

Article

Soil Type-Dependent Interactions of P-Solubilizing Microorganisms with Organic and Inorganic Fertilizers Mediate Plant Growth Promotion in Tomato

Isaac Kwadwo Mpanga ^{1,*} , Harrison Kwame Dapaah ², Joerg Geistlinger ³, Uwe Ludewig ¹ and Günter Neumann ¹

¹ Institute of Crop Science (340h), Universität Hohenheim, Fruwirthstraße 20, 70593 Stuttgart, Germany; u.ludewig@uni-hohenheim.de (U.L.), gd.neumann@t-online.de (G.N.)

² School of Agriculture and Technology, University of Energy and Natural Resources, Sunyani P. O. Box 214, Ghana; hkdapaah@yahoo.com

³ Institute of Bioanalytical Sciences, Anhalt University of Applied Sciences, 06406 Bernburg, Germany; joerg.geistlinger@hs-anhalt.de

* Correspondence: mpangaisaac75@gmail.com or isaac.mpanga@uni-hohenheim.de; Tel.: +49-15217489408

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Abstract: The use of plant growth-promoting microorganisms (PGPMs) as bio-effectors (BEs) to improve the nutrient acquisition of crops has a long history. However, limited reproducibility of the expected effects still remains a major challenge for practical applications. Based on the hypothesis that the expression of PGPM effects depends on soil type and the properties of the applied fertilizers, in this study, the performance of selected microbial inoculants was investigated for two contrasting low-fertility soils supplied with different organic and inorganic fertilizers. Greenhouse experiments were conducted with tomato on an alkaline sandy loam of pH 7.8 and an acidic loamy sand of pH 5.6 with limited phosphate (P) availability. Municipal waste compost, with and without poultry manure (PM), rock phosphate (RP), stabilized ammonium, and mineral nitrogen, phosphorus and potassium (NPK) fertilization were tested as fertilizer variants. Selected strains of *Bacillus amyloliquefaciens* (Priest et al. 1987) Borriss et al. 2011 (FZB42) and *Trichoderma harzianum* Rifai (OMG16) with proven plant growth-promoting potential were used as inoculants. On both soils, P was identified as a major limiting nutrient. Microbial inoculation selectively increased the P utilization in the PM-compost variants by 116% and 56% on the alkaline and acidic soil, while RP utilization was increased by 24%. This was associated with significantly increased shoot biomass production by 37–42%. Plant growth promotion coincided with a corresponding stimulation of root growth, suggesting improved spatial acquisition of soluble soil P fractions, associated also with improved acquisition of nitrogen (N), potassium (K), magnesium (Mg), and calcium (Ca). There was no indication for mobilization of sparingly soluble Ca phosphates via rhizosphere acidification on the alkaline soil, and only mineral NPK fertilization reached a sufficient P status and maximum biomass production. However, on the moderately acidic soil, FZB42 significantly stimulated plant growth of the variants supplied with Ca–P in the form of RP + stabilized ammonium and PM compost, which was equivalent to NPK fertilization; however, the P nutritional status was sufficient only in the RP and NPK variants. The results suggest that successful application of microbial biofertilizers requires more targeted application strategies, considering the soil properties and compatible fertilizer combinations.

Keywords: bio-effector (BE); compost; tomato; phosphate mobilization; phosphorus recovery efficiency (PRE); poultry manure (PM); biofertilizer; nitrogen; *Trichoderma harzianum*; *Bacillus amyloliquefaciens* FZB42

1. Introduction

The world's total mineral NPK fertilizer consumption is projected to reach approximately 225,000,000 metric tons by 2030, representing an average increase of 20% against levels recorded in 2010 [1], with particularly high demands in horticultural production systems. The sole dependency on synthetic mineral fertilizers threatens the environment by high energy requirements for fertilizer production from limited natural resources, and unwanted losses by leaching, runoff, and volatilization, contributing to eutrophication and greenhouse gas emissions [2,3]. Moreover, in developing countries, many farmers are smallholders without much income, and they frequently face problems with covering the costs for mineral fertilizers. Approaches to convert waste materials into resources, and using fertilizers based on organic and inorganic waste materials could offer more sustainable and cost-saving perspectives. Products of interest comprise composts, digestates, manures, products of waste water recycling, slags, and ashes but also less processed fertilizers, including rock phosphates. However, apart from potential contaminants (i.e., heavy metals, antibiotics, abundance of antibiotic resistance genes, or pathogenic microorganisms), the low solubility of plant nutrients, and the large proportions of nutrients sequestered in organic binding forms that are not readily available for plant uptake, are major challenges for the use of organic and inorganic waste materials as fertilizers in agricultural and horticultural practice [4,5]. Therefore, a fertilization management plan that is adapted to the crop demand is even more complicated as compared with mineral fertilizers, and is associated with a high risk of nutrient losses into the environment [4] and low fertilizer use efficiency. The use of microbial inoculants with root growth-promoting and nutrient-mobilizing properties as an option for improving plant nutrient acquisition has been discussed for decades [6–9], and may therefore also contribute to the acquisition of nutrients from fertilizers based on organic and inorganic waste materials [4]. However, a lack of reproducibility of the expected effects, particularly under field conditions, still remains a major challenge for practical applications [10]. There is increasing evidence for selective interactions between the form and the amount of fertilizers and microbial inoculants. In a meta-study covering 171 publications, Schütz et al. [11] demonstrated that P-solubilizing microorganisms as plant inoculants were mainly effective in soils with moderate available P levels (25–35 kg P ha⁻¹) but not on low P soils or at higher P availability. In an investigation with *Bacillus*-, *Pseudomonas*-, and *Trichoderma*-based inoculants in maize on six different soils in five countries with eight different types of fertilizers based on recycling products from organic and inorganic waste materials, Thonar et al. [12] reported superior performance particularly in combination with composted animal manures. Nkebiwe et al. [13,14] found beneficial effects by combination of microbial inoculants with placement of stabilized ammonium fertilizers.

Based on these findings, we hypothesised that the selection of suitable combinations of microbial inoculants with organic or inorganic fertilizers could be a key factor for the development strategies to improve the fertilizer use efficiency with support of microbial inoculants. Therefore, in this study we compared the performance of greenhouse tomato, supplied with different types of fertilizers (municipal waste compost, poultry manure, rock phosphate, stabilized ammonium) in combination with selected microbial inoculants with proven plant growth-promoting and phosphate-solubilizing properties, pre-selected in the studies of Thonar et al. [12] and Nkebiwe et al. [13,14]. To also consider the potential impact of different soil properties, the experiments were conducted on two contrasting soils from Ghana with low P availability, and moderately acidic and alkaline pH in face of the significance of soil pH for P fixation in soils.

2. Materials and Methods

2.1. Soil Properties

The experiments were conducted under greenhouse conditions on two contrasting soils from Ghana with pH 5.6 and 7.8 with low P availability (Table 1). Soil samples of the top 10 cm horizon, collected from Dormaa Ahenkro and Atebubu in the Brong-Ahafo Region in Ghana, were used

for the experiments. Chemical and physical soil properties are summarized in Table 1. Soil characterization was performed according to the **Association of German Agricultural Research and Research Institutes** (VDLUFA) instructions for soil analysis [15].

Table 1. Physical and chemical properties of the experimental soils.

