cycle. *Encycl. Ocean Sci.* 4, 401–412.
- Sadaf, N., Haider, M.Z., Iqbal, N., Abualreesh, M.H., Alatawi, A., 2022. Harnessing the Phytase production potential of soil-borne fungi from wastewater irrigated fields based on eco-cultural optimization under shake flask method. *Agriculture* 12, 103.
- Saha, M., Sarkar, S., Sarkar, B., Sharma, B.K., Bhattacharjee, S., Tribedi, P., 2016. Microbial siderophores and their potential applications: a review. *Environ. Sci. Pollut. Res.* 23, 3984–3999.
- Sand, W., 1997. Microbial mechanisms of deterioration of inorganic substrates—a general mechanistic overview. *Int. Biodeterior. Biodegradation* 40, 183–190.
- Scavino, A.F., Pedraza, R.O., 2013. The role of siderophores in plant growth-promoting bacteria, in: *Bacteria in Agrobiolgy: Crop Productivity*. Springer, pp. 265–285.
- Sharma, S., Kumar, V., Tripathi, R.B., 2011. Isolation of phosphate solubilizing microorganism (PSMs) from soil. *J. Microbiol. Biotechnol. Res.* 1, 90–95.
- Sharma, S.B., Sayyed, R.Z., Trivedi, M.H., Gobi, T.A., 2013. Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. *Springerplus* 2, 1–14.
- Sheng, Y., Dong, H., Coffin, E., Myrold, D., Kleber, M., 2022. The Important Role of Enzyme

- Adsorbing Capacity of Soil Minerals in Regulating β -Glucosidase Activity. *Geophys. Res. Lett.* 49, e2021GL097556.
- Silva, L.I. da, Pereira, M.C., Carvalho, A.M.X. de, Buttrós, V.H., Pasqual, M., Dória, J., 2023. Phosphorus-Solubilizing Microorganisms: A Key to Sustainable Agriculture. *Agriculture* 13, 462.
- Sindhu, S.S., Phour, M., Choudhary, S.R., Chaudhary, D., 2014. Phosphorus cycling: prospects of using rhizosphere microorganisms for improving phosphorus nutrition of plants. *Geomicrobiol. Biogeochem.* 199–237.
- Singh, B., Satyanarayana, T., 2011. Microbial phytases in phosphorus acquisition and plant growth promotion. *Physiol. Mol. Biol. Plants* 17, 93–103.
- Sisti, L.S., Flores-Borges, D.N.A., Andrade, S.A.L. de, Koehler, S., Bonatelli, M.L., Mayer, J.L.S., 2019. The role of non-mycorrhizal fungi in germination of the mycoheterotrophic orchid *pogoniopsis schenckii* cogn. *Front. Plant Sci.* 10, 1589.
- Soares, E. V., 2022. Perspective on the biotechnological production of bacterial siderophores and their use. *Appl. Microbiol. Biotechnol.* 106, 3985–4004.
- Solangi, F., Zhu, X., Khan, S., Rais, N., Majeed, A., Sabir, M.A., Iqbal, R., Ali, S., Hafeez, A., Ali, B., 2023. The Global Dilemma of Soil Legacy Phosphorus and Its Improvement Strategies under Recent Changes in Agro-Ecosystem Sustainability. *ACS omega* 8, 23271–23282.
- Souza, R.M. de, Sobral, L.F., Oliveira Junior, A. de, 2020. Maize yield influenced by the residual effects of sedimentary phosphates in high-calcium soil. *Rev. Bras. Eng. Agrícola e Ambient.* 24, 735–740.
- Stewart, J.W.B., Tiessen, H., 1987. Dynamics of soil organic phosphorus. *Biogeochemistry* 4, 41–60. <https://doi.org/10.1007/BF02187361>
- Stumm, W., 1995. *The inner-sphere surface complex: A key to understanding surface reactivity.* ACS Publications.
- Syers, J.K., Johnston, A.E., Curtin, D., 2008. Efficiency of soil and fertilizer phosphorus use. *FAO Fertil. plant Nutr. Bull.* 18, 5–50.
- Tamburini, F., Pfahler, V., Bünemann, E.K., Guelland, K., Bernasconi, S.M., Frossard, E., 2012. Oxygen isotopes unravel the role of microorganisms in phosphate cycling in soils. *Environ. Sci. Technol.* 46, 5956–5962.
- TAO, G.-C., TIAN, S.-J., CAI, M.-Y., XIE, G.-H., 2008. Phosphate-Solubilizing and -Mineralizing Abilities of Bacteria Isolated from Soils. *Pedosphere* 18, 515–523. [https://doi.org/https://doi.org/10.1016/S1002-0160\(08\)60042-9](https://doi.org/https://doi.org/10.1016/S1002-0160(08)60042-9)
- Tian, J., Ge, F., Zhang, D., Deng, S., Liu, X., 2021. Roles of phosphate solubilizing microorganisms from managing soil phosphorus deficiency to mediating biogeochemical P cycle. *Biology (Basel).* 10, 158.
- Tian, P., Razavi, B.S., Zhang, X., Wang, Q., Blagodatskaya, E., 2020. Microbial growth and enzyme kinetics in rhizosphere hotspots are modulated by soil organics and nutrient availability. *Soil Biol. Biochem.* 141, 107662.
- Tiessen, H., 2008. Phosphorus in the global environment 1–7. https://doi.org/10.1007/978-1-4020-8435-5_1
- Tiessen, H., Stewart, J.W.B., Cole, C. V., 1984. Pathways of Phosphorus Transformations in Soils of Differing Pedogenesis. *Soil Sci. Soc. Am. J.* 48, 853–858. <https://doi.org/10.2136/sssaj1984.03615995004800040031x>
- Timofeeva, A., Galyamova, M., Sedykh, S., 2022. Prospects for using phosphate-solubilizing microorganisms as natural fertilizers in agriculture. *Plants* 11, 2119.
- Violante, A., Pigna, M., Ricciardella, M., Gianfreda, L., 2002. Adsorption of phosphate on variable charge minerals and soils as affected by organic and inorganic ligands, in: *Developments in Soil Science.* Elsevier, pp. 279–295.
- Wang, C., Pan, G., Lu, X., Qi, W., 2023. Phosphorus solubilizing microorganisms: potential promoters of agricultural and environmental engineering. *Front. Bioeng. Biotechnol.* 11, 1–5. <https://doi.org/10.3389/fbioe.2023.1181078>
- Wang, X., Phillips, B.L., Boily, J.-F., Hu, Y., Hu, Z., Yang, P., Feng, X., Xu, W., Zhu, M., 2019.

