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Are native phosphate solubilizing Bacteria a relevant alternative to mineral fertilizations for crops? Part II: PSB inoculation enables a halving of P input and improves the microbial community in the rapeseed rhizosphere

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Highlights

- P Solubilizing Bacteria screened from three crops were tested on rapeseed growth.
- PSB strain isolated from Faba bean (F23) allowed a 50% decrease of P fertilization.
- F23 inoculation increased alkaline phosphatase activity in soil surrounding roots.
- Effects on plant growth could also result from induced shifts in microbial community.

Abstract: In this study, three PSB strains belonging to the *Pseudomonas* genus, isolated from soil harvested under rapeseed (Pnapus), winter pea (*Pisum sativum*, Ppea), and faba bean (*Vicia faba*, Pfaba), were tested on rapeseed growth. This test was performed in a greenhouse experiment with four plant replicates in pots per modality during the vegetative stage of rapeseed (10 weeks), in combination with a gradient of P fertilization (P100, P50, P25 and P0 corresponding to 125, 62.5, 31.5 and 0 mg P₂O₅ per plant, respectively). Contrasted results were observed for the three tested strains. PSB from rapeseed (Pnapus) showed no effect on plant biomass or P status. Ppea and Pfaba increased the biomass and P amount in roots when P fertilization was decreased to 50%, but winter pea PSB (Ppea) dramatically decreased plant biomass below this P input threshold. For fertilization at P50 with Ppea and Pfaba strains, an increase in alkaline phosphatase activity was concomitantly observed in the rhizosphere. The structure and diversity of the soil microbial community was then assessed to explore possible indirect effects of Pfaba application. Relative abundance of several bacterial genera potentially involved in biological control against phytopathogens were decreased (*Sphingomonas*, *Acidobacteria* Gp6, *Lysobacter*, *Thermomonas*, and a genus belonging to the *Spartobacteria* class), whereas relative abundance of other genera involved in the nitrogen and sulphur cycles were increased (*Ramlibacter*, *Nocardioides*, *Inhella*, *Bradyrhizobium*₂ and *Thiobacillus*), possibly sustaining even more plant access to essential nutrients.

Keywords: Plant nutrition, phosphorus, biointrans, plant growth-promoting rhizobacteria, beneficial microbiome.

1. Introduction

Rapeseed (*Brassica napus*), a major worldwide oleoproteaginous crop, is characterized by high P requirements (Rashid and Bughio, 1993), leading to massive need of P mineral fertilizer input to cope with the scarcity of available orthophosphate in soil. Reducing this P input to limit cost of production, high reliance on chemical P, and improve the environmental balance of such a crop constitutes an essential prerequisite for agrosystem sustainability (Zaidi et al., 2009). The benefit of using Phosphate Solubilizing Bacteria (PSB), because of their ability to decomplex phosphate from calcium, iron or aluminium P particles (Alori et al., 2017) but also mineralize organic P source (Menezes-Blackburn et al., 2017), represents a reliable alternative in order to promote plant P uptake and growth (Ambreetha and Balachandar, 2019; Kalayu, 2019).

Numerous studies performed PSB screening in various environments, with *in vitro* characterization of P solubilisation abilities of isolated microorganisms and evaluation of possible other traits of Plant Growth Promotion (PGP) (Kalayu, 2019; Khan et al., 2009; Kishore et al., 2015; Zaidi et al., 2009). Further deployment by inoculation of PSB strains, during greenhouse assays, highlighted the promising potential as biofertilizers, with multiple positive effects on P nutrition of various plant species as well as biomass and yield enhancing, leading to a global increase of crop productivity (Bargaz et al., 2021; Kalayu, 2019). However, despite high P solubilization abilities *in vitro* or even beneficial effect on plant growth in controlled conditions, the success of such PSB bioinoculants often lacked sufficient reliability and reproducibility when transferred in soil and even more in field (Arora et al., 2011; Kishore et al., 2015; Martínez-Hidalgo et al., 2019). Improvement of PSB efficiency is now necessary to favour the transfer of this new practice and particularly to ensure the global adhesion of farmers.

One of the major constraints rely on the effective colonization of plant rhizosphere by the PSB strain after inoculation. Because PSB inoculants are often non-native, their effective implementation is largely affected by local soil characteristics and also plant host specificity (Gyaneshwar et al., 1998; Kishore et al., 2015; Patel et al., 2011). Furthermore, colonization of the rhizosphere by the introduced PSB could also be adversely impaired because of high competition and antagonism with the native microbial pool initially present in soil (Arora et al., 2011; Kishore et al., 2015). To maximise the implementation of the inoculated PSB in soil, the choice of relevant microbial strains with suitable rhizospheric competences, adapted to the local soil typology and the specificity of the targeted plant species, could be one critical factor of success (Kishore et al., 2015). Choice of the explored environment for PSB screening and deployment of adequate indicators allowing a pertinent evaluation of strains abilities could constitute an important way of improvement.

Relying exclusively on P solubilization abilities of PSB towards recalcitrant P pool in soil, converted in its available form for plants, could also weaken field-efficiency and success reproducibility of these biofertilizers. Several studies showed that a combined approach based on a reduced input of P mineral fertilizer supported by PSB inoculation was more relevant and could achieved similar crop performance that situation with the recommended P mineral dose (Khan et al., 2009). Thus, in the case of maize, half dose of mineral fertilizer supplemented with PSB inoculation allowed to reach equivalent yield as full P mineral input (Jilani et al., 2007). Similarly, Adnan et al. (2020) highlighted the benefit for maize growth and P nutrition of PSB inoculation combined with application of several mineral and organic P sources, also allowing to mitigate abiotic stress. For potato, El-Yazied and Selim (2007) showed that reducing mineral P input to 70 % of the recommended dose in association with inoculation of microbial biofertilizers enabled to achieve equal yield, with tubers of higher quality. The

exploration of the optimal balance between reduced P input and PSB deployment, to fit crop P requirement in its local soil environment, could constitute an important lever to improve biofertilizers efficiency.

Besides the potential benefit of PSB inoculation on crop growth, assessing the influence of these microbial inoculants on the indigenous soil microbiome, and the possible consequences on soil performance, appears essential in an agroecological context (Martínez-Hidalgo et al., 2019). Although still little studied, several studies highlighted significant shift of soil microbial community consequently to PSB addition (Estrada-Bonilla et al., 2021; Jeong et al., 2013; Liu et al., 2020; Song et al., 2021; Wang et al., 2021). Unfortunately, such microbial modifications could be potentially deleterious, leading to impaired functioning of nutrient cycling (van Elsas et al., 2015). On the contrary, PSM inoculation could result in the stimulation of other indigenous beneficial microbial populations, acting together to promote plant growth (Zaidi et al., 2009). The deployment of microbial biofertilizers in field required the global comprehension of the entire system and to identify the contribution of all factors that could influence crop health.

So, the aim of this study was (1) to assess *in planta* the PSB potential of three strains, previously screened from soils collected under rapeseed (*Brassica napus*), winter pea (*Pisum sativum*) and faba bean (*Vicia faba*) crops (Amy et al., 2022), and combined with a gradient of phosphate fertilization in order to promote P uptake and rapeseed growth, (2) to determine the existence of optimal PSB-fitness profile, (3) to identify processes potentially related to the rapeseed growth enhancement and/or P status and 4) to evaluate the incidence of PSB inoculation on the native microbial community associated with plants.

2. Materials and methods

2.1 PSB screening

A PSB screening has been previously performed from soils taken on plots presenting three different cultivations: rapeseed, winter pea and faba bean. Briefly, soil suspensions were made by mixing 5 g of fresh soil to 45 mL NaCl 0.09%. Serial dilutions of these suspensions were then plated on Pikovskaya (Pikovskaya, 1948) and NBRIP (Nautiyal, 1999) media. Several Plant Growth Promoting Rhizobacteria (PGPR) traits were assessed on each isolated bacterial strain. Siderophore production was evaluated on a Chrome-Azurol-S (CAS) medium as described by Lakshmanan et al. (2015). Indole-3-acetic acid (IAA, auxin) production, ACC deaminase activity and hydrogen cyanide (HCN) production were measured as described by (Kumar et al., 2012). Ability to solubilize phytate were also tested on a phytate screening medium as described by Howson and Davis (1983). Molecular identification was performed by the Sanger sequencing (Genoscreen, Lille, France) of a 16S amplicon obtained from PCR amplification using 63f (5'-CAGGCCTAACACATGCAAGTC-3') (Marchesi et al., 1998) and BU16S4 (5'-CTGCTGCCTCCCGTAGG-3') derived from 341F. Briefly, 2 µL of bacterial cultivation, 1 X of GoTaq® Green Master Mix (Promega, USA) and 1 µM of each primer were mixed in a final volume of 50 µL. PCR reaction was performed in a GeneAmp® PCR System 9700 thermocycler (Applied Biosystems, USA). The amplification program was 2 min at 94°C, followed by 35 cycles of 1 min at 94°C, 1 min at 55°C and 1 min at 72°C. A final extension step was performed for 7 min at 72°C. PCR product quality was verified by electrophoresis on agarose gels stained with ethidium bromide. Obtained sequences were finally aligned using the sequence match tool from the Ribosome Database Project (RDP; Cole et al., 2014). Outcomes about this bacterial screening, and particularly the observed differences in PSB populations between the three explored rhizosphere is described in the article “Are native phosphate

solubilizing bacteria a relevant alternative to mineral fertilizations for crops? Part I. when rhizobacteria meet plant P requirements (Amy et al., 2022).

2.2 Description, cultivation and inoculum preparation of PSB strains

The three tested PSB strains were chosen according to their solubilization abilities and provenance. The five most efficient solubilizers on Pikovskaya or NBRIP media for each native soil were first selected. The quantitative P solubilization of these 15 strains were monitored during 5 days as described by Kumar et al., 2012. Medium pH was also measured in parallel. Finally, one PSB for each soil origin was selected according to their potential of quantitative P solubilization and auxin production abilities. The three selected strains, initially labelled C12, PW2 and F23 during screening experiment, would be thereafter named Pnapus, Ppea and Pfaba, respectively, for better reader's understanding. Potential metabolic phenotypes of these 3 PSB regarding diverse carbon sources were assessed using the Biolog GEN III system (BIOLOG, Inc, Hayward, CA, USA) according to the manufacturer's procedure.

For application on plants, PSB strains were cultivated for 24 h at 30°C in Luria broth. The optical density at 580 nm was measured to evaluate the CFU quantity per mL. Cultivations were then centrifuged and resuspended in NaCl 0.9% to inoculate 1×10^6 CFU in 1 mL directly near the crown of plants.

2.3 Greenhouse experiment

Soil was collected from a cropped field in the Pays de Bray in Normandy, France (49°46'24.197"N, 1°31'45.569"E). The physico-chemical characterization of the soil was previously determined to target a specifically phosphorus-deprived soil. The chosen soil was superficial clay-limestone (USDA) with a water pH of 8.3, deprived of available phosphorus

(Olsen P_2O_5 , 26 ppm) and with a nitrogen content of 1.66 g kg^{-1} dry soil. Soil was sieved then at 5 mm prior to be used in a pot experiment. Rapeseed (*Brassica napus*, cv. Aviso) seeds were surface sterilized and directly sown in 10 L pots containing 4 kg of soil at 70% of its water retention capacity. All plants received 125 mg N (ammonium nitrate 33.5%) and 63 mg KCl, according to French fertilization recommendation from COMIFER (COMIFER, 1995) and the requirements of the planted crop. Four levels of P fertilization were then studied: P100, P50, P25 and P0 corresponding to 125, 62.5, 31.5 and 0 mg P_2O_5 per plant, respectively. The highest level of P fertilization (P100) also corresponds to fertilization recommendations, i.e. 125 mg P_2O_5 brought as a superphosphate (TSP 45%). P50, P25 and P0 modalities were separately inoculated with the PSB strains Pnapus, Pfaba and Ppea or without PSB inoculant at sowing and 15 days after plants emergence. Four replicates of plants (one plant per pot) were achieved for each tested modality. Replicates of all modalities were randomly distributed in the greenhouse space. Plant growth conditions were 16 h days at 20°C and 8 h nights at 12°C with 80% relative humidity. Water irrigation was 30 mL per day, manually completed by weighing, if necessary, to maintain 70% of water retention capacity. A final harvest was done after 10 weeks of cultivation. For each replicate, plants were carefully taken out of pots. Bulk soil and rapeseed roots were first coarsely separated. Remaining soil neighbouring roots were collected as the extended rhizospheric area. This soil was then homogenized and sieved at 2 mm. Soil samples were either stored at -80°C after freezing in liquid nitrogen, for molecular and biochemical analyses, or air-dried, for classical chemical characterization. Shoots and roots were separated, dried at 70°C and grounded after weighing.

2.4 Elemental analyses of plant tissues

Phosphorus content in shoots and roots was directly measured after fine grinding by X-ray fluorescence (XRF, S1 Titan, Bruker, Billerica, MA, USA). Carbon and nitrogen content were assessed by elemental spectroscopy (FlashSmart CHN, ThermoFisher Scientific, Waltham, MA, USA) after the weighing of grinded plant tissue into tin capsules.

2.5 Physico-chemical soil properties assays

Physico-chemical properties of soil samples were assayed by standard methods. Water-pH was determined using the NF ISO 10390 method; soil total and organic carbon were assayed using a total organic carbon (TOC) analyser (Shimadzu SSM-5000A/TOC-VCSH Carbon, Shimadzu, Kyoto, Japan). Soil total nitrogen was determined by the Kjeldahl method (Kjeldahl, 1883). Active calcium carbonate content was assayed following the method described in standard NF-X31-106 (2002). Available P was determined using the Olsen method (NF ISO 11263).

2.6 Enzymatic activities assays

Urease, arylamidase, total phosphatase, acid phosphatase and alkaline phosphatase were assayed in microplates using standard ISO 20130:2018. Briefly, 4 g of soil were weighed and mixed with 25 mL deionized water. Corresponding substrates were added (urea, L-Leucine β -naphthylamide hydrochloride and p-nitro-phenylphosphate disodium salt hexahydrate), except in control wells. Plates were incubated at 37°C for 2 h for arylamidase, 30 min for phosphatases and 25°C during 3 h for urease. Reactions were stopped with the adjunction of ethanol (96%) for urease, 25% ethanol in Trizma (0.1M) pH 12 for phosphatases and a mix of salicylate and cyanurate for urease. Substrate was then added in control wells. Plates were then centrifuged 5 min at 1500 g. Supernatant was transferred to a new plate and read by photometric measurement ($\lambda = 650$ nm for urease, $\lambda = 540$ nm for arylamidase and $\lambda = 405$ nm for phosphatases).

2.7 DNA extraction and quantification

Total DNA was extracted from soil samples using the FastDNA SPIN Kit for soil (MP-Biomedicals, Santa Ana, CA, USA) and following the manufacturers' recommendations. Soil DNA concentrations were assayed by fluorimetry using the Fluorescent DNA Quantitation Kit Hoechst 33258 (Biorad, Hercules, CA, USA) and following the manufacturer's recommendations. DNA samples were then stored at -20°C until further analyses.

2.8 Illumina MiSeq sequencing and amplicon-metagenomics data processing:

Bacterial community composition and structure was assessed by the sequencing of the V3 and V4 regions of the 16S rRNA gene, whereas fungal community structure was assessed by the sequencing of the internal transcribed spacer (ITS 2) region of the 18S rRNA gene. The estimation of the absorbance ratio A_{260} / A_{280} attested DNA purity (with values between 1.96 to 2.16, data not showed). Two-step Nextera PCR libraries using the primer pair 341F (5'- CCT ACG GGN GGC WGC AG-3') (Miran et al., 2017) and 805R (5'- GAC TAC HVG GGT ATC TAA TCC -3') (Miran et al., 2017) and the primer pair gITS7 (5'- GTG AAT CAT CGA RTC TTT G-3') (Ihrmark et al., 2012) and ITS4 (5'- TCC GCT TAT TGA TAT GC-3') (White et al., 1990) were created for the bacterial and fungal communities, respectively. Subsequently, an Illumina MiSeq platform and a v3-600 cycles kit were used to sequence the PCR libraries (Microsynth France, Vaulx-en-Velin, France). The produced paired-end reads that passed Illumina's chastity filter were subjected to the de-multiplexing and trimming of Illumina adaptor residuals using Illumina's bcl2fastq software version v2.20.0.422. The quality of the reads was checked with the software FastQC version 0.11.8, and sequencing reads that fell below an average Q-score of 20 or had any uncalled bases (N) were removed from further analysis. The locus-specific V3-V4 primers and ITS2 primers were trimmed from the

sequencing reads with the software Cutadapt v3.2. Paired-end reads were discarded if the primer could not be trimmed. Trimmed forward and reverse reads of each paired-end read were merged to *in-silico* reform the sequenced molecule considering a minimum overlap of 15 bases using the software USEARCH version 11.0.667. Merged sequences were again quality filtered, allowing a maximum of one expected error per merged read and discarding those containing ambiguous bases. From the remaining reads, the fungal ITS2 sub regions were extracted with help of the ITSx software suite v1.1.3 and its included database. Reads that contain ambiguous bases or that were outliers regarding the amplicon size distribution were also discarded. Samples that resulted in less than 5,000 merged reads were discarded to not distort the statistical analysis. The remaining reads were denoised using the UNOISE algorithm implemented in USEARCH to form operational taxonomic units (OTUs) discarding singletons and chimeras in the process. The resulting OTU abundance table was then filtered for possible barcode bleed-in contaminations using the UNCROSS algorithm. OTU sequences were compared to the reference sequences of the RDP 16S, and the UNITE database and taxonomies were predicted considering a minimum confidence threshold of 0.5 using the SINTAX algorithm implemented in USEARCH. The computational data analysis described in this section was performed by Microsynth AG (Balgach, Switzerland).