Soil Properties	Soil Origin	
	Atebubu	Dormaa Ahenkro
Soil pH (CaCl ₂)	5.6	7.8
Total Nitrogen [%]	0.05	0.30
NO ₃ -N [mg kg ⁻¹ soil]	2.4	44.2
Plant available P [mg kg ⁻¹ soil]	7.22 (P CAL)	2.22 (P Olsen)
Total P (ICP-OES) [mg kg ⁻¹ soil]	90	473
K (CAL extract) [mg kg ⁻¹ soil]	33.2	357
Mg (CaCl ₂) [mg kg ⁻¹ soil]	110	250
Total Ca [mg kg ⁻¹ soil]	632	10,523
Fe (CAT extract) [mg kg ⁻¹ soil]	56.5	29.0
Zn (CAT extract) [mg kg ⁻¹ soil]	<1	4.0
Mn (CAT extract) [mg kg ⁻¹ soil]	188.0	27.3
Cu (CAT extract) [mg kg ⁻¹ soil]	0.54	1.14
Total Carbon [%]	0.75	4.82
Humus [%]	1.23	7.89
Sand (63–2000 µm) %	66.4	44.4
Silt (2–63 µm) %	28.6	38.3
Clay (<2 µm) %	5.0	17.3

CAL: Calcium acetate-lactate extract, CAT: Calciumchloride/-Diethylene triamine pentaacetic acid extract, ICP-OES: Inductively Coupled Plasma Optical Emission Spectrometry.

2.2. Test Plant

The tomato (*Solanum lycopersicum* L.) variety Promodoro UC 82-B (Bonanza seeds international, Yuba City, CA, USA) was used for the experiments.

2.3. Culture Conditions

A screen house experiment was installed at the School of Agriculture and Technology, University of Energy and Natural Resources in Sunyani, Ghana. In contrast to a greenhouse, the walls and roofs of screen houses consist of plastic mesh to protect the plants from animals and extreme weather conditions without artificial lighting and heating devices. Solar vents were installed to provide the screen house with good ventilation. The average temperature, relative humidity, and dew-point in the screen house was 31 °C, 60%, and 18% respectively. Tomato plants were cultivated until 49 days after sowing (DAS) on the alkaline soil (experiment 1) and for 35 DAS on the acidic soil (experiment 2). Watering of the plants was performed gravimetrically once per day during the first two weeks, and increased to two times in the subsequent weeks until harvest to reach a moisture content equivalent to 70% of the substrate water holding capacity.

2.4. Fertilization

In general, application of fertilizers was adapted according to nutrient availability indicated by the soil analysis (Table 1). This implicates differences in the fertilization management on the two investigated soils.

2.4.1. Alkaline Soil (Experiment 1)

In experiment 1 conducted on the pH 7.8 soil, P was identified as major limiting nutrient with available Olsen P levels (Table 1) far below the critical range between 10 and 30 mg kg⁻¹ soil reported in the literature [16]. Therefore, the P fertilization level was adjusted to 100 mg P kg⁻¹ soil in all

treatments. Organic fertilization was performed with a commercially available compost produced from municipal waste, or the same compost amended with poultry manure (PM compost) (Accra Compost and Recycling Plants Ltd., Accra, Ghana). The N, P, and K composition comprised 2% N, 1% P and 1% K for compost and 0.95% N, 1.35% P and 0.94% K for compost amended with poultry manure. The fertilization rate was 10 g kg⁻¹ soil for the compost fertilizer (corresponding to 200 mg N, 100 mg P and 200 mg K kg⁻¹ soil), while poultry manure-amended compost was applied with 7.4 g kg⁻¹ soil (corresponding to 71 mg N, 100 mg P and 70 mg K kg⁻¹ soil), and mixed thoroughly with the substrates. A negative control without fertilization and a positive control fertilized with superphosphate (single super phosphate, 18% P₂O₅, Triferto, Gent, Belgium, 100 mg P kg⁻¹ soil and calcium nitrate, n was also included.

2.4.2. Acidic Soil (Experiment 2)

The acidic soil from Atebubu with pH 5.6 was also characterized by extremely low P availability (Table 1), far below the recommended range for maximum yield between 61 and 120 mg CAL-P kg⁻¹ soil [17]. Therefore, fertilization was performed with the two compost types and with triple superphosphate as positive control at an application rate of 100 mg P kg⁻¹ soil. In face of the lower soil pH, acid-soluble rock phosphate (Granuphos 18% P₂O₅ (Landor, Birsfelden Switzerland) was included as additional variant. In this case, 150 mg N kg⁻¹ soil of stabilized ammonium sulfate (Novatec Solub, Compo-Expert, Münster, Germany) was added as nitrogen source to promote rock-P solubilization via ammonium-induced rhizosphere acidification. In face of the extremely low N content of the acidic soil (Table 1), and limited N supply recorded in experiment 1 for the organic fertilizers, additional N supplementation in form of stabilized ammonium (150 mg N kg⁻¹ soil) was also performed in the case of the organic fertilizers. Calcium nitrate (150 mg N kg⁻¹ soil) was added to the positive control supplied with soluble superphosphate.

2.5. Bioeffectors (BEs)

2.5.1. Alkaline Soil

In experiment 1, Combifactor B (CFB), a microbial consortium product based on *Trichoderma harzianum* OMG16 and *Bacillus amyloliquefaciens* FZB42 (also referred as *Bacillus velezensis* FZB42, ABITEP GmbH, Berlin, Germany), enriched with zinc (Zn) and manganese (Mn) (Hochschule Anhalt, Bernburg, Germany), was used as an inoculant. The combination product with micronutrients was selected as an inoculant since the soil nutrient analysis (Table 1) indicated low Mn availability [18]. The CFB product was applied three times in the form of *Bacillus* spores (2×10^9 cfu kg⁻¹ soil) and *Trichoderma* spores (2×10^8 cfu kg⁻¹ soil), in a formulation supplemented with 2 mg Mn, and 2 mg Zn kg⁻¹ soil as a suspension in water, first at the two-leaf stage of tomato seedlings during the nursery stage in small pots (50 mL), secondly, one week later during transplantation into bigger pots (2500 mL, 2 kg soil), and finally, one week after transplanting by drenching close to the stem base. In the variants without BE application, (NoBE), an equivalent amount of water was applied by weight.

2.5.2. Acidic Soil

In experiment 2 on the acidic soil, only the *Bacillus* FZB42-product was used as a single-strain inoculant with the same application schedule as in experiment 1.

2.6. Plant Biomass and Root Length

At final harvest, the dry biomass of the shoots was determined for both experiments after 3 d oven drying at 65 °C. The roots in each pot were washed out from the soil substrate after carefully shaking off the rhizosphere soil, and they were stored in 30% (v/v) ethanol. The roots were later separated, submerged in a water film in transparent Perspex trays, and subsequently digitalized using a flat-bed scanner (Epson Expression 1000 XL, Tokyo, Japan). Subsequently, the root length of the digitalized

samples was measured using the WinRHIZO root analysis system (Reagent Instruments, Quebec, QC, Canada). Thereafter, the root samples were oven dried for 2 d at 65 °C for the determination of dry matter.