Phosphate sorption speciation and precipitation mechanisms on amorphous aluminum hydroxide. *Soil Syst.* 3, 20.

Webb, E.C., 1992. Enzyme nomenclature 1992. Recommendations of the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology on the Nomenclature and Classification of Enzymes. Academic Press.

Withers, P.J.A., Rodrigues, M., Soltangheisi, A., De Carvalho, T.S., Guilherme, L.R.G., Benites, V.D.M., Gatiboni, L.C., De Sousa, D.M.G., Nunes, R.D.S., Rosolem, C.A., Andreote, F.D., Oliveira, A. De, Coutinho, E.L.M., Pavinato, P.S., 2018. Transitions to sustainable management of phosphorus in Brazilian agriculture. *Sci. Rep.* 8, 1–13. <https://doi.org/10.1038/s41598-018-20887-z>

Yadav, A.N., 2022. Phosphate-solubilizing microorganisms for agricultural sustainability. *J. Appl. Biol. Biotechnol.* 10, 1–6.

Yadav, A.N., Yadav, N., Sachan, S.G., Saxena, A.K., 2019. Biodiversity of psychrotrophic microbes and their biotechnological applications. *J. Appl. Biol. Biotechnol.* 7, 99–108.

Yang, L., Liu, Y., Cao, X., Zhou, Z., Wang, S., Xiao, J., Song, C., Zhou, Y., 2017. Community composition specificity and potential role of phosphorus solubilizing bacteria attached on the different bloom-forming cyanobacteria. *Microbiol. Res.* 205, 59–65.

ANNEX

Annex 1. Multivariate Permutational Analysis of Variance of bacterial community structure using the Adonis test based on Bray Curtis distance (Permutação = 999).

	Df	SumOfSqs	MeanSqs	F Model	R²	p-value
Source P	1	0.62	0.62	3.61	0.23	0.001**
Inoculation	1	0.23	0.28	1.66	0.10	0.098
Source vs Inoculation	1	0.45	0.45	2.60	0.16	0.029 *
Residual	8	1.37	0.17		0.50	
Total	11	2.72			1.00	

Df: degrees of freedom, SumOfSqs: sum of squares, MeanSqs: Mean of squares, **: $p \leq 0.1$, *: $p \leq 0.05$, ns: no significance. RRP: reactive natural phosphate. TSP: triple superphosphate

Annex 2. db-RDA results

db-RDA results				
	Df	SumOfSqs	F	p-value
Model	11	2.72	1.09	0.017**
Residual	18	7.18		

Df: degrees of freedom, SumOfSqs: sum of squares, **: $p < 0.05$

Annex 3. Variance partition in the RDA table

Variance partitioning in RDA			
General metrics	n ^o . of observations	Variance	Total variance
	12	0.50573	5.563
Partition Table			
Tabelas explicativas	Df	R.squared	Adj.R.squared
Source P	1	0.20352	0.12387
Inoculation	1	0.09964	0.00960
Source P + Inoculation	2	0.30316	0.14830
Individual fractions			
Source P Inoculation	1	-	0.13
Inoculation Source P	1	-	0.02
Waste			0.85

Df: degrees of freedom, R.squared: R^2 , Adj.R.squared: R^2 adjusted according to the Bonferroni correction