2.9 Statistical analyses

All data were analysed using R_{x64} software (4.0.2 version). One-way analysis of variance (ANOVA) of the ‘multcomp’ package was performed for every assay to compare all modalities for each measured variable, followed by a post-hoc LSD Fisher test using the function `LSD.test` from the R package ‘agricolae’. Two-way ANOVA was performed to assess the effects of the PSB application, P fertilization level and interaction of PSB and P fertilization using the `aov`

function of the ‘multcomp’ package. Significant difference was considered for p value < 0.05 . Principal component analysis (PCA) was performed to compare physico-chemical properties of soils, plant profiles and analysis of bacterial and fungal community structures according to the applied fertilization and PSB application, using the package ‘Factoshiny’. Biodiversity indexes were calculated from OTU matrices using the diversity function of the ‘Vegan’ package. Heatmaps for bacterial and fungal phyla and classes were performed using the heatmap function. A Bray–Curtis dissimilarity matrix was calculated using the function `vegdist` of the ‘Vegan’ package.

3. Results

3.1 Characterization of the 3 tested bacterial isolates

Among PSB isolated from soils collected in the vicinity of rapeseed, faba bean and winter pea, the three selected strains (Pnapus, Pfaba and Ppea), belonged to the *Pseudomonas* genus. Their 3 corresponding 16S rRNA gene sequences were deposit in GenBank (Pfaba or F23: OL721595; Ppea or PW2: OL721596; Pnapus or C12: OL721597). They showed solubilization abilities on both the Pikovskaya and NBRIP media (Table 1). Solubilized P on the Pikovskaya medium was comprised between 152.1 and 207.3 $\mu\text{g mL}^{-1}$, combined with a pH decrease for the Pnapus and Ppea strains. On the NBRIP medium, solubilized P was comprised between 49.3 and 104.5 $\mu\text{g mL}^{-1}$, and all strains showed a pH decrease (Table 1). Both Pfaba and Ppea produced gluconic acid in the two media, whereas Pnapus did not show gluconic acid production on the Pikovskaya medium (Table 1). By contrast, the maximal gluconic acid amount in NBRIP medium was observed for Pnapus (17.2 $\mu\text{mol mL}^{-1}$) compared to Pfaba and Ppea (8.3 and 5.4 $\mu\text{mol mL}^{-1}$, respectively). The three strains were able to solubilize phytate on

the PSM medium and produced siderophores and IAA (Table 1). Ppea produced a significantly higher amount of IAA compared to Pfaba and Pnapus. Meanwhile, only Pnapus showed an ability to synthesize HCN. Both Pfaba and Pnapus showed an ACC deaminase activity, but Ppea strain did not (Table 1).

Potential metabolic profiles of these strains regarding the degradation of C sources were determined using the Biolog GENIII system (Figure S1). The Pfaba strain showed a wider metabolic profile with 41 wells showing bacterial growth, compared to 20 positive wells for Pnapus and Ppea. Pfaba showed high capacities in degrading carboxylic acids and amino acids.

3.2 *Effects of P fertilization and PSB adjunction on growth and P and N status of shoots*

The level of P fertilization had a highly significant effect on shoot dry biomass (Table S1, Table 2). As expected, plants receiving total P fertilization (P100) presented the highest shoot dry biomass ($2.80 \text{ g plant}^{-1}$), opposed to plants without P fertilization (P0) (Table 2). Considering P25 fertilization, shoot dry biomasses tended to be intermediate with no significant differences compared to P100 or P0 fertilization. Surprisingly, the application of strain Ppea in combination with P25 fertilization led to a strong decrease in shoot biomass (Table 2). For P50 fertilization, shoot dry biomass fluctuated between $2.11 \text{ g plant}^{-1}$ (Pnapus) and $3.08 \text{ g plant}^{-1}$ (Pfaba) and were not significantly different from P100 fertilization (Table 2). However, under P50 fertilization, the addition of the isolate Pfaba led to the significant increase of the dry biomass of shoots compared to Pnapus.

The P fertilization level and PSB application also had a highly significant effect on P status in shoots (Table S1). As expected, the P amount globally increased with P fertilization rates in the absence of PSB (Table 2). Interestingly, the combination of P50 fertilization with the application of Pfaba or Ppea strains allowed reaching values of P close to that of P100. On the

contrary, a dramatic decrease was observed for P25 fertilization with the inoculation of Ppea strain (7.89 mg).

No significant difference in the N content of shoots was observed whatever the level of P fertilization and the addition of PSB (Table S1, Table 2).

3.3 *Effects of P fertilization and PSB adjunction on the growth and P and N status of roots*

The level of P fertilization alone and in interaction with PSB application, significantly affected root growth (Table S1). Root dry biomass was significantly higher after the application of Pfaba combined with P50 (302.5 mg plant⁻¹ on average), especially when compared to P100 (Table 3). Similarly, after Ppea application combined with P50, root dry biomass tended to be higher than that for P100 (Table 3).

Variations of P amount in roots were mainly explained by P fertilization and also by the interaction between P fertilization and PSB application (Table S1). As expected, and already observed in shoot, the P amount globally increased with P fertilization rates in absence of PSB (Table 3). PSB application with P25 fertilization did not affect the root P amount, except for Ppea. Interestingly, as observed in shoots, only the combination of P50 fertilization with Pfaba or Ppea application allowed to reach similar values to P100 fertilization (Table 3). Contrary to shoots, P fertilization alone or in interaction with PSB application significantly impacted the N status of roots (Table 3). Ppea application tended to decrease the N amount in roots for P25 treatment (1.75 mg). Inversely, for P50 fertilization, the N amount in roots tended to increase with the application of the Pfaba or Ppea strains (5.33 mg and 5.03 mg, respectively) when compared to all other modalities (Table 3).

Taken together, data of the morphometric and elemental contents for P50 treatments, P0 (without PSB) and P100 control were gathered to determine a global profile of plants (Figure S2). PCA showed similar profiles for plants with P50 supplemented with the Pfaba and Ppea strains and closed to P100. Moreover, these profiles were distinct from the global profile obtained by P50 supplement with Pnapus application or no P fertilization (P0).

3.4 Evaluation of soil physico-chemical properties after 10 weeks of cultivation

Soil physico-chemical properties, summarized in Table 4, were measured when plants were harvested, i.e. after 10 weeks of cultivation. Soil pH appeared to be significantly affected by the level of P fertilization alone or combined with PSB application. However induced modifications stayed tenuous (Table 4) and could not be considered as sufficiently conclusive. No significant differences for total carbon (TC) and total organic carbon (TOC) were found among all modalities (Table 4).

By contrast, the level of P fertilization strongly influenced soil total N content (Table 4). P100 fertilization led to higher soil total N (1459.30 ppm) compared to P0, P25 and P50 controls without PSB. Interestingly, the adjunction of PSB, whatever the level of P fertilization, tended to increase the total N content of the soil in comparison to their respective control (Table 4).

Regarding the measure of Olsen P, both P fertilization and PSB application, considered alone or in interaction, strongly influenced available P in soils (Table S1). P25 control showed lower values compared to other controls for all levels of P fertilization (Table 4). Pnapus application significantly increased Olsen P for all P fertilization levels (Table 4), compared to their corresponding control, and even to P100. Similarly, Ppea application increased Olsen P in soil when combined to P0 and P25 treatments.

No clear influence of the level of P fertilization or PSB application on the content of soil calcium carbonate could be highlighted (Table S1). But some tenuous difference could be mentioned. For P25 treatment, application of P_{pea} strain significantly increased calcium carbonate content (64.42%) compared to P25 control (61.41%). Similarly, for P50 treatments, each applied strain significantly increased calcium carbonate values compared to the corresponding control. However, no difference of calcium carbonate content in soil was observed for all tested modalities compared to P100 fertilization.

PCA of soil physico-chemical properties for P50 treatments and P0 and P100 controls showed a slight separation of the global soil pattern for these 3 levels of P fertilizations, in absence of PSB, mainly explained by higher N, calcium carbonate and pH values (Figure 1). P100 and P50 controls also showed higher Olsen P values compared to P0 control. When focused on P50 modalities, soil physico-chemical properties after the application of P_{napus} or P_{faba} strains were clearly separated from the corresponding control, because of higher N, CaCO₃ and pH values. Soil physico-chemical properties of P50+P_{faba} appeared to be highly similar to those from P100 fertilization (Figure 1).

3.5 *Measurement of enzymatic activities in soil*

Several enzymatic activities implied in the functioning of biogeochemical cycles in soil were investigated to evaluate the modifications induced by P fertilization modalities, linked to PSB application.

Interaction of PSB application and P fertilization had a significant influence on urease activity (Table S1). The absence of P fertilization and PSB application (P0 control) led to a strong reduction of urease activity in soil (0.03 mmol NH₄ min⁻¹ g⁻¹), whereas all other fertilizations and PSB applications allowed to maintain a similar level to P100 (Table S2). Arylamidase

activity was also significantly affected by both PSB application and the level of P fertilization, considered alone and in interactions (Table S1). P100 and P0 control showed significantly lower activity when compared to all other fertilization modalities (Table S2). Level of P fertilization, and to a lesser extent in interaction with PSB application, significantly affected phosphatase activity measured at pH 7 (Table S1). A higher value for uninoculated P0 control was found (55.10 nmol PNP min⁻¹ g⁻¹), significantly different of all other controls (Table S2). Pfaba application seemed to decrease phosphatase activity when combined with P0 and P25, comparatively to the corresponding control. Similarly, this enzymatic activity was also decreased for P25+Ppea (47.08 nmol PNP min⁻¹ g⁻¹). No noticeable difference could be found regarding acid phosphatase activity, considering all modalities. On the contrary, alkaline phosphatase activity was clearly affected by PSB application, likewise also in interaction with level P fertilization. No difference was observed between all uninoculated control treatments (Table S2). However, alkaline phosphatase activity tended to be higher following Pfaba or Ppea application in combination with P50, particularly when compared to the P100 control (93.84 nmol PNP min⁻¹ g⁻¹).

Enzymatic profiles were then calculated as the geometric mean of the combination of all previously described enzymatic activities (Table S2). P0, P25 and P50 control showed the highest values, whereas the lowest values were obtained for P100, P25+Ppea and P50+Ppea.

3.6 *P fertilization and PSB effects on bacterial and fungal abundance*

Bacterial abundance, assessed by real-time PCR targeting a portion of 16S rDNA, showed no difference among all levels of P fertilization or after PSB application (Table S3). Meanwhile, fungal abundance, assessed by real-time PCR targeting a portion of 18S rDNA, showed tenuous differences. No noticeable difference was found by comparing P fertilization controls (Table

S3). Pnapus application had no impact on fungal abundance, regardless of the level of P fertilization. Pfaba application decreased fungal abundance when no P fertilization (P0) was provided (1.04×10^6 18S copies of 18S rRNA gene g^{-1} dry soil). On the contrary, Ppea application tends to slightly increase fungal abundance in P25 fertilization (3.46×10^6 copies of 18S rRNA gene g^{-1} dry soil).

3.7 Effects of the level of P fertilization and the inoculation of Pfaba on bacterial and fungal community structure

Because of interesting results on plant growth observed after Pfaba application combined with P50 fertilization, a focus on the diversity and the structure of microbial community associated with the plant rhizosphere was more particularly done for P100, P0, P0+Pfaba, P25, P25+Pfaba and P50 fertilization.

3.7.1 Diversity and richness indices

After the demultiplexing and trimming of raw reads for bacterial and fungal analyses (realized by Microsynth), the number of high-quality sequences retained for each studied modality were summarized in Table S4. Rarefaction curves were drawn and reached the plateau for all samples in both bacterial and fungal analyses (data not shown), making these results sufficiently representative of the associated microbiomes.

Bacterial and fungal richness (Table S5) were identical for all tested modalities. Regarding the diversity Shannon index, the results stayed closed, but some differences were highlighted. Inoculated P50 control showed the lowest observed Shannon index for bacterial communities. Fungal Shannon indexes showed globally no differences, except between P50 with Pfaba

application and P25 control. Same results were found concerning evenness with the Pielou index (Table S5) in both bacterial and fungal communities.

3.7.2 *Microbiome composition*

3.7.2.1 *Bacterial community*

All sequences were assigned at a different taxonomic level, from bacterial phylum to species (Table S6). Among the bacterial community, the dominant phyla (Table 5) were Proteobacteria, followed by Acidobacteria and Actinobacteria. PCA of the overall OTU matrix (Figure 2) depicted a high variation in bacterial community structure and diversity for uninoculated P50 control. However, Pfaba application combined with P50 fertilization appears totally separated from this control as well as from inoculated P0 and P100. All treatments with Pfaba application, whatever the level of P fertilization, appeared to be grouped together, whereas control treatments are more dispersed (Figure 2). Nevertheless, no such clear patterns were found at the phylum and class scales on heatmap analyses (Figures S3 and S4). Here, we decided to focus more precisely on the incidence of Pfaba application on the bacterial community associated with rhizospheric soil, in comparison to the corresponding uninoculated control, at each level of P fertilization. Considering the phyla scale, at P0, both relative proportions of Gemmatimonadetes and Nitrospirae were significantly higher following Pfaba addition (Table 5). At P25, relative proportions of Acidobacteria and Firmicutes were significantly lower following Pfaba addition (Table 5). On the contrary, the relative proportion of Gemmatimonadetes was significantly higher. At P50 fertilization, the relative proportions of Acidobacteria and Verrucomicrobia were significantly lower following Pfaba addition (Table 3). On the contrary, the relative proportion of Bacteroidetes was significantly higher compared to uninoculated HF control. When comparing all Pfaba-inoculated modalities together, only the

relative proportions of Firmicutes were significantly influenced by the level of P fertilization, with a higher proportion for P50+Pfaba (0.49%) compared to 0.42% for both P0 and P25 with the Pfaba strain (Table 5).

At the genus scale, some differences could also be highlighted (Figure 3, Table S7). At P0, compared to non-inoculated control, Pfaba application significantly increased relative abundances of the genera *Gemmatimonas*, *Thermodesulforhabdus* and *Allocatelliglobospora* but decreased those of *Sphingomonas*, *Ferruginibacter*, *Lysobacter*, *Sphingosinicella*, *Thermomonas* and *Spartobacteria* (Table S7). At P25, Pfaba application increased relative abundances of the genera *Gemmatimonas*, *Methyloversatilis*, *Dokdonella*, *Thermodesulforhabdus*, *Bradyrhizobium* and *Kitasatospora* but decreased those of *Acidobacteria-GP6*, *Ferruginibacter*, *Pleomorphobacterium* and *Sorangium* (Table S7). At P50, Pfaba application increased relative abundances of the genera *Ramlibacter*, *Nocardioides*, *Inhella*, *Bradyrhizobium* and *Thiobacillus* but decreased those of *Sphingomonas*, *Acidobacteria-GP6*, *Lysobacter*, *Thermomonas* and *Spartobacteria*. When comparing all Pfaba-inoculated modalities together, relative abundances of some bacterial genera were influenced by the level of associated P fertilization. Proportions of *Angiococcus* and *Thiobacillus* were lower for P0+Pfaba, whereas those of *Lysobacter* were higher. Those of *Ramlibacter* and *Pleomorphobacterium* were lower for P25+Pfaba. Proportions of *Thermodesulforhabdus* were lower for P50+Pfaba, whereas those of *Labilithrix* were higher.