2.7. Shoot N, P, K, and Mg Concentration and Content

For both experiments, plant mineral nutrient analysis was performed as follows: tomato shoot N was measured with a Vario Max CN macro-elemental analyser (Elementar Analysensysteme, Hanau, Germany). For P, K, Ca, and Mg, a microwave digestion method was employed for the wet ashing of finely ground dry plant materials (250 mg) in 1 mL of deionized water, 2.5 mL conc. HNO₃ (1:3), and 2 mL H₂O₂ (30%). Digestion was performed in a microwave digestion system (Ethos, MLS, Leutkirch, Germany) for 1 h and allowed to cool for 30 min. Approximately 5 g activated charcoal was added for sample decolouration, mixed well by shaking, and allowed 15 min to settle. The samples were then filtered with ashless MG 640d Blue ribbon filter paper (Macherey & Nagel, Düren, Germany). Phosphate was estimated spectrophotometrically (Hitachi Ltd., Tokyo, Japan) according to Gericke and Kurmis [19]. Magnesium and calcium were measured by atomic absorption spectrophotometry (iCE 3000 series, Thermo Fischer, Dreieich, Germany) and K by flame emission spectrophotometry (Eppendorf-ELEX6361, Netheler+Hinz, Hamburg, Germany).

2.8. Rhizosphere Soil pH

The collected rhizosphere soil samples were air-dried and sub-sampled for pH analysis. Soil pH was measured after 1 h shaking of a soil suspension in 0.01 M CaCl₂ in a 1:1 ratio ratio (Digital pH-Meter E532, Metrohm Harisau, Switzerland).

2.9. Phosphorous Recovery Efficiency

Phosphorus recovery efficiency (PRE) was calculated based on the formula below:

$$= \left[\frac{\text{ShootPcontent}(\text{fertilized}) - \text{Negativecontrol}(\text{unfertilized}) \text{ (mg pot}^{-1}\text{)}}{\text{Fertilizerapplied} \text{ (mg pot}^{-1}\text{)}} \right] \times 100 \quad (1)$$

2.10. Experimental Setup and Data Analysis

The experiments were established in a completely randomized design with five replicates per treatment in experiment 1, and four replicates per treatment in experiment 2. Statistical analysis was performed in SAS 9.4 (2016) (SAS Institute Inc., Cary, NC, USA), with the treatments as fixed variables and the measured parameters as random variables. The normality of the data was tested using a Q-Q Plot with fit diagnostics. The proc glimmix procedure was performed to give a general overview for the comparison of specific fertilizer types to no fertilization (negative control) or soluble P (positive control) variants. The pairwise t-test was employed, to test for specific differences within fertilizer types between inoculated and non-inoculated variants at $p < 0.05$. (supplementary Table S1).

3. Results

3.1. Experiment 1, Alkaline Soil (pH 7.8)

3.1.1. Plant Growth and Rhizosphere pH

At 49 DAS, both, compost and PM-compost fertilizers increased plant growth (Figure 1) and shoot biomass production (Figure 2) to a similar extent (+82%) as compared with the unfertilized control (NoFert) variant, but did not reach the biomass of the plants supplied with mineral nitrate + superphosphate (NP) fertilization (+346%).

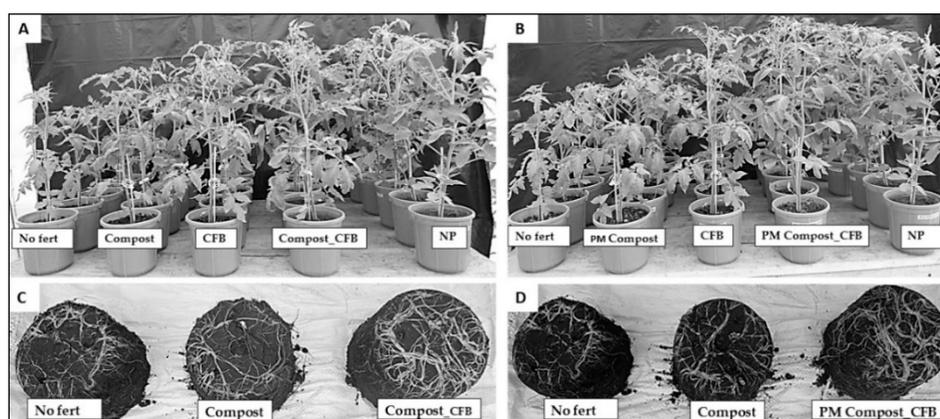


Figure 1. (A) Plant growth of 7-week-old tomatoes on pH 7.8 soil supplied with CFB and compost, mineral NPK fertilization (NP) or without fertilization; (B) CFB and PM compost; and root development with (C) CFB and compost; (D) CFB and PM compost. (CFB = Combifactor B, PM = poultry manure, NP = calcium nitrate plus superphosphate, Nofert = unfertilized).

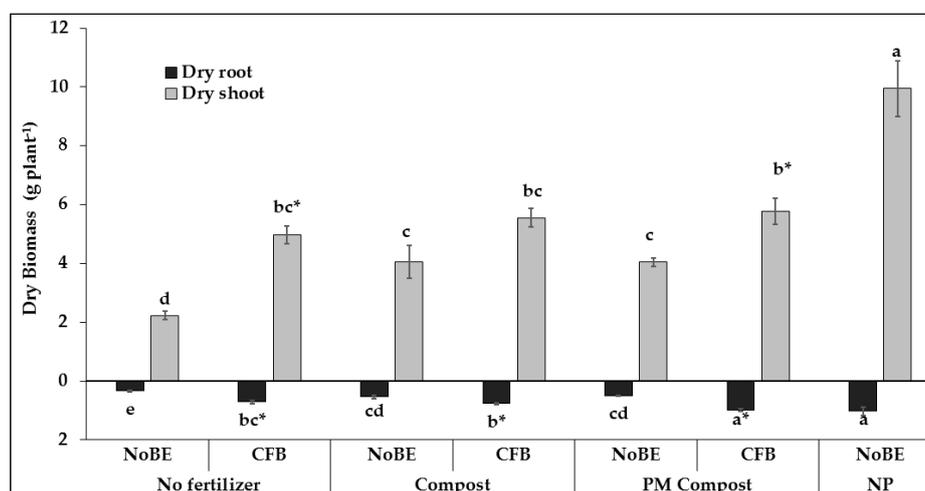


Figure 2. Shoot and root dry weight of 7-week-old tomatoes on pH 7.8 soil (CFB = Combifactor B, PM = poultry manure, NP = calcium nitrate plus superphosphate). Means and SE of five replicates. Significant treatment differences are indicated by different letters (T-grouping (LSD) at $p = 0.05$). * = significant difference compared with the non-inoculated control (NoBE) within the same fertilizer treatment (t -test at $p < 0.05$).

Compared with the non-inoculated control treatments (NoBE), application of the microbial inoculants (CFB) significantly increased the shoot biomass production by 123% in the unfertilized variants, and by 42% in the compost amended with poultry manure ($p < 0.05$). This was associated with significant increases, also in root biomass (Figure 2) and root length (Figure 3). The total root length of plants supplied with PM-compost and CFB inoculation was finally comparable with the plants receiving full mineral (NP) fertilization. However, in all treatments without CFB inoculation, root growth inhibition was observed in comparison with the NP variant (Figure 3).

Tomato is a plant species with a well-documented potential for rhizosphere acidification under conditions of P limitation, which is known to mediate the solubilization of Ca-phosphates in alkaline soils, while the release of carboxylates with P-solubilizing potential is negligible [20,21]. Therefore, pH measurements were conducted with root-adhering rhizosphere soil samples, collected by shaking of the root systems at final harvest. However, the maximum decline in rhizosphere soil pH recorded on the pH 7.8 soil reached only 0.1 pH units without any significant treatment differences (Supplementary data, Figure S1).