3.7.2.2 Fungal community

All sequences were assigned at different taxonomic levels, from bacterial phylum to species (Table S8). Regarding fungal phyla relative abundance (Table 6), Ascomycota was highly represented, followed by Mortierellomycota and Basidiomycota. Proportions of other fungal

phyla were comprised between 0.14% and 2.90%. Unidentified fungi represented 2% to 3.96% of all fungal OTUs. PCA of fungal OTUs (Figure 4) showed that all modalities with Pfaba inoculation were grouped together, as observed on PCA analysis for bacterial OTUs. However, the distinctions from uninoculated modalities were not clearly observed, such as those for the P0 and P25 levels. Only the P50 fertilization level presented a tenuous separation between uninoculated control and Pfaba application, according to the second axis (Figure 4). In the same way as the bacterial analysis, no such patterns were found on heatmaps at the phylum and class scales (Figures S5 and S6). Once more, as for the bacterial community, we focused on the description of the incidence of Pfaba application on the fungal community associated with rhizospheric soil, in comparison to the corresponding uninoculated control, at each level of P fertilization. Considering the phyla scale, no clear significant differences could be noticed following Pfaba application in comparison to each corresponding uninoculated control, excepted a higher proportion of Glomeromycota for P25+ Pfaba compared to P25 control, P0+Pfaba and P50+Pfaba. A higher variability of fungal phyla's relative abundance was observed compared to bacterial phyla. Interestingly, the global pattern of fungal phyla observed for uninoculated P25 control tended to be tenuously dissimilar from all other modalities (Table 6). Indeed, P25 presented the lowest relative abundance of Ascomycota (77.17%) and the highest relative abundances of Mortierellomycota (11.19%) and Basidiomycota (7.42%).

Regarding the relative abundance of fungal genera, some significant differences could be noted (Figure 5, Table S9). At P0, compared to non-inoculated control, Pfaba application significantly lowered the relative abundance of unidentified *Pucciniomycetes* (Table S9). At P25, Pfaba application significantly decreased the relative abundance of *Agaricales* but increased those of *Myrothecium*, *Podospora* and *Stachybotrys* (Table S9). Under P50 fertilization, Pfaba application significantly lowered the relative abundance of unidentified *Pyrenochaeta* but

increased that of *Rhynchogastrema* (Table S9). When comparing all Pfaba-inoculated modalities together, the relative abundances of some fungal genera were influenced by the level of associated P fertilization. Thus, the relative abundance of *Cladosporium*, *Podospora* and *Rhynchogastrema* were higher for P0+Pfaba, P25+Pfaba and P50+Pfaba, respectively. On the contrary, the proportion of *Podospora* was lower for P25+Pfaba (Figure 5).

4. Discussion

4.1 Key factors of success for PSB strain application

The three tested PSB strains were first selected according to their *in vitro* potential of P solubilization and also considering their associated PGPR traits. Ppea and Pfaba strains showed contrasted solubilization efficiency according to nutrient ratios and forms, between the richer Pikovskaya and the more stringent NBRIP medium. This suggests a metabolic change according to cultivation conditions (Manzoni et al., 2017; Nautiyal, 1999). Even if only a small proportion of soil bacteria were currently successfully cultivated on artificial media (Steen et al., 2019), the isolation of these three *Pseudomonas* isolates among all possible cultivated strains could figure that they were probably not rare members of the bacterial community. Indeed, the rhizosphere of the three investigated crops could potentially harbour abundant *Pseudomonas* populations (pea: Chaudhari et al., 2020; faba bean: Siczek et al., 2020 and rapeseed: Jiménez-Gómez et al., 2020). However, the evaluation of their specific abundance in the initial microbial pool from the sampled field could be interesting for a better characterization and comprehension of the agrosystem.

When these PSB strains were added to soil after rapeseed emergence, a wide variety of plant responses was observed. Pnapus strain, isolated from rapeseed rhizospheric soil, which

presented high *in vitro* solubilization efficiency on both tested media (Pikovskaya and NBRIP) and also interesting PGPR traits, showed no effect on rapeseed growth. On the contrary, the Pfaba strain, isolated from faba bean rhizospheric soil, with lower *in vitro* solubilization abilities on both Pikovskaya and NBRIP media but higher production of gluconic acid and more efficiency to use phytate, presented a promising ability to promote plant growth. Its application on rapeseed seedlings led to an increased biomass in roots compared to plants receiving a total mineral P fertilization (P100) without any adjunction of PSB strains. As Pfaba, Ppea strain tends to increase root growth. However, dramatical deleterious effects were observed when the level of P fertilization associated with Ppea treatment was lower. Furthermore, combined with P25 fertilization, the application of Ppea strain increased the global fungal biomass, suggesting a possible preferential cooperation between Ppea and fungi regarding P acquisition (Zhang et al., 2016). These results highlight the importance of considering the surrounding environmental conditions, particularly the importance of a minimal available P input to activate the PSB potentiality. Indeed, the absence of PSB or PGPR effect on rapeseed growth for P25 fertilization suggests that the initial threshold of available P may not have been sufficient (i) for PSB development and colonization, (ii) for the expression of its PSB potential and (iii) for P solubilization to the benefit of rapeseed growth (i.e. cooperation preference with fungi for microbial P uptake). Another hypothesis is that the response of rapeseed to P starvation could have raised a deleterious crosstalk between plants and microorganisms through plant immune system remodelling (Isidra-Arellano et al., 2021). Indeed, Morcillo et al. (2020) found P starvation-like immune system modulation in *Arabidopsis thaliana* turned the PGPR strain *Bacillus amyloliquefaciens* into a pathogenic phenotype. As some *Pseudomonas* could be plant pathogens (Carezzano et al., 2017; Gislason and de Kievit, 2020), the immune status of plants,

particularly linked to nutrient conditions, must be considered to ensure the success of PSB deployment.

As tested strains were originated from soils under the influence of roots of three different crops, the Pnapus strain originating from the rapeseed environment could be expected to induce superior growth promotion of this crop, because of higher adaptation to the specificity of the host and superior rhizospheric competences allowing efficient colonization and interaction. But this was ultimately not verified here. When regarding the general metabolism of various C sources evaluated through the determination of the substrate utilization pattern of the three tested strains by Biolog analysis, the Pfaba strain showed a more diverse and efficient profile, particularly in carboxylates and amino-acid consumption. As these C forms are known to be abundant in root exudates (Jones, 1998; Mimmo et al., 2011), the PGPR ability to consume these compounds could represent a crucial advantage for their competitiveness with the autochthonous microbial community and thus an efficient colonization of the ecological niche. Indeed, one of the proposed keys of success of PGPR inoculation relies on the efficient colonization of the root environment, including bacterial mobility and the ability of microorganisms to recognize plants recruitment signals, escape immune defences, produce biofilm and produce metabolites of interest for plant growth if surrounding physico-chemical conditions are favourable (Pang et al., 2021; Trivedi et al., 2020).

Malate has been proposed as an important signal metabolite to recruit PGPR in the rhizosphere (Rudrappa et al., 2008). The ability of the three tested PSB strains to degrade it represents a strong clue of their potential of closed specific interaction with rapeseed. Pfaba strain is distinguishable by its capability to use citric acid and succinic acid, exuded by rapeseed

(Shahbaz et al., 2006). Here, we assume that the promising effect of the Pfaba isolate relies on its wider metabolic profile, allowing better colonization of the plant rhizosphere. This strain constitutes the best candidate to enhance rapeseed growth, despite the apparently lower *in vitro* solubilization efficiency. Moreover, these results also highlighted the importance of a minimal combined threshold of available P in a P-deprived soil. Here, in the chosen soil with a low P content, P fertilization could be decreased to a half level compared to the recommended dose, combined with Pfaba application. Because mineral phosphate is generally quickly complexed into unavailable forms when applied to soil (Helfenstein et al., 2018), the combined solution of half-reduced P input and PSB inoculation could also be the optimal one in order to minimize nutrient loss through boosted P solubilization (Elhaissofi et al., 2021). This appears to be a promising alternative to reduce chemical fertilizers. Nevertheless, the chosen soil showed particular characteristics, including a high level of free calcium carbonate content and thus a particularly low available phosphate content (Castro and Torrent, 1994). So, transposition of such results has to be confirmed in another pedoclimatic context. Nevertheless, our results now need to be confirmed on a complete cultivation cycle of rapeseed, particularly to evaluate the consequence on yield and production quality. They also need to be validated through field experiments.

4.2 Biological processes sustaining rapeseed growth

Changes in the bioavailability of nutrients constitutes the main goal for PSB deployment. The initial contribution of the three tested *Pseudomonas* isolates to P solubilization as indigenous microbiota in the sampled field was not known but could have been interesting to investigate deeper. In this greenhouse experiment, both Pnapus and Pfaba strains, combined with P50 fertilization level, led to interesting modifications of soil chemical property profiles, indicating

an action of microorganisms on nutrient availability. Regarding Pnapus application, no effect was observed on plant growth, so no modifications of soil chemical properties were expected. However, following Pnapus inoculation, soil chemical profile was characterized by a slightly higher amount of available P. As free calcium carbonate increased, available P was probably provided by solubilization. Because phosphatase activities are known to be repressed by surrounding P availability (Sinsabaugh and Follstad Shah, 2012), this increase in available P could be sufficient to inhibit organic P mineralization. This phenomenon could explain why alkaline phosphatase activity was still maintained similarly to P50 control (without PSB inoculation), impairing plant growth promotion because of P starvation. On the contrary, rapeseed growth enhancement following Pfaba inoculation occurred concomitantly with modifications of soil chemical properties. Furthermore, Pfaba application led to a modification of the chemical profile of soil, reaching the total fertilization profile. Interestingly, Ppea application did not significantly modify the soil chemical profile compared to P50 control. Meanwhile, as free calcium carbonate increased in the soil following the inoculation of these two strains, we assume that P solubilization occurred. As our results show soils at the final stage, we hypothesize that solubilized P was consumed by plants and/or soil microorganisms. In parallel, microbial alkaline phosphatase activity was increased for P50 fertilization combined with Ppea and Pfaba inoculation. The immediate consumption of the newly available P by plants and microorganisms allowed maintaining activity of organic P mineralization and thus an interesting equilibrium state and continuous P flow.

Even if soil total N tended to increase with PSB inoculation (significantly with Ppea application), no significant increase in the activities of N-mineralizing enzymes was found. Previous studies showed that P availability increased N mineralization (Chen et al., 2016;

Hobbie, 2015), which was not the case here. As peptides and amino acids could be rapidly mineralized (Ma et al., 2017), a past increase of arylamidase activity could not be totally excluded. Other N and P enzymes, such as N-acetyl-glucosaminidase, nucleases or phytases, could provide more information on nutrient sources. Disentangling P solubilization processes and PGPR activities remains sometimes difficult at our current state of knowledge. Here, some evidence of P solubilization activity seemed to be observed but were not systematically correlated with P uptake by plants. Microbial-mediated biological processes did not explain the modifications of the chemical properties of soils, and no coupling between N and P cycles has been demonstrated here. Although some evidence of P solubilization has been observed, its effects on rapeseed growth could also have been the result of PGPR traits such as IAA production or even a combined effect of PGPR and PSM abilities.

4.3 Modifications of the microbial community structure to the benefit of rapeseed growth

PGPR inoculation in soil can induce significant shifts in the structure of the native microbial community (De Zutter et al., 2021), with deleterious or in the contrary beneficial possible consequences (Martínez-Hidalgo et al., 2019; Zaidi et al., 2009). Here, PSB inoculation showed no effect on global indicators for bacteria or fungi abundance. However, microbial abundance was well known to fluctuate according to numerous abiotic environmental factors and through temporal scales (Leitner et al., 2021; Nguyen et al., 2021; Silva et al., 2012). Bacterial and fungal abundances observed here were determined once, at the final plant harvest, and so had to be carefully considered as only mirroring the situation at this specific time of sampling. A deeper exploration was then performed by analyzing the microbiome composition of the different modalities using the strain Pfaba, which appeared to be the most promising actor. Indeed, the Pfaba strain actually modified the associated bacterial communities by increasing

their diversity and evenness when combined with P50 fertilization. On the contrary, no such global effect could be observed for fungal indicators. When comparing all studied modalities, only minor shifts were found regarding the relative abundance of fungal genera. The relative abundance of *Pyrenochaeta* appeared to decrease in the modality P50+Pfaba,. *Pyrenochaeta* had already been described as an infectious agent causing root rots on a wide host spectrum, such as rapeseed, tomato and onion (Bennett et al., 2020; Elsiddig and Hassan, 2017; Testen et al., 2019). Conversely, the relative abundance of *Rhynchogastrema* increased with the treatment P50+Pfaba. The role of *Rhynchogastrema* in soils and plants remained unclear except for its mycoparasitic lifestyle (Begerow et al., 2017). The modulation of the structure of the fungal community after Pfaba inoculation was more tenuous compared to the bacterial compartment. Rhizospheric microbial communities associated with Brassicaceae are well known to be very constrained and principally linked to the production of glucosinolates by plants. In particular, Bressan et al. (2009) showed a stronger impact of these specific compounds on fungi, which was only marginally modified by Pfaba inoculation. Metabarcoding analyses were realized from total genomic DNA directly extracted from soil. Because of fungal forms' multiplicity in soil, such as spores or hyphae, OTUs relative abundance observed here had to be considered with caution. Nevertheless, equality of treatment between modalities, and so shared similar limits of the methodology, made the comparison still relevant.

The impacts of Pfaba application on the bacterial community structure was more conclusive. OTU analysis showed that bacterial community structures were very close following Pfaba inoculation, whatever the P fertilization level, and differentiated from controls with no PSB. These results suggest that plant growth enhancement could also be due to the modification of bacterial community structure following the Pfaba inoculation. However, this positive effect

seemed to be expressed only when the P threshold in soil was sufficient (i.e. for P50 fertilization, particularly). Bacterial relative abundance shifts were significantly observed on some interesting genera, as already described in several studies focused on PGPR inoculations (Chen et al., 2021; De Zutter et al., 2021; Fu et al., 2017; Y. Zhang et al., 2019b). The abundance of 5 genera decreased with PSB under P50 fertilization: *Sphingomonas*, *Acidobacteria-Gp6*, *Lysobacter*, *Thermomonas* and a genus belonging to the *Spartobacteria* class. These genera have already been described as potential biocontrol strains (Innerebner et al., 2011; Zhao et al., 2020) or involved in soil suppressiveness (Seccareccia et al., 2015) but also sometimes as PGPR involved in root growth (Visioli et al., 2018), resistance to osmotic stresses (Videira et al., 2009) and endophytic N₂ fixation (Asaf et al., 2017). Conversely, the bacterial genera *Ramlibacter*, *Nocardioides*, *Inhella*, *Bradyrhizobium* and *Thiobacillus* increased. *Ramlibacter* and *Nocardioides* had already been described as presenting PGPR potential in recent studies (Meena et al., 2020; Zhang et al., 2019a). Indeed, *Ramlibacter* genus, the abundance of which was increased following the application of a PGPR cocktail, positively contributed to disease mitigation and plant growth (Zhang et al., 2019a). The production of growth-enhancing phytohormones has been demonstrated in *Nocardioides*, which could play a role in mitigating salinity stress and promote plant growth in this condition (Meena et al., 2020). To our knowledge, less is known in the literature about the contribution of *Inhella* to plant growth. It had been recently suggested that *Inhella* is involved in cadmium remediation in soils (Xu et al., 2020). *Thiobacillus* was already described as a potentially PGPR enhancing transformation of sulphur in sulphate, contributing to their availability for plants (Gilani et al., 2021). *Bradyrhizobium* is well known as N₂-fixing bacteria, including as free-living fixators (Tao et al., 2021). Taken together, these results highlight a potentially interesting shift in the bacterial vicinity of rapeseed by decreasing the bacteria involved in disease control to the benefit of other

PGPR more involved in plant growth and the N and sulphur cycles. More, sulphur improvement in the vicinity of rapeseed roots could lead to a higher production in glucosinolates (Zhao et al., 1997), suggesting a shift towards this specific plant defense strategy, combined with a modulation of microbiota from biocontrol to nutrition specialization. Interestingly, cadmium-remediating bacteria were enhanced, and the use of mineral P fertilizers was often correlated with metal trace elements, including cadmium (Taylor and Percival, 2001). Because no change in enzyme activities involved in N cycle were found here, the tendency to an enhancement in free-living *Bradyrhizobium* populations following Pfaba application could also explain the observed increase of the content of soil total N.

Because extracellular DNA from dead microorganisms could persist from weeks to years in soil, possibly impacting description on microbial diversity (Carini et al., 2017), all conclusions done here had to be considered with caution. However, all samples probably shared the same previous background of relic DNA, reflecting the previous soil history. Moreover, even if the description of microbial composition and structure observed here after 10 weeks of plant growth might potentially compile both DNA from intact and dead cells, this final microbial diversity eventually constituted the legacy of all induced modifications and so stayed pertinent to discuss to our opinion.

5. Conclusion

In this study, three PSB strains isolated from rapeseed (Pnapus), winter pea (Ppea) and faba bean (Pfaba) were tested on early stages of rapeseed growth combined to a gradient of phosphate fertilization. Despite some clues being found showing a potential of phosphate solubilization in the soil for the Pnapus strain, no effect could be noticed on plant growth. Even if Ppea strain enhanced the growth and P amount in roots when rapeseed received 50% of the

recommended P inputs, this strain was highly deleterious to rapeseed growth below this fertilization rate, which could be very limiting for field deployment. The global benefits of Pfaba strain for sustaining rapeseed growth and P amount in roots under low P fertilization were more relevant, making this strain a promising alternative for the development of an inoculum combined with half-reduced P mineral input. Enhanced plant growth following the application of Pfaba strain could be due to several processes, such as the expression of its own phosphate-solubilizing and PGPR potential through metabolites production, sustained by its successful colonization of rapeseed rhizosphere because of wider range of carbon source catabolism. But this benefit on rapeseed growth could also be the resultant of induced beneficial shifts in the structure of bacterial communities, promoting other bacterial genera known as PGPR and involved in the N and sulphur cycles. The obtained results also emphasized the importance of a minimal required P threshold to initiate the desired positive effects of the phosphate-solubilizing/PGPR isolates on plant growth. The direct impact of Pfaba inoculation on rapeseed root architecture and growth traits in contrast to P and N fertilization could now be investigate, in relation to N and P fertilization. Moreover, the Pfaba effect will have to be validated on the complete cultivation cycle of rapeseed, particularly in field experiments.

A promising perspective could be to explore an approach based on the construction of SynComs, i.e. synthetic microbial communities, allowing to combine all desirable PGPR traits from several microorganisms. Besides artificial PSB inoculation, another alternative could also be to maximise the balance to native beneficial microbial partners associated with plants. Future investigations should now focus on optimizing and shaping plant–microorganisms interactions. Research has to be engaged to better understand the influence of rapeseed exudation on PGPR recruitment, their maintenance in the rhizosphere and their expression of a beneficial phenotype for plants.

Declaration of Interests:

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

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Table 1: *In vitro* characterization of PGPR traits for the 3 tested PSB isolates: P solubilization and pH on Pikovskaya and NBRIP media; production of gluconic acid on both media; phytate solubilization; siderophores, HCN (hydrogen cyanide) and IAA (indole-3-acetic acid) productions; and molecular identification. Results are expressed as the mean value \pm standard deviation. SI: Solubilization Index.

Strain designation		Pnapus	Pfaba	Ppea
Pikovskaya medium	SI	2.3	2.3	1.3
	solubilized P ($\mu\text{g/mL}$)	197.5 ± 1.5	152.1 ± 0.9	207.3 ± 5.2

	medium pH	4.3 ± 0.1	6.7 ± 0.1	4.3 ± 0.0
	Gluconic acid production (µmol/mL)	0.0 ± 0.2	11.0 ± 0.4	6.4 ± 0.4
NBRIP	SI	2.5	1.4	2.1
	solubilized P (µg/mL)	104.5 ± 0.6	49.3 ± 0.3	55.8 ± 0.3
	medium pH	3.7 ± 0.1	3.8 ± 0.1	4.1 ± 0.1
	Gluconic acid production (µmol/mL)	17.2 ± 1.6	8.3 ± 0.1	5.4 ± 0.8
Other traits	Phytates SI	2.3	3.5	2.5
	Siderophore production	+	+	+
	HCN production	+	-	-
	IAA production (ng/µL)	10.6 ± 1.1	18.8 ± 3.7	33.7 ± 3.4
	ACC deaminase activity	+	+	-

		Biomass (g plant ⁻¹)			P content (mg)			N content (mg)		
Table 2: Dry biomass and elemental contents	P100	Ctrl	2.80 ± 0.30	<i>ab</i>	18.52 ± 3.25	<i>a</i>	103.60 ± 15.52	<i>ab</i>		
		Ctrl	1.82 ± 0.07	<i>def</i>	8.95 ± 0.78	<i>cde</i>	74.37 ± 8.26	<i>b</i>		
		P0	Pnapus	1.90 ± 0.24	<i>cdef</i>	9.29 ± 1.51	<i>cde</i>	73.23 ± 4.77	<i>b</i>	
			Pfaba	1.75 ± 0.12	<i>def</i>	8.65 ± 0.85	<i>cde</i>	79.12 ± 0.91	<i>b</i>	
	P25	Ppea	1.68 ± 0.18	<i>ef</i>	7.44 ± 0.72	<i>e</i>	86.47 ± 10.87	<i>ab</i>		
		Ctrl	2.31 ± 0.17	<i>bcde</i>	12.23 ± 1.04	<i>bc</i>	83.03 ± 6.64	<i>b</i>		
		Pnapus	2.41 ± 0.41	<i>abcde</i>	12.48 ± 2.38	<i>bc</i>	84.20 ± 5.63	<i>ab</i>		
		Pfaba	2.35 ± 0.30	<i>abcde</i>	12.83 ± 1.31	<i>bc</i>	87.05 ± 3.70	<i>ab</i>		
	P50	Ppea	1.53 ± 0.22	<i>f</i>	7.89 ± 1.27	<i>de</i>	76.03 ± 5.49	<i>b</i>		
		Ctrl	2.50 ± 0.28	<i>abcd</i>	14.67 ± 2.69	<i>ab</i>	82.12 ± 1.89	<i>b</i>		
		Pnapus	2.11 ± 0.21	<i>bcdef</i>	12.05 ± 1.54	<i>bcd</i>	88.99 ± 6.53	<i>ab</i>		
		Pfaba	3.08 ± 0.38	<i>a</i>	17.41 ± 1.84	<i>a</i>	86.63 ± 1.94	<i>ab</i>		
	Ppea	2.61 ± 0.35	<i>abcd</i>	15.03 ± 2.25	<i>ab</i>	80.96 ± 5.23	<i>b</i>			

(P, N, C) in rapeseed shoots, for each level of fertilization (total, zero, quarter and half) and each tested PSB (control: no PSB inoculation and inoculation with Pnapus, Pfaba or Ppea). Results are expressed as the mean value ± standard deviation. Letters indicate significant differences obtained by post-hoc LSD Fisher test using the function LSD.test from the R package “agricolae” (p -value < 0.05).

Table 3: Dry biomass and elemental contents in roots of rapeseed, for each level of fertilization (total, zero, quarter and half) and each tested PSB (control: no PSB inoculation and inoculation with Pnapus, Pfaba or Ppea). Results are expressed as the mean value ± standard deviation. Letters indicate significant differences obtained by post-hoc LSD Fisher test using the function LSD.test from the R package “agricolae” (p -value < 0.05).

		Biomass (mg plant ⁻¹)			P content (mg)			N content (mg)		
P100	Ctrl	165.00	± 29.01	<i>bcd</i>	0.62	± 0.16	<i>abc</i>	3.46	± 0.69	<i>abcd</i>
	Ctrl	85.00	± 18.49	<i>de</i>	0.18	± 0.04	<i>ef</i>	2.72	± 0.65	<i>bcd</i>
	Pnapus	122.50	± 31.19	<i>cde</i>	0.25	± 0.08	<i>def</i>	2.90	± 0.68	<i>bcd</i>
	Pfaba	87.50	± 16.52	<i>de</i>	0.18	± 0.05	<i>ef</i>	2.17	± 0.36	<i>bcd</i>
	Ppea	82.50	± 12.50	<i>de</i>	0.18	± 0.04	<i>ef</i>	1.96	± 0.21	<i>cd</i>
	Ctrl	162.50	± 53.60	<i>bcd</i>	0.37	± 0.12	<i>cdef</i>	3.58	± 0.93	<i>abcd</i>
P25	Pnapus	200.00	± 65.45	<i>abc</i>	0.47	± 0.16	<i>bcde</i>	3.90	± 1.16	<i>abc</i>
	Pfaba	155.00	± 20.21	<i>bcd</i>	0.38	± 0.05	<i>cdef</i>	3.07	± 0.18	<i>bcd</i>
	Ppea	65.00	± 15.55	<i>e</i>	0.14	± 0.03	<i>f</i>	1.75	± 0.38	<i>d</i>
	Ctrl	177.50	± 30.38	<i>bcd</i>	0.50	± 0.13	<i>bcd</i>	3.98	± 0.56	<i>ab</i>
	Pnapus	85.00	± 15.55	<i>de</i>	0.19	± 0.03	<i>def</i>	2.16	± 0.31	<i>bcd</i>
	pfaba	302.50	± 51.70	<i>a</i>	0.87	± 0.02	<i>ab</i>	5.33	± 0.81	<i>a</i>
	Ppea	247.50	± 52.18	<i>ab</i>	0.74	± 0.18	<i>ab</i>	5.03	± 1.01	<i>a</i>

Table 4: Physico-chemical properties of sampled soils expressed as mean \pm standard deviation. P fertilization (P100), zero fertilization (P0), half fertilization (P50) with or without PSM application, Pnapus, Pfaba or Ppea. Letters indicate significant differences obtained by post-hoc LSD Fisher test using the function LSD.test from the R package “agricolae” (p -value < 0.05).

		pH		Total C (‰)		Organic C (‰)		Total N (ppm)		[P ₂ O ₅] _{Olsen} (ppm)		CaCO ₃ (%)	
P100	Ctrl	8.39 \pm 0.04	<i>abc</i>	86.10 \pm 0.29	<i>a</i>	20.78 \pm 1.47	<i>a</i>	1459.30 \pm 150.02	<i>bce</i>	27.60 \pm 0.77	<i>cde</i>	63.28 \pm 1.93	<i>abc</i>
	Ctrl	8.33 \pm 0.05	<i>e</i>	86.00 \pm 0.43	<i>a</i>	19.58 \pm 0.67	<i>a</i>	1193.15 \pm 339.43	<i>a</i>	25.15 \pm 2.70	<i>efg</i>	62.78 \pm 0.75	<i>abc</i>
P0	Pnapus	8.33 \pm 0.01	<i>cde</i>	86.08 \pm 0.96	<i>a</i>	20.27 \pm 1.59	<i>a</i>	1587.99 \pm 68.28	<i>ac</i>	30.78 \pm 2.32	<i>b</i>	63.35 \pm 0.38	<i>abc</i>
	Pfaba	8.36 \pm 0.01	<i>bcde</i>	85.07 \pm 0.55	<i>a</i>	19.75 \pm 1.70	<i>a</i>	1565.87 \pm 134.79	<i>ab</i>	26.43 \pm 1.39	<i>ef</i>	62.22 \pm 1.51	<i>bc</i>
	Ppea	8.38 \pm 0.01	<i>abc</i>	85.49 \pm 0.92	<i>a</i>	20.48 \pm 1.07	<i>a</i>	1372.58 \pm 122.60	<i>ace</i>	35.23 \pm 3.23	<i>ab</i>	61.10 \pm 3.26	<i>c</i>
P25	Ctrl	8.41 \pm 0.03	<i>a</i>	86.29 \pm 1.01	<i>a</i>	20.18 \pm 0.98	<i>a</i>	1273.55 \pm 137.12	<i>ace</i>	22.10 \pm 0.55	<i>h</i>	61.41 \pm 3.53	<i>c</i>
	Pnapus	8.34 \pm 0.01	<i>cde</i>	86.22 \pm 0.45	<i>a</i>	20.81 \pm 1.24	<i>a</i>	1803.94 \pm 121.10	<i>ace</i>	24.80 \pm 1.44	<i>fg</i>	63.38 \pm 0.63	<i>abc</i>
	Pfaba	8.39 \pm 0.02	<i>ab</i>	86.16 \pm 0.52	<i>a</i>	20.17 \pm 0.53	<i>a</i>	1853.80 \pm 142.53	<i>acd</i>	23.33 \pm 0.72	<i>gh</i>	62.59 \pm 1.11	<i>abc</i>
	Ppea	8.40 \pm 0.03	<i>ab</i>	85.14 \pm 1.11	<i>a</i>	19.43 \pm 1.11	<i>a</i>	1705.50 \pm 67.55	<i>ace</i>	29.67 \pm 1.11	<i>bcd</i>	64.42 \pm 0.52	<i>ab</i>
P50	Ctrl	8.39 \pm 0.02	<i>abc</i>	85.12 \pm 0.98	<i>a</i>	19.40 \pm 1.58	<i>a</i>	1315.66 \pm 226.62	<i>ace</i>	27.19 \pm 0.95	<i>def</i>	61.33 \pm 1.67	<i>c</i>
	Pnapus	8.38 \pm 0.02	<i>abcd</i>	85.70 \pm 0.70	<i>a</i>	19.91 \pm 1.10	<i>a</i>	1459.30 \pm 150.02	<i>ce</i>	29.77 \pm 2.39	<i>bc</i>	64.59 \pm 0.61	<i>ab</i>
	Pfaba	8.37 \pm 0.04	<i>abcde</i>	86.43 \pm 0.87	<i>a</i>	20.50 \pm 1.08	<i>a</i>	1568.99 \pm 40.50	<i>de</i>	25.91 \pm 1.67	<i>ef</i>	64.92 \pm 0.90	<i>a</i>
	Ppea	8.33 \pm 0.06	<i>de</i>	85.65 \pm 0.47	<i>a</i>	19.78 \pm 1.35	<i>a</i>	1756.63 \pm 46.28	<i>e</i>	26.00 \pm 0.39	<i>ef</i>	64.11 \pm 1.32	<i>ab</i>

Table 5 : Bacterial phyla relative abundance expressed as percentages of total sequences obtained by Illumina sequencing. Letters indicate significant differences obtained by post-hoc LSD Fisher test using the function LSD.test from the R package “agricolae” (p-value < 0.05).

	P0		P100		P50		P25	
	Ctrl (mean ± sd)	Pfaba (mean ± sd)	Ctrl (mean ± sd)	Ctrl (mean ± sd)	Pfaba (mean ± sd)	Ctrl (mean ± sd)	Pfaba (mean ± sd)	
Acidobacteria	28.4 ± 0.78 <i>bc</i>	27.6 ± 1.06 <i>c</i>	28.7 ± 0.35 <i>abc</i>	29.3 ± 0.44 <i>ab</i>	27.7 ± 0.88 <i>c</i>	29.5 ± 0.83 <i>a</i>	28.0 ± 0.63 <i>c</i>	
Actinobacteria	21.2 ± 0.94 <i>b</i>	22.5 ± 0.91 <i>ab</i>	21.8 ± 0.91 <i>ab</i>	22.2 ± 1.54 <i>ab</i>	23.1 ± 0.61 <i>a</i>	22.4 ± 0.56 <i>ab</i>	23.0 ± 1.14 <i>a</i>	
Armatimonadetes	0.19 ± 0.01 <i>b</i>	0.18 ± 0.02 <i>ab</i>	0.2 ± 0.02 <i>ab</i>	0.18 ± 0.01 <i>ab</i>	0.2 ± 0.03 <i>a</i>	0.2 ± 0.02 <i>ab</i>	0.19 ± 0.02 <i>a</i>	
Bacteroidetes	3.85 ± 0.13 <i>ab</i>	3.83 ± 0.18 <i>ab</i>	4.03 ± 0.29 <i>ab</i>	3.57 ± 0.39 <i>b</i>	4.06 ± 0.16 <i>a</i>	3.74 ± 0.27 <i>ab</i>	3.7 ± 0.4 <i>ab</i>	
BRC1	0.12 ± 0.01 <i>ab</i>	0.11 ± 0.01 <i>ab</i>	0.11 ± 0.01 <i>ab</i>	0.11 ± 0.02 <i>ab</i>	0.13 ± 0.02 <i>a</i>	0.1 ± 0.02 <i>b</i>	0.12 ± 0.02 <i>ab</i>	
Candidate_div_WPS_1	0.82 ± 0.05 <i>a</i>	0.76 ± 0.03 <i>a</i>	0.76 ± 0.1 <i>a</i>	0.79 ± 0.11 <i>a</i>	0.76 ± 0.04 <i>a</i>	0.73 ± 0.05 <i>a</i>	0.74 ± 0.02 <i>a</i>	
Chloroflexi	0.44 ± 0.11 <i>a</i>	0.38 ± 0.02 <i>ab</i>	0.4 ± 0.04 <i>ab</i>	0.35 ± 0.05 <i>b</i>	0.39 ± 0.05 <i>ab</i>	0.4 ± 0.03 <i>ab</i>	0.39 ± 0.01 <i>ab</i>	
Cyanobacteria	0.24 ± 0.16 <i>a</i>	0.12 ± 0.04 <i>a</i>	0.16 ± 0.05 <i>a</i>	0.11 ± 0.03 <i>a</i>	0.18 ± 0.04 <i>a</i>	0.24 ± 0.07 <i>a</i>	0.18 ± 0.17 <i>a</i>	
Firmicutes	0.44 ± 0.03 <i>ab</i>	0.42 ± 0.03 <i>b</i>	0.46 ± 0.04 <i>ab</i>	0.45 ± 0.05 <i>ab</i>	0.49 ± 0.05 <i>a</i>	0.48 ± 0.03 <i>a</i>	0.42 ± 0.02 <i>b</i>	
Gemmatimonadetes	3.76 ± 0.14 <i>bcd</i>	4.07 ± 0.21 <i>a</i>	3.57 ± 0.17 <i>d</i>	4.01 ± 0.37 <i>ab</i>	3.94 ± 0.11 <i>abc</i>	3.65 ± 0.15 <i>cd</i>	4.22 ± 0.12 <i>a</i>	
Latescibacteria	1.01 ± 0.04 <i>a</i>	1.03 ± 0.13 <i>a</i>	0.98 ± 0.05 <i>a</i>	1.02 ± 0.08 <i>a</i>	1.03 ± 0.07 <i>a</i>	0.99 ± 0.09 <i>a</i>	1.06 ± 0.03 <i>a</i>	
Nitrospirae	0.84 ± 0.09 <i>bc</i>	1.02 ± 0.1 <i>a</i>	0.82 ± 0.14 <i>c</i>	1.01 ± 0.17 <i>ab</i>	0.96 ± 0.09 <i>abc</i>	1.02 ± 0.14 <i>ab</i>	1.06 ± 0.08 <i>a</i>	
Planctomycetes	0.7 ± 0.05 <i>a</i>	0.66 ± 0.02 <i>ab</i>	0.69 ± 0.05 <i>a</i>	0.66 ± 0.12 <i>ab</i>	0.65 ± 0.02 <i>ab</i>	0.6 ± 0.02 <i>b</i>	0.64 ± 0.03 <i>ab</i>	
Proteobacteria	34.3 ± 0.75 <i>ab</i>	33.6 ± 0.51 <i>ab</i>	33.5 ± 0.63 <i>ab</i>	32.3 ± 2.07 <i>b</i>	32.8 ± 0.73 <i>ab</i>	32.2 ± 0.64 <i>b</i>	32.7 ± 1.38 <i>ab</i>	
Verrucomicrobia	3.8 ± 0.26 <i>ab</i>	3.74 ± 0.21 <i>ab</i>	3.8 ± 0.17 <i>ab</i>	4.01 ± 0.4 <i>a</i>	3.68 ± 0.12 <i>b</i>	3.75 ± 0.03 <i>ab</i>	3.65 ± 0.17 <i>b</i>	

Table 6 : Fungal phyla relative abundance expressed as percentages of total sequences obtained by Illumina sequencing. Letters indicate significant differences obtained by post-hoc LSD Fisher test using the function LSD.test from the R package “agricolae” (p-value < 0.05).