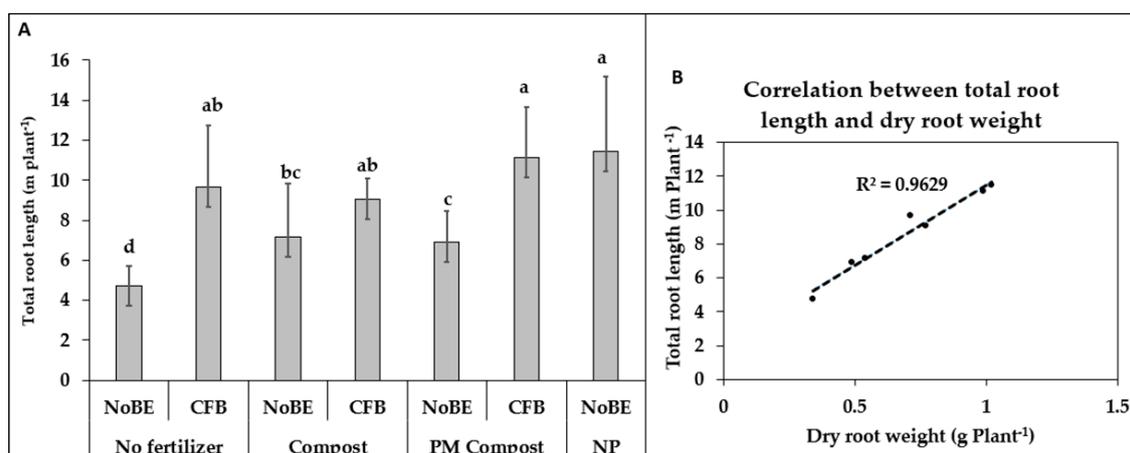


Figure 3. Total root length (A) and root length correlation with dry root biomass (B) of 7-week-old tomatoes on pH 7.8 soil. (CFB = Combifactor B, PM = Poultry manure, NP = calcium nitrate plus superphosphate). Means and SE of five replicates. Significant treatment differences are indicated by different letters (T-grouping (LSD) at $p = 0.05$). * = significant difference compared with the non-inoculated control (NoBE) within the same fertilizer treatment (t -test at $p < 0.05$).

3.1.2. Plant Nutritional Status

Although all fertilizer treatments stimulated shoot P accumulation as compared with the NoFert variant (Table 2), only mineral P fertilization (NP) was able to increase the shoot P concentrations above the deficiency threshold (Figure 4). For nitrogen, only the PM compost variants reached the sufficiency range (Figure 4) and N shoot accumulation increased in the order NoFert < compost < PM-compost < NP (Table 2).

Table 2. Effects of Combifactor B inoculation on the shoot accumulation of N, P, K, Mg, and Ca of 7-week-old tomatoes on pH 7.8 soil supplied with different fertilizers. (CFB = Combifactor B, PM = Poultry Manure, NP = calcium nitrate plus superphosphate). Means and SE of five replicates. Significant treatment differences are indicated by different letters (T-grouping (LSD) at $p = 0.05$). * = significant difference compared with the non-inoculated control (NoBE) within the same fertilizer treatment (t -test at $p < 0.05$).

	Shoot Mineral Content (mg Plant ⁻¹)				
	N	P	K	Mg	Ca
No fertilization	66.3 e	2.7 d	102.9 d	18.0 e	62.0 e
CFB	94.2 d *	7.5 b *	169.2 c *	25.1 cd *	102.9 cd *
Compost	86.6 d	6.3 bc	157.6 c	23.5 d	85.5 d
Compost_CFB	107.3 d *	7.4 b	192.3 bc *	28.3 cd *	110.7 c *
PM Compost	148.4 c	5.8 bc	181.2 c	31.2 bc	108.6 cd
PM Compost_CFB	213.6 b *	8.7 b *	232.2 b *	37.8 b *	143.2 b *
NP_	289.1 a	37.0 a	405.1 a	57.7 a	233.7 a

The microbial inoculants (CFB) further promoted P and N accumulation, with significant effects being detectable for the treatments without fertilization (NoFert) and for the PM-compost variants (Table 2). The nutritional status for potassium, magnesium, and calcium was sufficient in all treatments, with a trend for declining concentrations in the CFB variants (Figure 4). The CFB inoculation significantly increased K, Ca, and Mg shoot accumulation in the NoFert variant (Table 2). In the compost treatment, a significant CFB effect was recorded for Ca accumulation, and for K and Ca in the PM-compost variant.

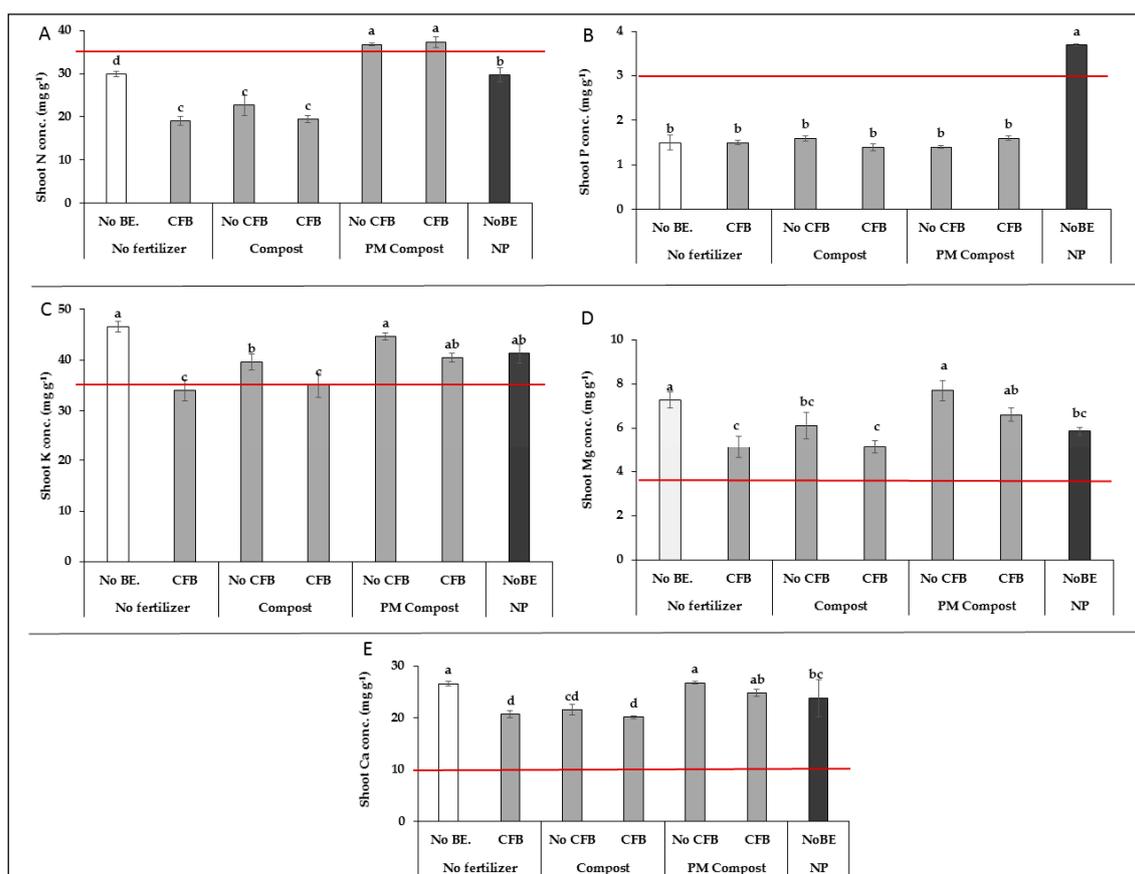


Figure 4. Effects of Combifactor B inoculation on shoot N (A), P (B), K (C), Mg (D), and Ca (E) concentrations of 7-weeks-old tomatoes on pH 7.8 soil supplied with different fertilizers (CFB = Combifactor B, PM = poultry manure, NP = calcium nitrate plus superphosphate). The red lines represent the deficiency thresholds for nutrient tissue concentrations [22]. Means and SE of five replicates. Significant treatment differences are indicated by different letters (T-grouping (LSD) at $p = 0.05$). * = significant difference compared with the non-inoculated control (NoBE) within the same fertilizer treatment (t -test at $p < 0.05$).

3.2. Experiment 2, Acidic Soil (pH 5.6)

In addition to the organic fertilizers tested in experiment 1, rock phosphate (RP) fertilization was included into experiment 2, as an alternative, low-cost P source with the potential to be used on acidic soils due to improved Ca-P solubility at low soil pH. By contrast, the application of RP on neutral to alkaline soils is considered to be largely ineffective [23,24]. Amendments of ammonium sulfate, stabilized with the nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP), were added to support RP solubilization by root-induced acidification in response to preferential N uptake in the ammonium form [25] and to improve the obviously sub-optimal nitrogen supply of the organic fertilizers (Figure 3). The *Bacillus amyloliquefaciens* strain FZB42 with the proven potential to solubilize sparingly soluble rock phosphate [14], and to promote nutrient acquisition from organic fertilizers in maize [12], was used as microbial inoculant.

3.2.1. Plant Growth and Development

At 35 DAS, the fertilizer applications significantly increased plant growth (Figure 5) and shoot biomass production (Figure 6) of tomato compared with the NoFert control, in the order NoFert < compost < PM-compost < RP < superphosphate (NP).

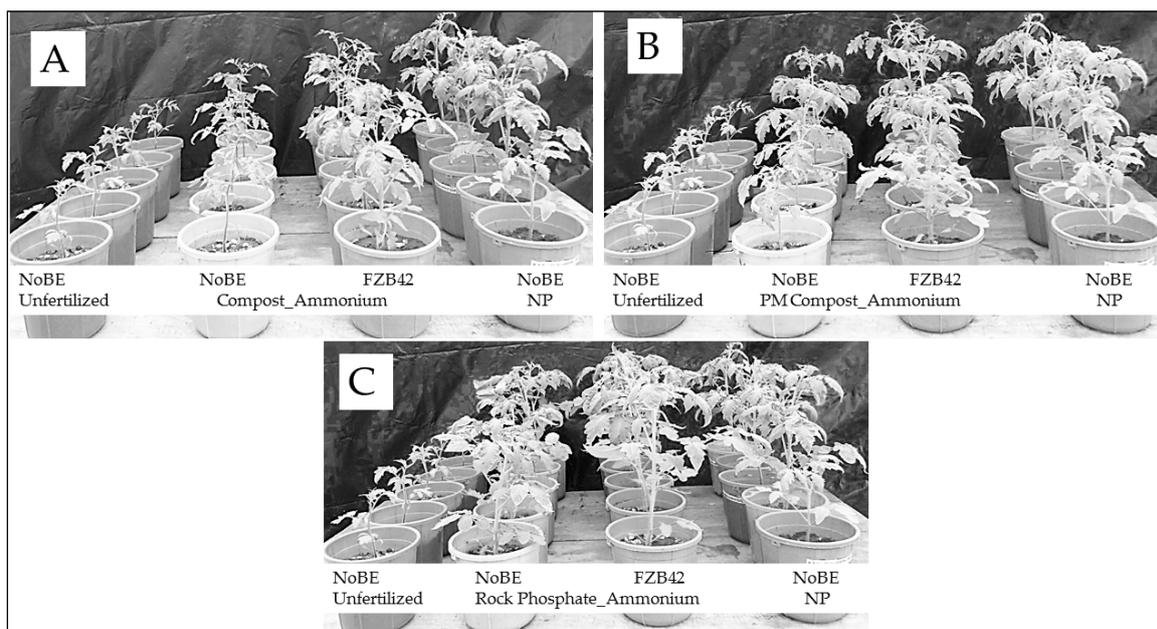


Figure 5. Plant growth of 4-week-old tomato on pH 5.6 soil supplied with different fertilizers and with and without *Bacillus velezensis* FZB42 inoculation (A) PM compost (B) and rock phosphate (C) (NoBE = no bioeffector, PM = poultry manure, NP = calcium nitrate plus superphosphate).

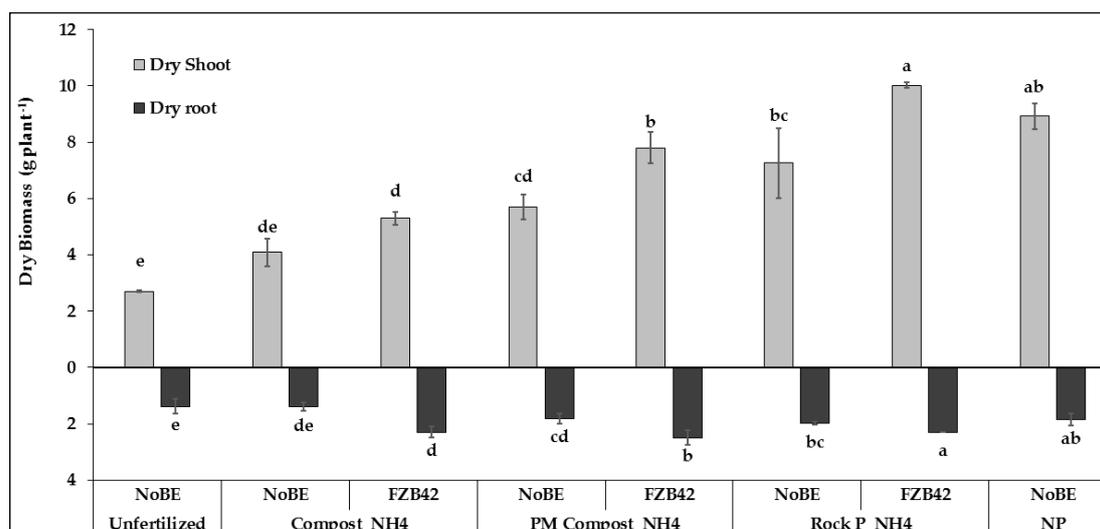


Figure 6. Shoot and root dry weight of 5-week-old tomatoes with FZB42 and different P sources and ammonium sulfate under acidic soil conditions (PM = poultry manure, NP = calcium nitrate plus superphosphate). Means and SE of four replicates. Significant treatment differences are indicated by different letters (T-grouping (LSD) at $p = 0.05$). * = significant difference compared with the non-inoculated control (NoBE) within the same fertilizer treatment (t -test at $p < 0.05$).

Inoculation with FZB42 significantly stimulated shoot and root biomass production in the PM-Compost and RP variants (Figure 6) to a level that was not significantly different from the plants that were supplied with mineral superphosphate fertilization (NP). FZB-induced shoot growth promotion increased in the order Compost < PM-Compost < RP, while the stimulation of root biomass production by FZB inoculation declined in the same order (Figure 6).