	P0		P100		P50		P25	
	Ctrl	Pfaba	Ctrl	Ctrl	Pfaba	Ctrl	Pfaba	
	(mean ± sd)	(mean ± sd)	(mean ± sd)	(mean ± sd)	(mean ± sd)	(mean ± sd)	(mean ± sd)	
Ascomycota	83.53 ± 5.97 <i>ab</i>	85.27 ± 2.01 <i>a</i>	81.76 ± 6.53 <i>ab</i>	83.87 ± 2.18 <i>ab</i>	83.03 ± 1.98 <i>ab</i>	77.17 ± 5.19 <i>b</i>	81.19 ± 7.40 <i>ab</i>	
Basidiomycota	5.81 ± 4.20 <i>ab</i>	3.63 ± 0.36 <i>b</i>	4.48 ± 1.53 <i>ab</i>	3.73 ± 0.68 <i>b</i>	3.98 ± 0.75 <i>b</i>	7.42 ± 2.05 <i>a</i>	4.30 ± 2.63 <i>ab</i>	
Chytridiomycota	0.20 ± 0.17 <i>a</i>	0.16 ± 0.15 <i>a</i>	0.16 ± 0.07 <i>a</i>	0.16 ± 0.06 <i>a</i>	0.24 ± 0.26 <i>a</i>	0.28 ± 0.25 <i>a</i>	0.67 ± 0.97 <i>a</i>	
Glomeromycota	0.30 ± 0.32 <i>ab</i>	0.19 ± 0.09 <i>b</i>	0.26 ± 0.25 <i>ab</i>	0.17 ± 0.07 <i>b</i>	0.14 ± 0.06 <i>b</i>	0.17 ± 0.07 <i>b</i>	0.60 ± 0.47 <i>a</i>	
Mortierellomycota	6.20 ± 0.83 <i>b</i>	7.20 ± 1.89 <i>b</i>	7.40 ± 1.60 <i>b</i>	6.70 ± 0.88 <i>b</i>	7.54 ± 2.22 <i>b</i>	11.19 ± 3.97 <i>a</i>	8.36 ± 3.22 <i>ab</i>	
Mucoromycota	0.20 ± 0.04 <i>a</i>	0.22 ± 0.09 <i>a</i>	0.27 ± 0.19 <i>a</i>	0.23 ± 0.13 <i>a</i>	0.24 ± 0.10 <i>a</i>	0.32 ± 0.04 <i>a</i>	0.20 ± 0.13 <i>a</i>	
Olpidiomycota	0.30 ± 0.18 <i>a</i>	1.10 ± 0.33 <i>a</i>	2.90 ± 4.05 <i>a</i>	1.18 ± 1.00 <i>a</i>	2.83 ± 1.30 <i>a</i>	1.43 ± 1.52 <i>a</i>	1.97 ± 2.24 <i>a</i>	
Unidentified_fungi	3.46 ± 3.32 <i>a</i>	2.22 ± 0.22 <i>a</i>	2.78 ± 1.09 <i>a</i>	3.96 ± 3.05 <i>a</i>	1.99 ± 0.44 <i>a</i>	2.00 ± 0.11 <i>a</i>	2.72 ± 1.09 <i>a</i>	

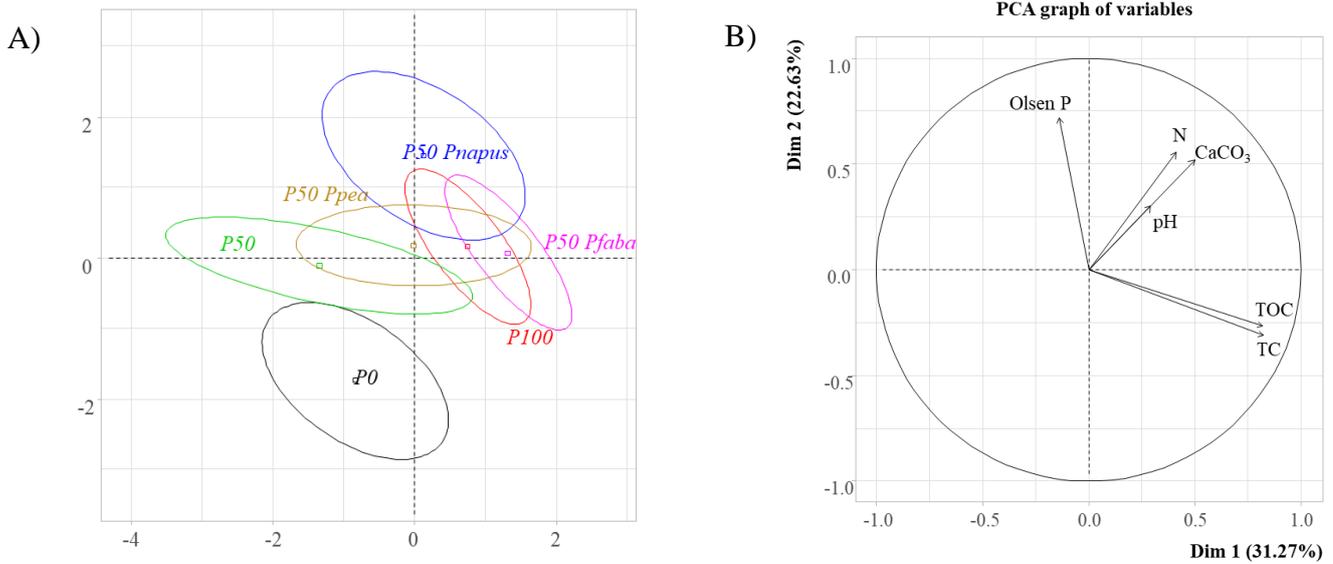


Figure 1: A) Principal Component Analysis of physico-chemical properties of soils receiving a total P fertilization (P100), zero fertilization (P0), half fertilization (P50) with or without PSM application, Pnapus, Pfaba or Ppapa. B) Correlation graphic of tested variables: N; Nitrogen, TOC; Total Organic Carbon, TC; Total Carbon

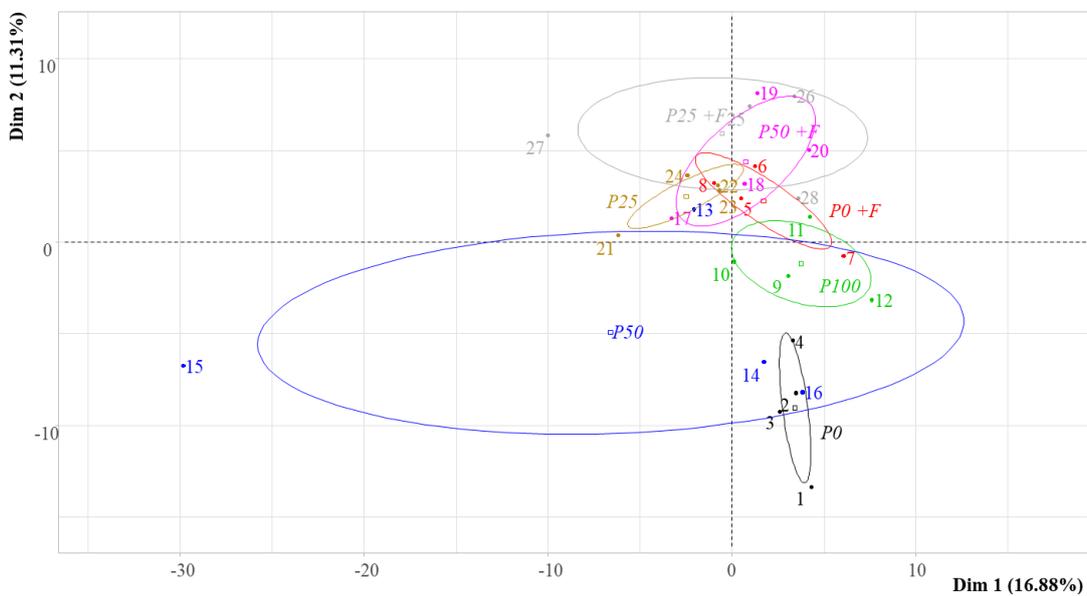


Figure 2: Principal Component Analysis generated from matrices of bacterial OTUs relative abundances, obtained by Illumina sequencing of four replicates per treatments. P0: zero P

fertilization, P100: total P fertilization, P50: half fertilization, P25: quarter fertilization, « +F » means Pfaba PSB application.

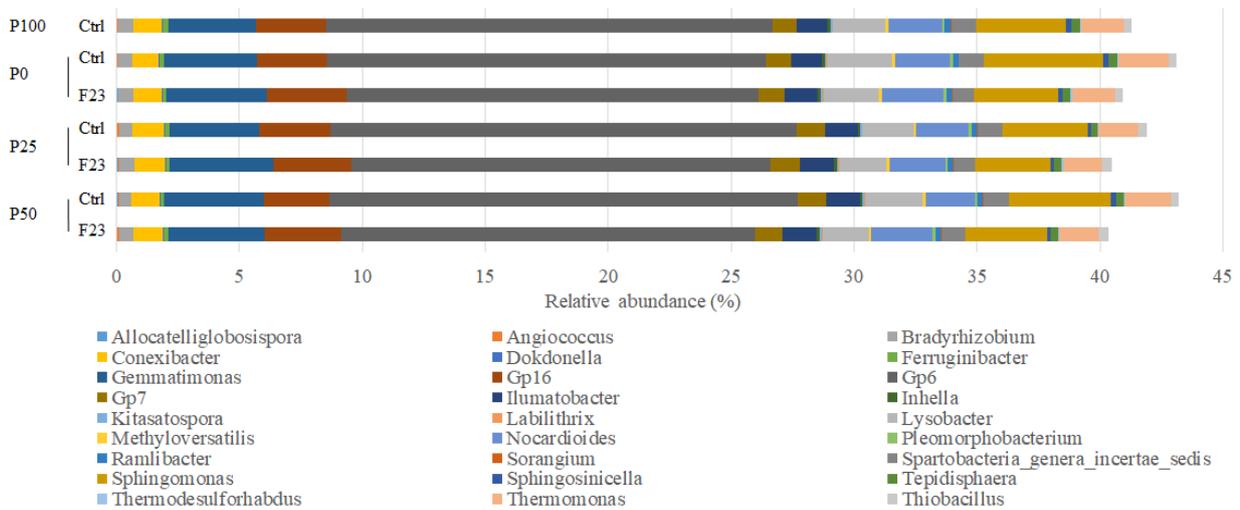


Figure 3: Relative abundance of bacterial genera expressed as percentages of total sequences obtained by Illumina sequencing. Only genera showing significant differences among the different treatments are showed here. P100: Total P fertilization, P0: zero P Fertilization, P25: quarter fertilization, P50: half fertilization, Ctrl: control samples receiving no PSB inoculum, F23: samples receiving the F23 strain

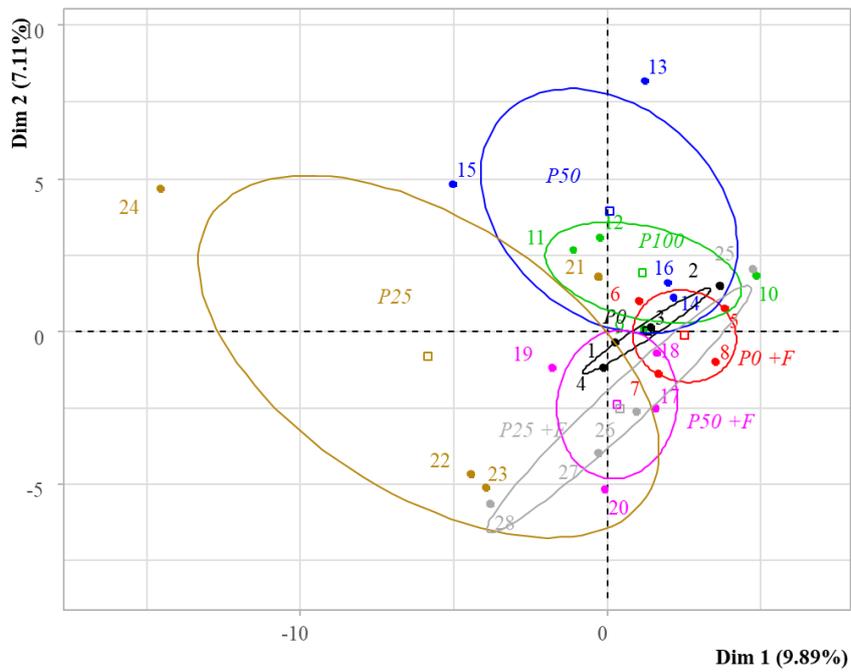


Figure 4: Principal Component Analysis generated from matrices of fungal OTUs relative abundances obtained by Illumina sequencing of four replicates per treatments. P0: zero P fertilization, P100: total P fertilization, P50: half fertilization, P25: quarter fertilization, « +F » means F23 PSB application

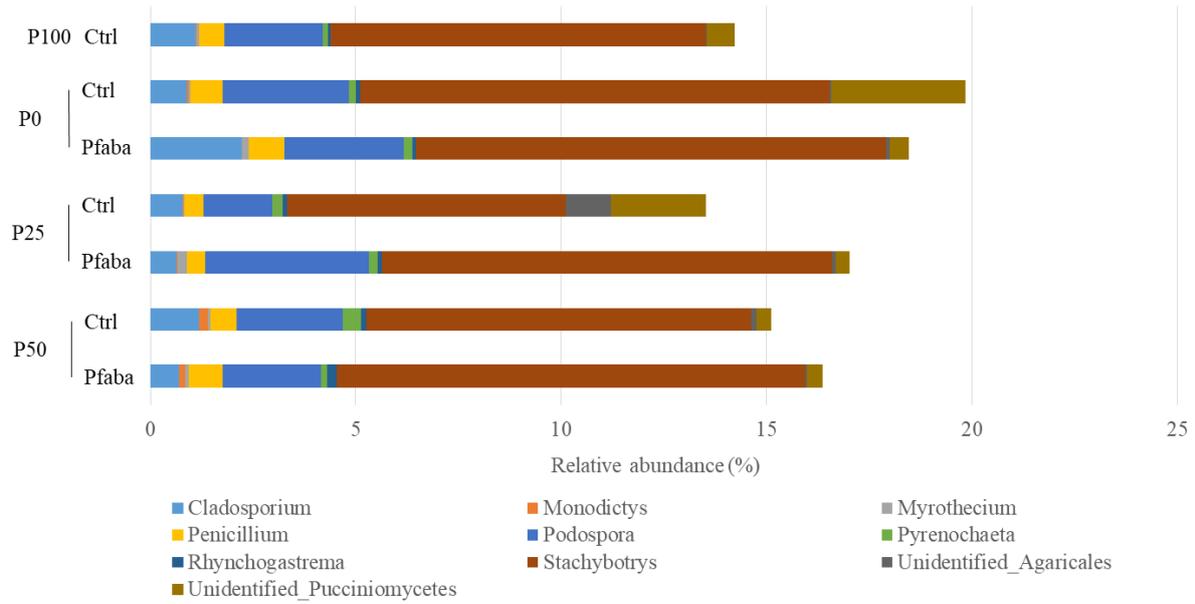


Figure 5: Fungal genera relative abundance expressed as percentages of total ITS sequences obtained by Illumina sequencing realized from total DNA extracted from soils. Only genera showing at least one significant difference among the tested treatments are presented here. P100: Total P fertilization, P0: zero P Fertilization, P25: quarter P fertilization, P50: half P fertilization; Ctrl: control samples receiving no PSB inoculum, F23: samples receiving the F23 strain.