3.2.2. Plant Nutritional Status

All fertilizer treatments increased N and P accumulation in the order: NoFert < Compost < PM-compost < RP < superphosphate (NP), with significant effects for PM-compost, RP, and NP (Table 3). However, only RP and NP treatments exceeded the P deficiency threshold, while sufficient N supply was recorded for PM compost, RP, and NP (Figure 7). The nutritional status of K, Mg, and Ca was sufficient, or at least very close the respective sufficiency thresholds in all treatments (Figure 7). Corresponding with plant growth stimulation (Table 3), the shoot accumulation of K, Mg, and Ca tended to increase in response to the fertilizer applications (Table 3) in the order: NoFert < compost < PM-compost < RP < NP, with significant effects for PM-Compost, RP and NP, but the tissue concentrations declined in the same order (Figure 7).

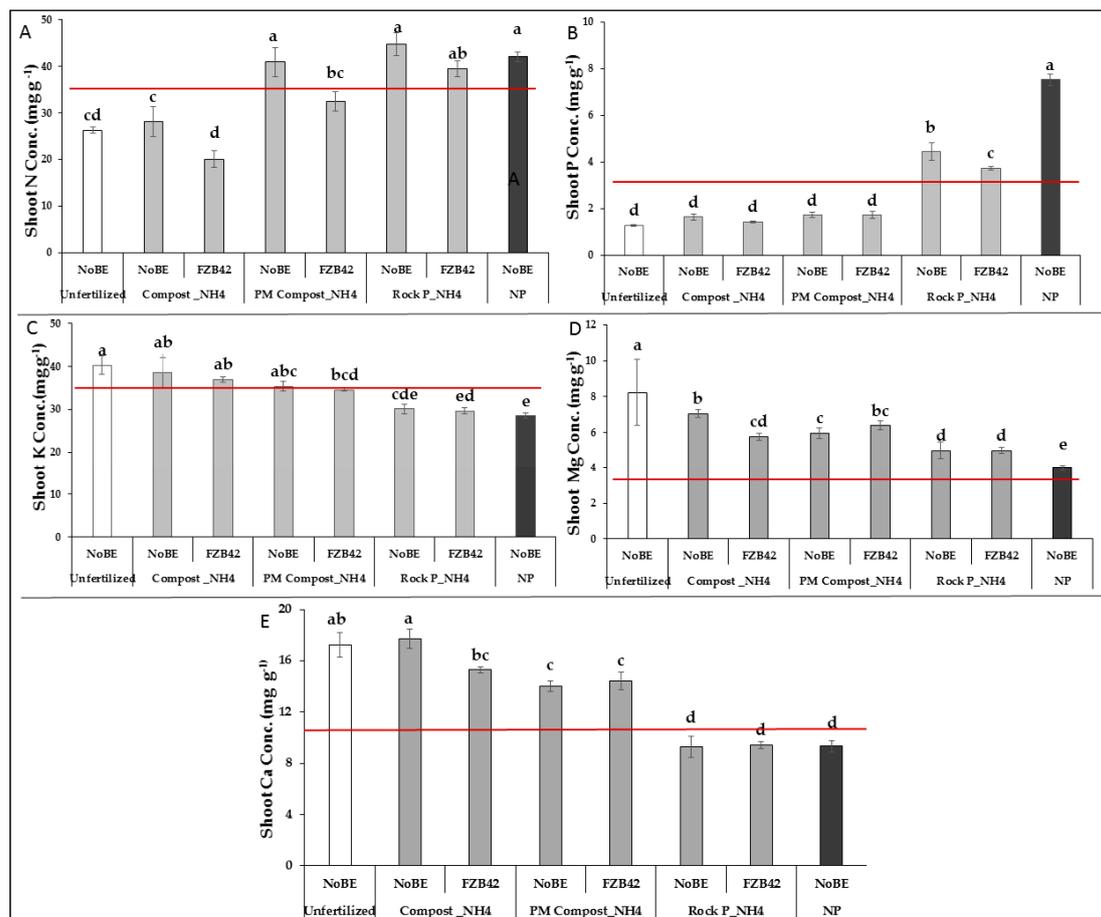


Figure 7. Effects of *Bacillus amyloliquefaciens* FZB42 inoculation on shoot N (A), P (B), K (C), Mg (D), and Ca (E) concentrations of 5-week-old tomatoes on pH 7.8 soil supplied with different fertilizers (Compost_NH₄ = municipal waste compost + stabilized ammonium sulfate, PM-Compost_NH₄ = municipal waste compost + poultry manure + stabilized ammonium sulfate, Rock P_NH₄ = rock phosphate + stabilized ammonium sulfate, NP = calcium nitrate plus superphosphate). The red lines represent the deficiency thresholds for nutrient tissue concentrations [22]. Means and SE of four replicates. Significant treatment differences are indicated by different letters (T-grouping (LSD) at $p = 0.05$).

Table 3. Effects of *Bacillus amyloliquefaciens* FZB 42 inoculation on shoot accumulation of N, P, K, Mg, and Ca of 5-week-old tomatoes on pH 5.6 soil supplied with different fertilizers. (Compost_NH₄ = municipal waste compost + stabilized ammonium sulfate, PM-Compost_NH₄ = municipal waste compost + poultry manure + stabilized ammonium sulfate, Rock P_NH₄ = rock phosphate + stabilized ammonium sulfate, NP = calcium nitrate plus superphosphate). Means and SE of four replicates. Significant treatment differences are indicated by different letters (T-grouping (LSD) at $p = 0.05$). * = significant difference compared with the non-inoculated control (NoBE) within the same fertilizer treatment (t -test at $p < 0.05$).

Shoot Minerals Content (mg Plant ⁻¹)					
	N	P	K	Mg	Ca
Unfertilized	71.2 d	3.4 f	108.9 e	22.2 c	46.6 d
Compost_NH ₄ ⁺	108.9 d	6.5 ef	159.9 de	28.5 bc	71.0 c
Compost_NH ₄ ⁺ _FZB42	106.2 d	7.5 ef	194.9 cd*	30.3 bc	81.0 bc
PM Compost_NH ₄ ⁺	228.3 c	9.7 de	199.9 c	33.7 b	79.6 bc
PM Compost_NH ₄ ⁺ _FZB42	247.7 c	13.2 d	268.9 a*	49.3 a *	111.0 a *
Rock P_NH ₄ ⁺	314.8 b	30.7 c	214.1 bc	34.8 b	65.0 c
Rock P_NH ₄ ⁺ _FZB42	394.1 a *	37.3 b	297 a	50.0 a *	94.7 b *
TSP_NO ₃ ⁻ (NP)	373.8 a	66.9 a	253.5 ab	35.5 b	80.4 bc

Inoculation with the FZB42 *Bacillus* strain significantly increased K, Mg, and Ca shoot accumulation in the PM-compost and RP variants, while significant stimulation of N and P accumulation was recorded only for the RP treatment with a similar trend under PM-compost fertilization (Table 3). However, N and P shoot concentrations declined after FZB inoculation (Figure 7).

4. Discussion

4.1. Fertilizer Effects

In this study, different types of alternative organic and inorganic fertilizers, based on compost, poultry manure, and rock phosphate, as well as conventional mineral superphosphate, were applied at the same P dosage for greenhouse tomato cultivation on two soils with limited P availability (17,18) and contrasting pH (Table 1). On both soils, all tested fertilizers showed beneficial effects on plant growth (Figures 1, 2, 5 and 6). However, in contrast to conventional mineral NPK fertilization (NP), P remained the major limiting nutrient, particularly in case of the organic fertilizers (Figures 4 and 7), with shoot P concentrations in the deficiency range [22], where any surplus of P supply was immediately transformed into biomass production. This was confirmed in the case of tomato plants supplied with municipal waste compost, where a sufficient N supply was achieved by increasing the N availability of the fertilizer via additions of poultry manure and/or mineral N (NH₄⁺) applications (Figures 4 and 7). In these cases, a significant stimulation of plant growth was detected only when it was possible to increase additionally the shoot P accumulation, e.g., by inoculation with the plant growth-promoting microorganisms (Tables 2 and 3). The status of the remaining macronutrients (K, Mg, Ca) was sufficient in all treatments [21], and shoot accumulation of these nutrients increased in response to stimulation of plant growth, induced by increasing the P supply (Table 3), while the tissue concentrations declined at the same time (Figure 7), as a consequence of a dilution effect.

4.2. PGPM Effects

On both soils, inoculation with the selected microbial inoculants with proven PGPM potential in maize [12,13], also improved the growth of tomato plants (Figures 1, 2, 5 and 6), and acquisition of P as major limiting nutrient, in a soil type-, and fertilizer-specific manner.

4.2.1. Alkaline Soil

On the alkaline pH 7.8 soil, plants without mineral P fertilization suffered from severe P limitation, as indicated by P shoot concentrations of approximately 1.5 mg g^{-1} DM (Figure 4), which was far below the published deficiency threshold of 3 mg g^{-1} DM [22], and by distinct inhibition of both, shoot and root growth (Figures 2 and 3).

The inoculation with the microbial combination product Combifactor B (CFB), based on strains of *Bacillus amyloliquefaciens* FZB42 and *Trichoderma harzianum* OMG16 in combination with Zn and Mn as stress-protective micronutrients [26,27], significantly increased plant growth (Figures 1 and 2) and shoot P accumulation (Table 2) in the unfertilized control (B), and the compost treatment amended with poultry manure (PM-compost). This effect was associated with a corresponding stimulation of root growth, reflected by increased root biomass and root length (Figures 2 and 3), suggesting an improved spatial acquisition of soluble soil P fractions. Root growth stimulation is a well-documented mechanism of plant growth promotion via microbial inoculants. This has been related with the microbial production of hormonal factors with root growth-stimulating properties, such as auxins or certain quorum-sensing molecules [9,28], but also with the ability to produce 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which counteracts excessive stress-induced ethylene accumulation with inhibitory effects on root growth [29,30], as a well-documented response to P deficiency in higher plants [31]. Accordingly, root growth inhibition in response to severe P limitation was recorded also in the present study (Figures 2 and 3), and both, the production of auxin and ACC deaminase have been reported for the *Bacillus* strain FZB42, which was used as an inoculant [32,33]. Similarly, auxin production and root growth stimulation is documented for *Trichoderma harzianum* [34].

Moreover, improved root development can support the establishment of arbuscular-mycorrhizal associations with important functions particularly for P acquisition, although this aspect has not been considered in the present study. In this context, it is worthwhile to mention that mycorrhizal helper functions supporting mycorrhizal root colonization have been documented for the inoculant strain *B. amyloliquefaciens* FZB42 [12,35]. More recently, it has been also demonstrated that changes in root morphology can significantly modify the rhizosphere microbial diversity [36] with yet unexplored consequences for soil health and nutrient turnover in the rhizosphere.

Soils with neutral to alkaline pH are frequently characterized by limited solubility of micronutrients, such as Fe, Zn, Mn, and Cu. Zinc limitation has been identified as a major factor for limiting plant growth via increased oxidative auxin degradation due to a lack of Zn as a cofactor for the enzymatic detoxification of reactive oxygen species [26,27]. Therefore, the supplementation of Zn via CFB application may have contributed to root growth stimulation by the inoculants on the moderately alkaline soil with pH 7.8. However, soil analysis revealed a plant-available Zn status of 4 mg kg^{-1} soil (Table 1), which is considered to be sufficient for plant growth [18], and accordingly, Zn limitation seems to be an unlikely scenario in this case. By contrast, the plant-available Mn concentration was suboptimal on this soil [18] but subsequent plant analysis revealed a sufficient Mn status [22] of $40\text{--}50 \text{ mg kg}^{-1}$ shoot dry matter, independent of the CFB treatment (data not shown). These findings suggest that the plant potential for Mn acquisition was sufficient to cover the Mn demand, and a further contribution via CFB inoculation was not required.

The observed stimulation of root growth may also impact on P acquisition via chemical changes in the rhizosphere: in response to P limitation, plants usually increase the release of root-secretory acid phosphatases [25], which can help to hydrolyze soluble organic P forms in the rhizosphere, which are particularly abundant after application of organic fertilizers. Accordingly, root secretion of acid phosphatases is also characteristic for tomatoes exposed to P starvation [37]. Moreover, the secretion of phosphatases into growth media with limited P availability has been similarly reported for *Bacillus amyloliquefaciens* FZB42 and *Trichoderma harzianum* [14]. Consequently, a bigger root system with increased activities of both, plant and microbial rhizosphere phosphohydrolases may represent a particular advantage for acquiring P, e.g., from organic fertilizers, which are rich in organic P forms. However, in this study, a significantly improved P acquisition from organic fertilizers due to increased

release of phosphatases by the microbial inoculants seems to be unlikely. This is indicated by the observation that the additional effect of BE inoculation on P accumulation (Table 2) and plant growth (Figure 2) was in same order of magnitude with and without the application of the organic fertilizers, or even higher in the unfertilized control. This finding suggests that improved spatial soil exploitation for the uptake of soluble mineral phosphates due to the microbial promotion of root growth (Figure 3), and not an increase in the mineralization of organic P forms supplied with the organic fertilizers represented the major contribution of the microbial inoculants to plant P acquisition on the moderately alkaline soil.

On neutral and alkaline soils, substantial amounts of mineral P are present in the form of sparingly-soluble Ca phosphates, which can be solubilized by rhizosphere acidification and/or organic chelators [25]. However, pH determinations of the rhizosphere soil revealed no indications for root-induced rhizosphere acidification (Figure S1), although tomato is a plant species with a known potential to acidify the rhizosphere under P limitation [20,21], and Ca–P mobilization via the acidification of artificial growth media has been similarly demonstrated both, for *Bacillus amyloliquefaciens* FZB42 and *Trichoderma harzianum* [14]. Possibly, a high pH buffering capacity of the moderately alkaline soil substrate counteracted the expression of significant rhizosphere acidification effects, which is a well-known problem for plant nutrient acquisition strategies based on proton extrusion on well-buffered alkaline soils [25]. Accordingly, a limited potential of the tested microbial inoculants to contribute to plant acquisition of Ca–P under real rhizosphere conditions has been reported also in earlier studies [12,38].

As a consequence of the general stimulation of shoot and root growth induced by the microbial inoculants; also the shoot accumulation of the non-limiting nutrients K, Mg, and Ca increased due to improved nutrient acquisition potential of a bigger root system (Table 2). Substantial differences were found also for the N availability of the applied fertilizers. Despite the lower total N application (see Section 2.4), the N status of plants supplied with PM-amended municipal waste compost was sufficient, while the N supply only by municipal waste compost was not adequate (Figure 4). This effect may be attributed to particularly high levels of readily plant-available N forms (particularly NH_4^+) reported for poultry manures [39]. Similar to K, Mg, and Ca accumulation, the microbial inoculants also increased N accumulation (Table 2), closely related with microbial effects on root growth (Figure 3). However, despite improved spatial nutrient acquisition by stimulation of root growth, the inoculant effect was not big enough to cover also the plant demand of P as a limiting nutrient. The P nutritional status remained in the deficiency range (Figure 4) and the plants could not express growth responses comparable with the variants supplied with soluble nutrients via mineral NPK fertilization (Figure 2).