Supplemental data:

Table S1: *p*-values of two-ways ANOVA showing potential effects of PSB application, P fertilization and interactions of PSB and fertilization on each tested variable. Statistical tests were performed using aov function of R (4.0.2) software.

		PSB	P fertilization	PSB x fertilization
Biomass and status of P and N in plants	Shoots dry biomass	0.128	0.001	0.148
	Roots dry biomass	0.328	0.002	0.003
	Total dry biomass	0.148	0.001	0.091
	P amount in shoots	0.023	5.07E-07	0.161
	P amount in roots	0.251	7.40E-05	0.004
	P content in shoots	0.042	9.06E-06	0.964
	P content in roots	0.014	6.73E-08	0.700
	N amount in shoots	0.855	0.031	0.783
	N amount in roots	0.588	0.012	0.014
Soil chemical properties	pH	0.127	0.027	0.013
	Olsen P	1.72E-07	1.45E-05	8.28E-07
	CaCO ₃	0.111	0.076	0.063
	Total Nitrogen	0.651	2.71E-07	0.247
	TOC	0.833	0.479	0.695
	TC	0.273	0.623	0.071
Enzyme activities	Arylamidase	7.60E-09	1.73E-06	2.82E-03
	Urease	0.084	0.122	0.029
	Alkaline phosphatase	0.004	0.273	0.004
	Acid phosphatase	0.948	0.056	0.175
	phosphatase pH 7	0.101	1.17E-04	0.010
Molecular biology analyses	bacterial abundance	0.685	0.280	0.717
	fungal abundance	0.087	0.080	0.532
	bacteria : fungi ratio	0.095	0.191	0.680
	bacterial Shannon index	0.004	0.231	0.192
	bacterial Pielou index	0.004	0.222	0.183
	<i>Sphingomonas</i> relative abundance	3.44E-06	5.86E-05	0.018
	<i>Ramlibacter</i> relative abundance	0.773	0.009	0.099
	Gp6 relative abundance	3.45E-04	0.396	0.536
	<i>Lysobacter</i> relative abundance	0.005	0.004	0.450
	<i>Bradyrhizobium</i> relative abundance	0.049	0.011	0.378
	<i>Thermomonas</i> relative abundance	0.019	0.008	0.211
	<i>Nocardioides</i> relative abundance	0.001	0.378	0.255
	<i>Inhella</i> relative abundance	0.148	0.041	0.050
	<i>Thiobacillus</i> relative abundance	0.089	0.010	0.307
	<i>Spartobacteria</i> relative abundance	0.001	0.325	0.528
	<i>Pyrenochaeta</i> relative abundance	0.466	0.362	0.159
<i>Rhynchogastrema</i> relative abundance	0.106	0.002	0.018	

Table S2: Enzymatic activities in soils linked to N and P cycles realized on four replicates. Enzymatic profiles are calculated as the geometric mean of enzymatic activities (mean \pm standard deviation). ds = dry soil. Letters indicate significant differences obtained by post-hoc LSD Fisher test using the function LSD.test from the R package “agricolae” (p -value < 0.05).

		Urease [mmol NH ₄ min ⁻¹ g ⁻¹ ds]			Arylamidase [nmol B-Naph min ⁻¹ g ⁻¹ ds]			Phosphatase [nmol PNP min ⁻¹ g ⁻¹ ds]			Acid Phosphatase [nmol PNP min ⁻¹ g ⁻¹ ds]			Alkaline Phosphatase [nmol PNP min ⁻¹ g ⁻¹ ds]			Enzymatic profile		
P100	Ctrl	0.14	\pm 0.06	<i>b</i>	2.00	\pm 0.27	<i>b</i>	49.37	\pm 0.39	<i>ac</i>	45.41	\pm 2.02	<i>a</i>	101.74	\pm 10.11	<i>ab</i>	9.06	\pm 1.2	<i>b</i>
P0	Ctrl	0.03	\pm 0.03	<i>c</i>	2.28	\pm 0.44	<i>b</i>	55.10	\pm 1.25	<i>ce</i>	48.39	\pm 3.41	<i>a</i>	107.12	\pm 5.32	<i>ab</i>	17.7	\pm 10.8	<i>a</i>
	Pnapus	0.15	\pm 0.02	<i>ab</i>	3.41	\pm 0.53	<i>a</i>	52.55	\pm 4.46	<i>bc</i>	50.26	\pm 5.13	<i>a</i>	107.84	\pm 6.70	<i>ab</i>	10.7	\pm 0.17	<i>ab</i>
	Pfaba	0.13	\pm 0.03	<i>ab</i>	3.58	\pm 0.66	<i>a</i>	49.82	\pm 1.87	<i>ac</i>	46.25	\pm 2.19	<i>a</i>	102.82	\pm 5.76	<i>ab</i>	10.1	\pm 0.32	<i>ab</i>
	Ppea	0.15	\pm 0.04	<i>b</i>	4.17	\pm 0.43	<i>a</i>	52.97	\pm 2.93	<i>bc</i>	49.53	\pm 6.32	<i>a</i>	109.78	\pm 5.04	<i>b</i>	11.2	\pm 0.56	<i>ab</i>
P25	Ctrl	0.11	\pm 0.01	<i>ab</i>	3.86	\pm 0.62	<i>a</i>	52.09	\pm 1.09	<i>ac</i>	46.82	\pm 2.20	<i>a</i>	106.51	\pm 6.18	<i>ab</i>	15.8	\pm 11.3	<i>ab</i>
	Pnapus	0.16	\pm 0.07	<i>a</i>	4.05	\pm 0.09	<i>a</i>	51.97	\pm 3.73	<i>ac</i>	46.72	\pm 2.24	<i>a</i>	113.54	\pm 2.84	<i>b</i>	11.1	\pm 0.75	<i>ab</i>
	Pfaba	0.13	\pm 0.01	<i>ab</i>	3.59	\pm 0.33	<i>a</i>	47.96	\pm 2.90	<i>ab</i>	44.04	\pm 2.87	<i>a</i>	105.04	\pm 4.30	<i>ab</i>	15.1	\pm 10.4	<i>ab</i>
	Ppea	0.10	\pm 0.06	<i>b</i>	4.19	\pm 0.41	<i>a</i>	47.08	\pm 0.84	<i>ab</i>	45.18	\pm 1.01	<i>a</i>	109.66	\pm 8.49	<i>b</i>	9.31	\pm 2.34	<i>b</i>
P50	Ctrl	0.13	\pm 0.06	<i>ab</i>	3.43	\pm 0.53	<i>a</i>	48.86	\pm 1.64	<i>ab</i>	46.52	\pm 1.34	<i>a</i>	93.84	\pm 5.77	<i>a</i>	14.3	\pm 8.95	<i>ab</i>
	Pnapus	0.11	\pm 0.01	<i>ab</i>	3.94	\pm 0.24	<i>a</i>	46.46	\pm 2.91	<i>a</i>	41.83	\pm 3.16	<i>a</i>	99.86	\pm 4.61	<i>ab</i>	10.3	\pm 1.52	<i>ab</i>
	Pfaba	0.16	\pm 0.03	<i>b</i>	4.38	\pm 0.34	<i>a</i>	49.33	\pm 2.01	<i>ac</i>	47.86	\pm 4.25	<i>a</i>	109.99	\pm 3.93	<i>b</i>	11.1	\pm 0.75	<i>ab</i>
	Ppea	0.13	\pm 0.05	<i>ab</i>	4.06	\pm 0.26	<i>a</i>	50.03	\pm 0.71	<i>ac</i>	45.42	\pm 6.21	<i>a</i>	114.22	\pm 4.93	<i>b</i>	9.23	\pm 2.48	<i>b</i>

Table S3: Microbial abundance of sampled soils obtained by real-time PCR targeted 16S and 18S rRNA genes, according to the fertilization treatments and PSB inoculation. Results are expressed as mean \pm standard deviation of four replicates, ds = dry soil. Letters indicate significant differences obtained by post-hoc LSD Fisher test using the function LSD.test from the R package “agricolae” (p -value < 0.05).

		Bacterial abundance (16S rRNA gene copies g ⁻¹ ds)			Fungal abundance (18S rRNA gene copies g ⁻¹ ds)			16S : 18 S ratio		
		Mean	SD	Signif.	Mean	SD	Signif.	Mean	SD	Signif.
P100	Ctrl	1.64x10 ⁸	± 6.45x10 ⁷	<i>a</i>	1.05x10 ⁶	± 4.57x10 ⁵	<i>ab</i>	164.38	± 65.14	<i>a</i>
P0	Ctrl	1.39x10 ⁸	± 3.62x10 ⁷	<i>a</i>	1.90x10 ⁶	± 5.14x10 ⁵	<i>abc</i>	85.06	± 24.72	<i>bc</i>
	Pnapus	1.19x10 ⁸	± 5.10x10 ⁷	<i>a</i>	1.40x10 ⁶	± 5.79x10 ⁶	<i>ab</i>	91.51	± 8.64	<i>bc</i>
	Pfaba	1.26x10 ⁸	± 1.91x10 ⁷	<i>a</i>	1.04x10 ⁶	± 2.10x10 ⁵	<i>b</i>	123.68	± 22.16	<i>ab</i>
	Ppea	1.17x10 ⁸	± 8.42x10 ⁷	<i>a</i>	1.65x10 ⁶	± 3.34x10 ⁵	<i>a</i>	94.90	± 28.05	<i>bc</i>
P25	Ctrl	2.49x10 ⁸	± 1.88x10 ⁸	<i>a</i>	1.77x10 ⁶	± 1.02x10 ⁶	<i>abc</i>	109.24	± 25.95	<i>abc</i>
	Pnapus	2.10x10 ⁸	± 2.59x10 ⁸	<i>a</i>	3.02x10 ⁶	± 3.13x10 ⁶	<i>abc</i>	61.17	± 54.60	<i>bc</i>
	Pfaba	1.64x10 ⁸	± 1.30x10 ⁸	<i>a</i>	1.42x10 ⁶	± 6.73x10 ⁵	<i>ab</i>	110.68	± 72.26	<i>abc</i>
	Ppea	3.83x10 ⁸	± 3.87x10 ⁸	<i>a</i>	3.46x10 ⁶	± 8.72x10 ⁵	<i>c</i>	57.96	± 21.77	<i>c</i>
P50	Ctrl	1.62x10 ⁸	± 1.65x10 ⁸	<i>a</i>	2.13x10 ⁶	± 1.01x10 ⁶	<i>abc</i>	90.22	± 16.48	<i>bc</i>
	Pnapus	1.39x10 ⁸	± 2.90x10 ⁷	<i>a</i>	1.83x10 ⁶	± 6.53x10 ⁵	<i>abc</i>	84.19	± 33.21	<i>bc</i>
	Pfaba	1.72x10 ⁸	± 8.58x10 ⁷	<i>a</i>	1.69x10 ⁶	± 4.20x10 ⁵	<i>ab</i>	103.82	± 44.37	<i>bc</i>
	Ppea	1.60x10 ⁸	± 5.44x10 ⁷	<i>a</i>	2.47x10 ⁶	± 1.30x10 ⁶	<i>a</i>	56.74	± 12.87	<i>c</i>

Table S4: Number of bacterial and fungal sequences in the sampled soil, obtained by Illumina sequencing and after bioinformatics filtration. P100: Total P fertilization, P0: zero P fertilization, P25: quarter P fertilization, P50: half P fertilization. Ctrl: control samples without PSB inoculum, F23: samples inoculating with the strain F23. Results are expressed as mean \pm standard deviation of four replicates.

		Bacterial high-quality sequences		Bacterial OTUs		Fungal high-quality sequences		Fungal OTUs	
P100		77 494.25	\pm 14 750.98	270.00	\pm 0.50	24 748.25	\pm 6 142.29	116.00	\pm 3.86
P0	Ctrl	91 016.25	\pm 7 861.78	270.00	\pm 0.50	42 539.00	\pm 10 079.94	120.00	\pm 2.52
	Pfaba	99 243.25	\pm 10 197.90	270.00	\pm 0.50	40 074.50	\pm 20 873.12	118.00	\pm 2.71
P25	Ctrl	71 108.25	\pm 11 608.10	270.00	\pm 0.50	12 671.75	\pm 7 948.08	109.00	\pm 21.70
	Pfaba	99 710.00	\pm 20 760.55	270.00	\pm 0.50	25 160.75	\pm 6 613.34	118.00	\pm 3.11
P50	Ctrl	80 106.00	\pm 25 204.59	271.00	\pm 0.58	15 334.00	\pm 9 629.58	113.00	\pm 9.83
	Pfaba	81 566.00	\pm 9 699.39	270.00	\pm 0.50	20 667.25	\pm 1 785.96	118.00	\pm 2.58

Table S5: Diversity indices calculated from OUT abundance obtained by Illumina sequencing in four replicates of sampled soils. Shannon index indicates specific diversity, Pielou index indicates the evenness and richness represents the total numbers of OTUs. P100: Total P fertilization, P0: zero P Fertilization, P25: quarter fertilization, P50: half fertilization, Ctrl: control samples without PSB inoculum, Pfaba : samples inoculating with the strain Pfaba. Letters indicate significant differences obtained by post-hoc LSD Fisher test using the function LSD.test from the R package “agricolae” (p -value < 0.05).

		P0		P100		P50		P25	
		Ctrl	Pfaba	Ctrl	Ctrl	Pfaba	Ctrl	Pfaba	
		(mean ± sd)	(mean ± sd)	(mean ± sd)	(mean ± sd)	(mean ± sd)	(mean ± sd)	(mean ± sd)	
Bacterial communities	Shannon Index	4.41 ± 0.02 <i>ab</i>	4.43 ± 0.03 <i>a</i>	4.41 ± 0.02 <i>ab</i>	4.36 ± 0.06 <i>c</i>	4.44 ± 0.04 <i>a</i>	4.37 ± 0.04 <i>bc</i>	4.42 ± 0.03 <i>ab</i>	
	Richness	270 ± 0.5 <i>a</i>	270 ± 0.5 <i>a</i>	270 ± 0.5 <i>a</i>	271 ± 0.58 <i>a</i>	270 ± 0.5 <i>a</i>	270 ± 0.5 <i>a</i>	270 ± 0.5 <i>a</i>	
	Pielou Index	0.7 ± 0.00 <i>ab</i>	0.7 ± 0 <i>a</i>	0.7 ± 0 <i>ab</i>	0.69 ± 0.01 <i>c</i>	0.71 ± 0.01 <i>a</i>	0.7 ± 0.01 <i>bc</i>	0.7 ± 0 <i>ab</i>	
Fungal communities	Shannon Index	3.37 ± 0.01 <i>ab</i>	3.43 ± 0.05 <i>ab</i>	3.46 ± 0.09 <i>ab</i>	3.43 ± 0.14 <i>ab</i>	3.28 ± 0.26 <i>b</i>	3.51 ± 0.05 <i>a</i>	3.43 ± 0.1 <i>ab</i>	
	Richness	120 ± 2.52 <i>a</i>	118 ± 2.71 <i>a</i>	116 ± 3.86 <i>a</i>	113 ± 9.83 <i>a</i>	118 ± 2.58 <i>a</i>	109 ± 21.7 <i>a</i>	118 ± 3.11 <i>a</i>	
	Pielou Index	0.62 ± 0 <i>ab</i>	0.63 ± 0.01 <i>ab</i>	0.64 ± 0.02 <i>ab</i>	0.63 ± 0.04 <i>ab</i>	0.6 ± 0.05 <i>b</i>	0.65 ± 0.03 <i>a</i>	0.63 ± 0.02 <i>ab</i>	

Table S6: Matrix of all bacterial OTUs detected in each sample, targeting the V3-V4 region of 16S rRNA gene. P100: Total P fertilization, P0: zero P fertilization, P25: quarter P fertilization, P50: half P fertilization, Pnapus: samples inoculating with the strain Pnapus, with 4 replicates per treatment (1,2,3,4).

(Separate file, in excel format)

Table S7: Relative abundance of bacterial genera expressed as percentages of total sequences obtained by Illumina sequencing. Only genera showing significant differences among the treatments are showed here. P100: Total P fertilization, P0: zero fertilization, P25: quarter fertilization, P50: half fertilization, Ctrl: control samples without PSB inoculum, Pnapus: samples inoculating with the strain Pnapus. Letters indicate significant differences obtained by post-hoc LSD Fisher test using the function LSD.test from the R package “agricolae” (p -value < 0.05)

	P0		P100		P50		P25	
	Ctrl	Pnapus	Ctrl	Ctrl	Ctrl	Pnapus	Ctrl	Pnapus
	(mean ± sd)	(mean ± sd)	(mean ± sd)	(mean ± sd)	(mean ± sd)	(mean ± sd)	(mean ± sd)	(mean ± sd)
Sphingomonas	4.82 ± 0.61 <i>a</i>	3.44 ± 0.07 <i>cd</i>	3.68 ± 0.23 <i>c</i>	4.15 ± 0.26 <i>b</i>	3.33 ± 0.10 <i>cd</i>	3.45 ± 0.32 <i>cd</i>	3.05 ± 0.29 <i>d</i>	
Gemmatimonas	3.76 ± 0.14 <i>bcd</i>	4.07 ± 0.21 <i>a</i>	3.57 ± 0.17 <i>d</i>	4.01 ± 0.37 <i>ab</i>	3.94 ± 0.11 <i>abc</i>	3.65 ± 0.15 <i>cd</i>	4.22 ± 0.12 <i>a</i>	
Ramlibacter	0.23 ± 0.02 <i>ab</i>	0.23 ± 0.02 <i>ab</i>	0.27 ± 0.03 <i>a</i>	0.19 ± 0.07 <i>b</i>	0.26 ± 0.00 <i>a</i>	0.20 ± 0.02 <i>b</i>	0.20 ± 0.03 <i>b</i>	
Gp6	17.86 ± 0.54 <i>ab</i>	16.74 ± 1.20 <i>b</i>	18.16 ± 0.50 <i>ab</i>	19.02 ± 1.66 <i>a</i>	16.79 ± 1.15 <i>b</i>	18.99 ± 0.84 <i>a</i>	17.09 ± 0.51 <i>b</i>	
Ferruginibacter	0.19 ± 0.01 <i>a</i>	0.15 ± 0.02 <i>bc</i>	0.19 ± 0.01 <i>a</i>	0.17 ± 0.03 <i>ab</i>	0.15 ± 0.01 <i>bc</i>	0.17 ± 0.03 <i>ab</i>	0.13 ± 0.02 <i>c</i>	
Methyloversatilis	0.12 ± 0.01 <i>bcd</i>	0.14 ± 0.01 <i>ab</i>	0.15 ± 0.02 <i>a</i>	0.11 ± 0.01 <i>c</i>	0.11 ± 0.01 <i>c</i>	0.11 ± 0.03 <i>c</i>	0.15 ± 0.00 <i>a</i>	
Lysobacter	2.61 ± 0.16 <i>a</i>	2.24 ± 0.15 <i>b</i>	2.12 ± 0.36 <i>bc</i>	2.33 ± 0.08 <i>ab</i>	1.87 ± 0.16 <i>c</i>	2.06 ± 0.30 <i>bc</i>	1.89 ± 0.30 <i>c</i>	
Dokdonella	0.05 ± 0.01 <i>ab</i>	0.05 ± 0.00 <i>ab</i>	0.05 ± 0.01 <i>ab</i>	0.03 ± 0.01 <i>c</i>	0.04 ± 0.00 <i>abc</i>	0.04 ± 0.01 <i>bc</i>	0.06 ± 0.02 <i>a</i>	
Sphingosinicella	0.24 ± 0.03 <i>a</i>	0.16 ± 0.01 <i>c</i>	0.21 ± 0.03 <i>ab</i>	0.19 ± 0.04 <i>bc</i>	0.16 ± 0.02 <i>c</i>	0.16 ± 0.02 <i>c</i>	0.16 ± 0.01 <i>c</i>	
Thermodesulforhabdus	0.04 ± 0.01 <i>c</i>	0.07 ± 0.02 <i>a</i>	0.05 ± 0.01 <i>bc</i>	0.05 ± 0.01 <i>bc</i>	0.05 ± 0.01 <i>bc</i>	0.04 ± 0.01 <i>c</i>	0.06 ± 0.01 <i>ab</i>	
Bradyrhizobium	0.56 ± 0.06 <i>bcd</i>	0.58 ± 0.04 <i>abc</i>	0.64 ± 0.08 <i>a</i>	0.51 ± 0.04 <i>c</i>	0.60 ± 0.05 <i>abc</i>	0.55 ± 0.01 <i>bc</i>	0.64 ± 0.05 <i>a</i>	
Ilumatobacter	1.25 ± 0.04 <i>c</i>	1.33 ± 0.03 <i>abc</i>	1.26 ± 0.05 <i>bc</i>	1.36 ± 0.05 <i>a</i>	1.38 ± 0.04 <i>a</i>	1.35 ± 0.08 <i>ab</i>	1.38 ± 0.11 <i>a</i>	
Thermomonas	2.07 ± 0.10 <i>a</i>	1.75 ± 0.11 <i>bc</i>	1.73 ± 0.16 <i>bc</i>	1.87 ± 0.20 <i>ab</i>	1.61 ± 0.19 <i>c</i>	1.59 ± 0.09 <i>c</i>	1.58 ± 0.28 <i>c</i>	
Angiococcus	0.04 ± 0.02 <i>ab</i>	0.04 ± 0.01 <i>b</i>	0.03 ± 0.01 <i>b</i>	0.05 ± 0.03 <i>ab</i>	0.06 ± 0.01 <i>a</i>	0.06 ± 0.01 <i>a</i>	0.05 ± 0.00 <i>ab</i>	
Nocardioides	2.23 ± 0.18 <i>ab</i>	2.48 ± 0.17 <i>a</i>	2.16 ± 0.16 <i>b</i>	2.01 ± 0.05 <i>b</i>	2.46 ± 0.30 <i>a</i>	2.13 ± 0.22 <i>b</i>	2.26 ± 0.12 <i>ab</i>	
Gp16	2.85 ± 0.11 <i>ab</i>	3.24 ± 0.16 <i>a</i>	2.87 ± 0.09 <i>ab</i>	2.71 ± 0.69 <i>b</i>	3.12 ± 0.17 <i>ab</i>	2.88 ± 0.18 <i>ab</i>	3.15 ± 0.16 <i>a</i>	
Inhella	0.11 ± 0.01 <i>ab</i>	0.11 ± 0.01 <i>ab</i>	0.13 ± 0.02 <i>a</i>	0.10 ± 0.02 <i>b</i>	0.14 ± 0.02 <i>a</i>	0.11 ± 0.01 <i>b</i>	0.12 ± 0.02 <i>ab</i>	
Pleomorphobacterium	0.13 ± 0.02 <i>abcd</i>	0.14 ± 0.01 <i>abc</i>	0.10 ± 0.01 <i>d</i>	0.12 ± 0.03 <i>bcd</i>	0.14 ± 0.01 <i>ab</i>	0.14 ± 0.01 <i>a</i>	0.11 ± 0.02 <i>cd</i>	
Labilithrix	0.04 ± 0.01 <i>ab</i>	0.04 ± 0.02 <i>ab</i>	0.02 ± 0.01 <i>c</i>	0.04 ± 0.02 <i>ab</i>	0.05 ± 0.01 <i>a</i>	0.04 ± 0.01 <i>bc</i>	0.03 ± 0.01 <i>bc</i>	
Thiobacillus	0.31 ± 0.01 <i>b</i>	0.31 ± 0.00 <i>b</i>	0.35 ± 0.05 <i>ab</i>	0.31 ± 0.02 <i>b</i>	0.36 ± 0.04 <i>a</i>	0.35 ± 0.03 <i>ab</i>	0.38 ± 0.04 <i>a</i>	
Spartobacteria_genera_incertae_sedis	1.04 ± 0.07 <i>a</i>	0.85 ± 0.04 <i>c</i>	0.96 ± 0.10 <i>abc</i>	1.04 ± 0.13 <i>a</i>	0.92 ± 0.05 <i>bc</i>	0.97 ± 0.06 <i>ab</i>	0.88 ± 0.09 <i>bc</i>	
Kitasatospora	0.07 ± 0.02 <i>abcd</i>	0.09 ± 0.01 <i>a</i>	0.06 ± 0.01 <i>bc</i>	0.07 ± 0.01 <i>abc</i>	0.08 ± 0.02 <i>ab</i>	0.06 ± 0.01 <i>c</i>	0.08 ± 0.01 <i>a</i>	
Tepidisphaera	0.35 ± 0.04 <i>a</i>	0.33 ± 0.01 <i>a</i>	0.33 ± 0.03 <i>a</i>	0.34 ± 0.08 <i>a</i>	0.31 ± 0.02 <i>ab</i>	0.27 ± 0.01 <i>b</i>	0.31 ± 0.01 <i>ab</i>	
Conexibacter	1.07 ± 0.11 <i>c</i>	1.16 ± 0.09 <i>abc</i>	1.15 ± 0.07 <i>bc</i>	1.14 ± 0.16 <i>bc</i>	1.20 ± 0.06 <i>abc</i>	1.29 ± 0.08 <i>a</i>	1.24 ± 0.04 <i>ab</i>	
Allocatelliglobospora	0.03 ± 0.01 <i>b</i>	0.04 ± 0.00 <i>a</i>	0.03 ± 0.01 <i>ab</i>	0.05 ± 0.02 <i>a</i>	0.04 ± 0.00 <i>ab</i>	0.04 ± 0.01 <i>ab</i>	0.04 ± 0.00 <i>a</i>	
Sorangium	0.02 ± 0.01 <i>c</i>	0.03 ± 0.01 <i>bc</i>	0.06 ± 0.03 <i>ab</i>	0.04 ± 0.01 <i>abc</i>	0.03 ± 0.01 <i>bc</i>	0.07 ± 0.04 <i>a</i>	0.04 ± 0.01 <i>bc</i>	
Gp7	1.03 ± 0.07 <i>bc</i>	1.11 ± 0.06 <i>ab</i>	0.96 ± 0.09 <i>c</i>	1.20 ± 0.13 <i>a</i>	1.12 ± 0.05 <i>ab</i>	1.13 ± 0.10 <i>ab</i>	1.19 ± 0.12 <i>a</i>	

Table S8: Matrix of all fungal OTUs detected in each sample, targeting the V3-V4 region of 16S rRNA gene. P100: Total P fertilization, P0: zero P fertilization, P25: quarter P fertilization, P50: half P fertilization, Pnapus: samples inoculating with the strain Pnapus, with 4 replicates per treatment (1,2,3,4).

(Separate file, in excel format)

Table S9: Fungal genera relative abundance expressed as percentages of total sequences obtained by Illumina sequencing. Only genera showing significant differences among the different treatments are showed here. P100: Total P fertilization, P0: zero fertilization, P25: quarter fertilization, P50: half fertilization, Ctrl: control samples without PSB inoculum, F23: samples inoculating with the strain F23. Letters indicate significant differences obtained by post-hoc LSD Fisher test using the function LSD.test from the R package “agricolae” (p -value < 0.05).

	P0		P100		P50		P25	
	Ctrl	Pnapus	Ctrl	Pnapus	Ctrl	Pnapus	Ctrl	Pnapus
	(mean ± sd)	(mean ± sd)						
Cladosporium	0.89 ± 0.27 <i>ab</i>	2.35 ± 2.49 <i>a</i>	1.02 ± 0.33 <i>ab</i>	1.19 ± 0.49 <i>ab</i>	0.70 ± 0.07 <i>b</i>	0.79 ± 0.19 <i>ab</i>	0.64 ± 0.18 <i>b</i>	
Monodictys	0.05 ± 0.09 <i>ab</i>	0.06 ± 0.09 <i>b</i>	0.02 ± 0.04 <i>b</i>	0.20 ± 0.17 <i>a</i>	0.15 ± 0.18 <i>ab</i>	0.02 ± 0.02 <i>b</i>	0.01 ± 0.01 <i>b</i>	
Myrothecium	0.04 ± 0.02 <i>b</i>	0.09 ± 0.07 <i>ab</i>	0.12 ± 0.14 <i>b</i>	0.06 ± 0.06 <i>b</i>	0.08 ± 0.05 <i>b</i>	0.03 ± 0.02 <i>b</i>	0.24 ± 0.22 <i>a</i>	
Penicillium	0.79 ± 0.08 <i>a</i>	0.88 ± 0.11 <i>a</i>	0.64 ± 0.10 <i>ab</i>	0.64 ± 0.38 <i>ab</i>	0.83 ± 0.15 <i>a</i>	0.45 ± 0.22 <i>b</i>	0.45 ± 0.06 <i>b</i>	
Podospora	3.07 ± 0.22 <i>ab</i>	2.90 ± 0.39 <i>ab</i>	2.60 ± 0.71 <i>bc</i>	2.59 ± 0.62 <i>bc</i>	2.39 ± 0.63 <i>bc</i>	1.68 ± 0.55 <i>c</i>	3.98 ± 1.70 <i>a</i>	
Pyrenochaeta	0.16 ± 0.05 <i>b</i>	0.22 ± 0.05 <i>ab</i>	0.14 ± 0.03 <i>b</i>	0.45 ± 0.43 <i>a</i>	0.14 ± 0.01 <i>b</i>	0.25 ± 0.21 <i>ab</i>	0.21 ± 0.10 <i>ab</i>	
Rhynchogastrema	0.12 ± 0.07 <i>bc</i>	0.07 ± 0.04 <i>bc</i>	0.07 ± 0.03 <i>c</i>	0.13 ± 0.01 <i>b</i>	0.24 ± 0.08 <i>a</i>	0.10 ± 0.05 <i>bc</i>	0.10 ± 0.04 <i>bc</i>	
Stachybotrys	11.43 ± 2.10 <i>a</i>	11.13 ± 0.77 <i>a</i>	9.56 ± 1.31 <i>ab</i>	9.37 ± 3.55 <i>ab</i>	11.40 ± 1.68 <i>a</i>	6.79 ± 4.00 <i>b</i>	10.97 ± 1.93 <i>a</i>	
Unidentified_Agaricales	0.04 ± 0.03 <i>b</i>	0.05 ± 0.01 <i>b</i>	0.06 ± 0.05 <i>b</i>	0.11 ± 0.03 <i>b</i>	0.05 ± 0.03 <i>b</i>	1.11 ± 1.18 <i>a</i>	0.08 ± 0.06 <i>b</i>	
Unidentified_Pucciniomycetes	3.26 ± 3.75 <i>a</i>	2.57 ± 4.08 <i>b</i>	0.60 ± 0.42 <i>b</i>	0.36 ± 0.15 <i>b</i>	0.39 ± 0.16 <i>b</i>	2.30 ± 0.55 <i>ab</i>	0.34 ± 0.13 <i>b</i>	

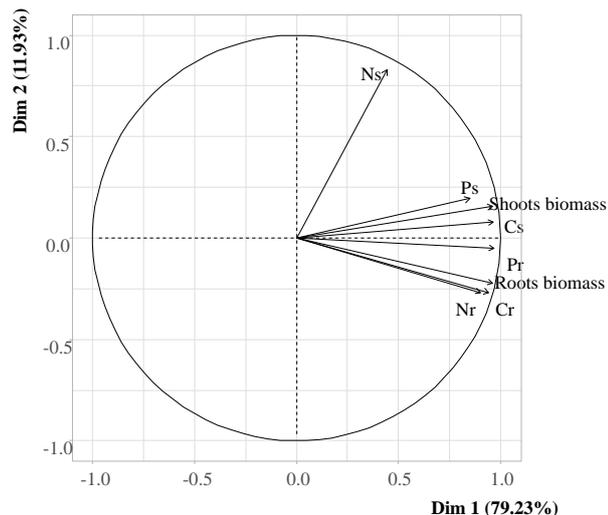
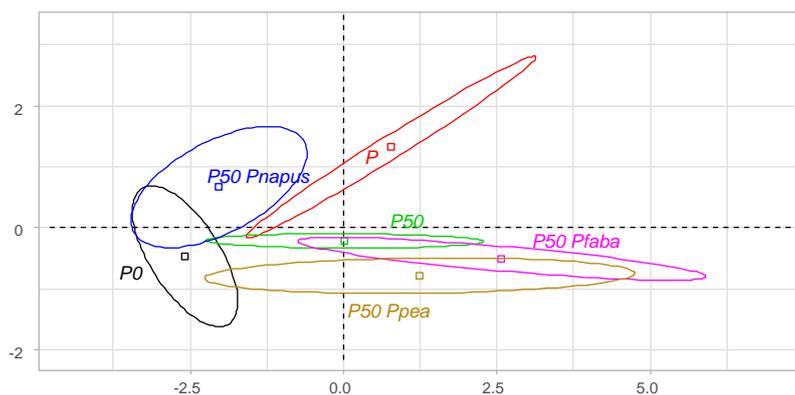


Figure S2: Principal Component Analysis of global plants profiles according to their morphometric characteristics and elemental contents obtained on four replicates. P0 = Zero P fertilization, P = Total P fertilization, P50 = Half fertilization; for the 3 tested isolates (Pnapus, Ppea, Pfaba). Ns = Nitrogen amount in shoots, Ps = Phosphorus amount in shoots, Cs = Carbon amount in shoots, Pr = P amount in roots, Cr = C amount in roots, Nr = N amount in roots.

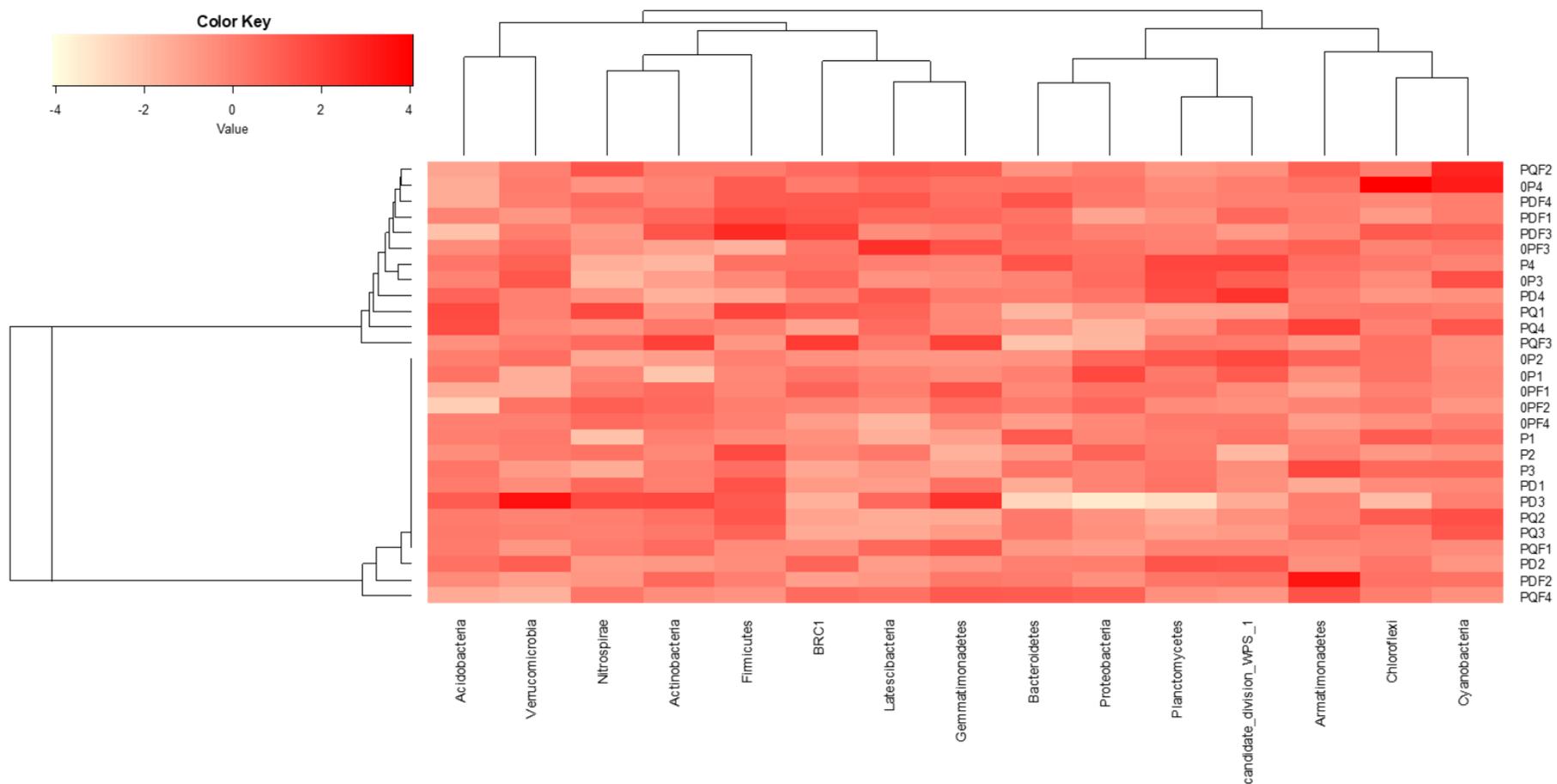


Figure S3: Heatmap of the relative abundance of bacterial phyla obtained by Illumina sequencing realized on four replicates of soils samples and performed following Z-score centered on the median. OP: No fertilization; OPF: no fertilization + Pfaba; PQ: quarter fertilization; PQF: quarter fertilization + Pfaba; PD: half fertilization; PDF: half fertilization + Pfaba; P: total fertilization.

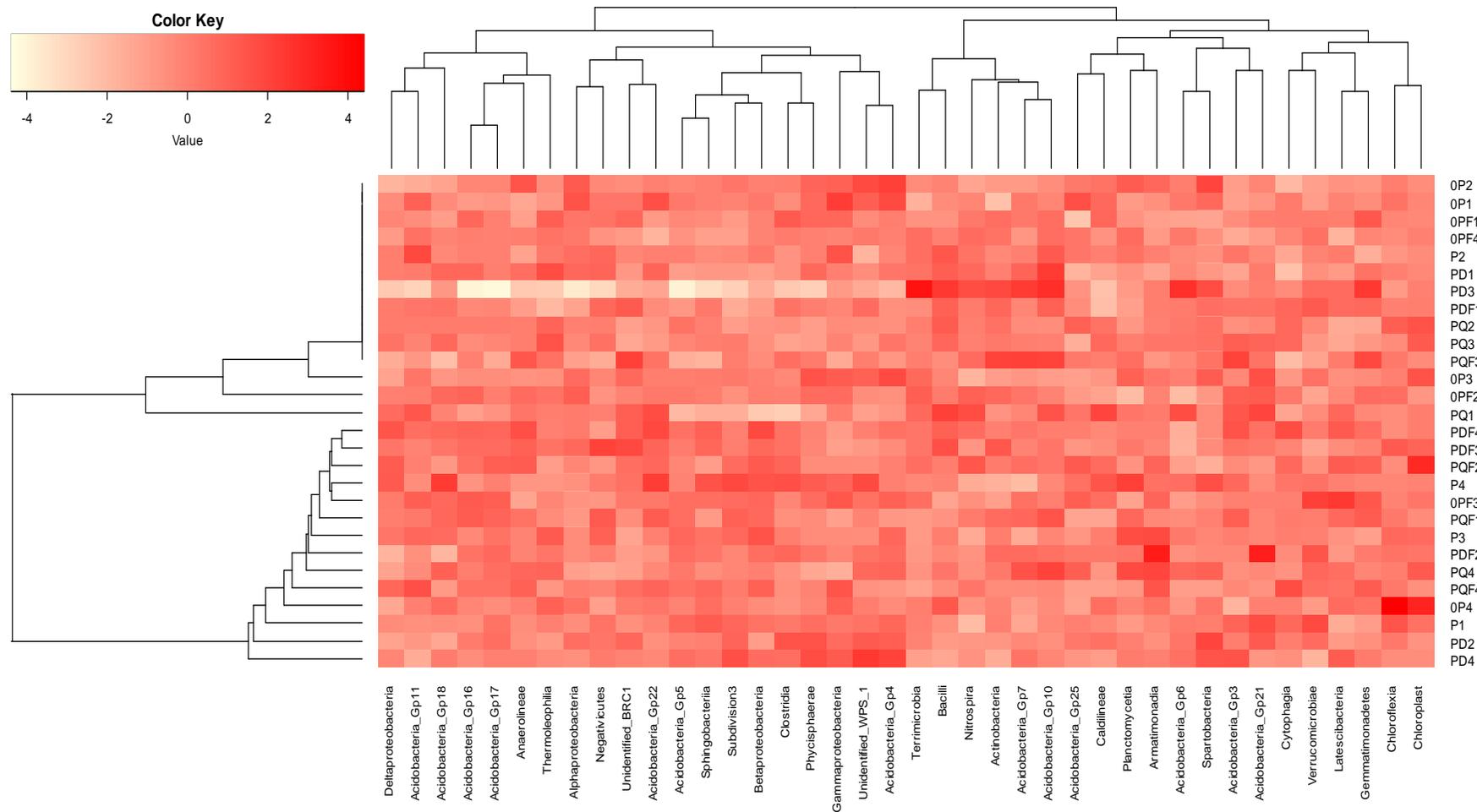


Figure S4: Heatmap of the relative abundance of bacterial classes obtained by Illumina sequencing realized on four replicates of soils samples and performed following Z-score centered on the median. 0P: No fertilization; 0PF: no fertilization + Pfaba; PQ: quarter fertilization; PQF: quarter fertilization + Pfaba; PD: half fertilization; PDF: half fertilization + Pfaba; P: total fertilization.

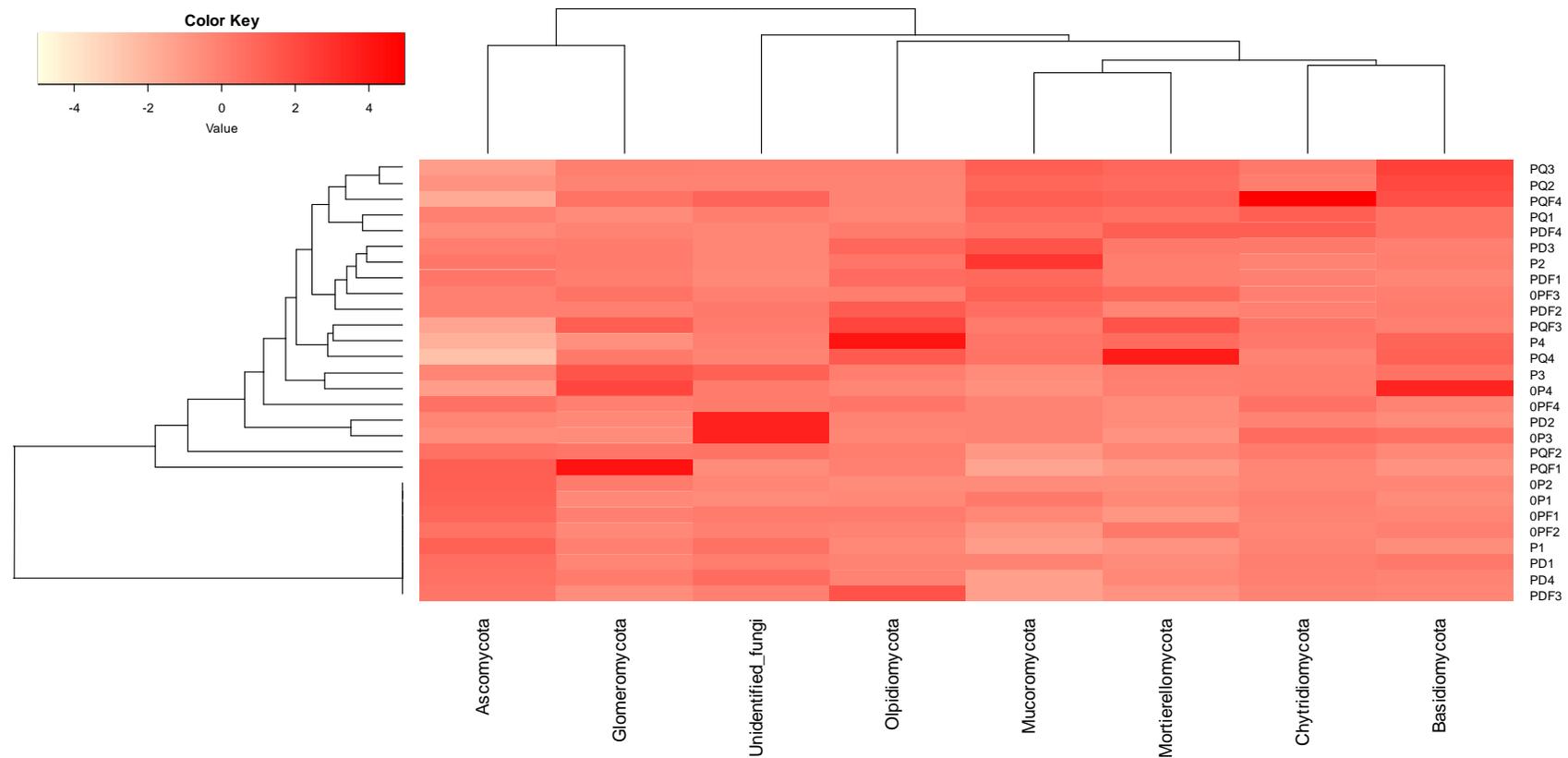


Figure S5: Heatmap of the relative abundance of fungal phyla obtained by Illumina sequencing realized on four replicates of soils samples and performed following Z-score centered on the median. 0P: No fertilization; OPF: no fertilization + Pfaba; PQ: quarter fertilization; PQF: quarter fertilization + Pfaba; PD: half fertilization; PDF: half fertilization + Pfaba; P: total fertilization

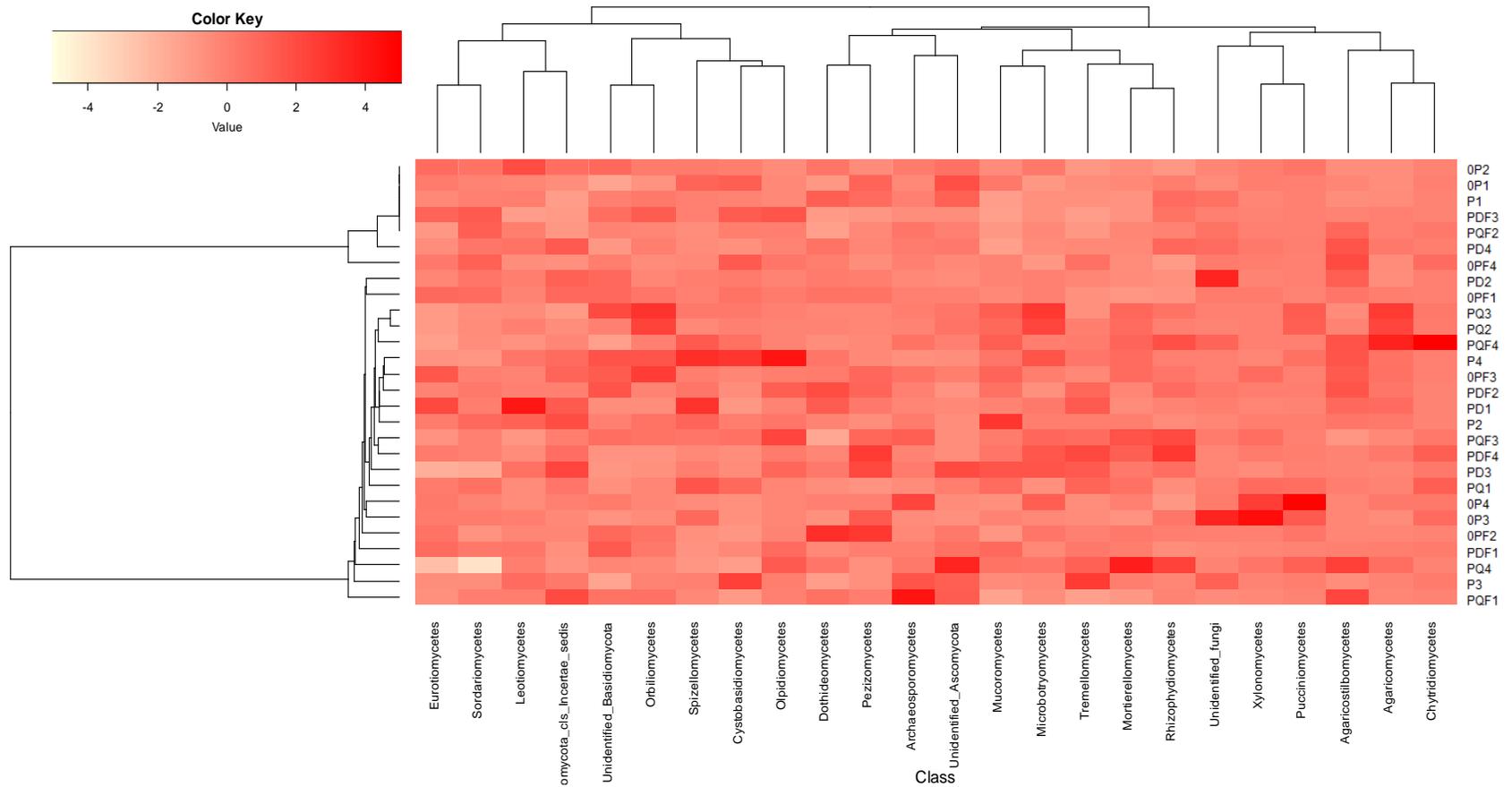


Figure S6: Heatmap of the relative abundance of fungal classes obtained by Illumina sequencing realized on four replicates of soils samples and performed following Z-score centered on the median. 0P: No fertilization; 0PF: no fertilization + Pfaba; PQ: quarter fertilization; PQF: quarter fertilization + Pfaba; PD: half fertilization; PDF: half fertilization + Pfaba; P: total fertilization