4.2.2. Acidic Soil

This scenario changed completely on the moderately acidic, sandy soil of pH 5.6 with a lower pH buffering capacity. On this soil, plants were able to acquire sparingly soluble Ca–P applied in the form of rock phosphate. In this case, stabilized ammonium sulfate was supplied as an N source to promote root-induced rhizosphere acidification, with beneficial effects on the solubilization of Ca–P [25]. Accordingly, the P and N status reached the sufficiency range (Figure 7), and compared with the unfertilized control, shoot and root biomass production increased by 163% and 44% (Figure 5), to a level that was not significantly different from plants with full mineral fertilization (CN). This is in line with earlier reports on the efficient use of rock phosphate fertilizers with acid-soluble Ca phosphates, particularly at lower soil pH [23,24]. On the weakly-buffered sandy soil, root extrusion of protons by the P-limited tomato plants [20,21], additionally stimulated by ammonium fertilization [25], obviously reached a degree of rhizosphere acidification that was sufficient for rock-P solubilization. This effect was further promoted by inoculation with *Bacillus amyloliquefaciens* FZB42. Compared with the non-inoculated control, shoot P accumulation increased significantly by 25% (Table 3), which was associated with root growth promotion by 15% (Figure 5), and which translated into an increase in shoot biomass production by 38% (Figure 5). This may indicate that the development of a larger acidifying root system induced by the microbial inoculant, at least partially contributed to improved

rock phosphate acquisition. However, in face of the documented potential of the FZB42 strain for rock phosphate mobilization [14,32], an additional contribution by microbial P mobilization cannot be excluded. Moreover, Ögüt et al. [40] demonstrated that various *Bacillus* strains are obviously able to stimulate root-induced proton extrusion of host plants supplied with ammonium fertilization, and thereby the rhizosphere acidification potential. On the other hand, in contrast to the beneficial effects of rock-P solubilization, rhizosphere acidification may also contribute to a stronger immobilization of P adsorbed to Fe, and Al oxides and hydroxides, as a dominant sparingly soluble P form in acidic soils [25]. However, in the face of the extremely low levels of plant-available P [17] and even total P in the respective soils (Table 1), this potentially negative impact on P availability was obviously largely overcompensated for by the beneficial effects of ammonium fertilization and plant growth-promoting rhizobacteria (PGPR) inoculation on the solubility of rock-P.

Similar to the experiment on the alkaline soil, PGPR inoculation had a selective impact on the utilization of the organic fertilizers. Compared with the unfertilized control, the municipal waste compost and the compost amended with poultry manure (PM compost) increased P accumulation and plant biomass without significant differences between the fertilizer treatments (Tables 2 and 3). The additional inoculation with FZB42 significantly stimulated plant growth (Figures 2 and 5) and nutrient accumulation compared with the non-inoculated (NoBE) control (Tables 2 and 3), only on the soil with PM compost, with a biomass production that was not significantly different from the plants supplied with full mineral P fertilization of the NP treatments. By contrast, due to the extremely low organic C content of the acidic soil (Table 1), a significant contribution of native organic soil P to plant P acquisition seems to be unlikely in this case.

Obviously, on both soils with P as the major limiting nutrient, the plant growth-promoting potential of the microbial inoculants was particularly expressed in combination with manure-based organic fertilizers, as similarly reported also in earlier studies with sweet pepper [23] or maize [12]. Accordingly, with the same amount of P supply, PGPM inoculation selectively increased P recovery efficiency on the manure-amended compost (Figure 8). The reasons for this selective effect may be directly related to differences in P forms and the availability of the applied organic fertilizers. However, indirect effects are also possible, due to a P-solubilizing potential that has been reported for manure components, or microbiome effects mediated by microorganisms that are involved in manure degradation [23]. The root growth-stimulating effect of the tested inoculants would consequently promote the acquisition of the increased available P fraction in the manure-amended variants.

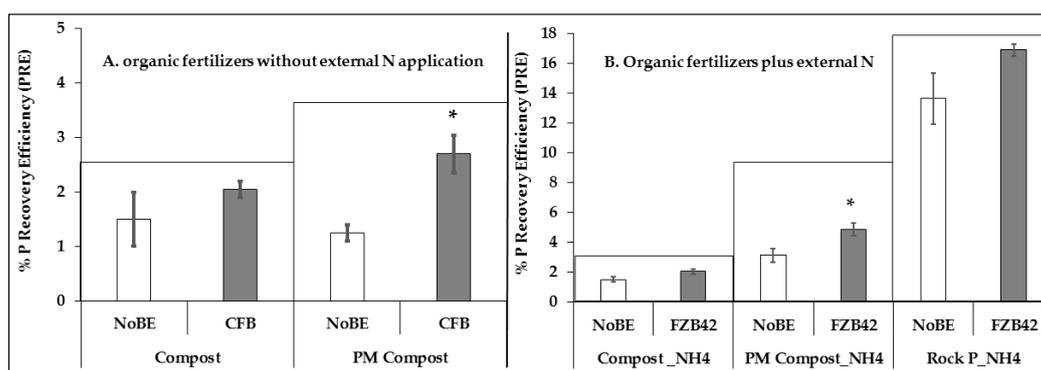


Figure 8. Phosphorus recovery efficiency [41] in tomatoes from different organic and inorganic fertilizers in moderately alkaline soil pH 7.8 (A), and moderately acidic soil pH 5.6 without (NoBE) and with microbial inoculation (B); CFB = Combifactor B FZB42 = *Bacillus amyloliquefaciens* FZB42, Compost_NH4 = Municipal waste compost + stabilized ammonium sulfate, PM-Compost_NH4 = Municipal waste compost + poultry manure + stabilized ammonium sulfate, Rock P_NH4 = rock phosphate + stabilized ammonium sulfate. * = significant *t*-test $p < 0.05$ compared with the non-inoculated control (NoBE) in each fertilizer variant.

4.3. Conclusions

The present study suggests that inoculation with plant growth-promoting microorganisms can provide an efficient tool to increase the use efficiency of alternative fertilizers based on waste recycling products or less well processed P fertilizers, such as rock phosphate, generating plant growth responses comparable with conventional mineral fertilization. Successful strategies may contribute to a better adaptation of fertilizer supply to the plant demands, reduce fertilizer inputs, the risk of unwanted nutrient losses with detrimental effects on the environment, thereby promoting zero waste" concepts turning waste into resources needed for crop production. However, obviously more targeted application strategies are required considering the impact of different soil properties and differences in the compatibility of the microbial inoculants with the selected fertilizer products. These interactions seem to be much more specific than currently assumed. This is reflected e.g., by the selective impact of the PGPM inoculants on P utilization from manure-amended compost as compared with sole application of the municipal waste compost, observed on both soils, or the specific effects on Ca–P utilization on the acidic soil only. However, the results also demonstrate that even the use of less-efficient fertilizers can be further improved by enrichment strategies with compatible fertilizer components, as demonstrated for poultry manure or stabilized ammonium in the present study. The results also demonstrate that consortium products do not necessarily exhibit a better performance than single-strain inoculants. A better understanding of the underlying mechanisms determining compatible interactions between fertilizers, soil properties, and PGPM inoculants may significantly contribute to the development of more reproducible application strategies, which still represents a major challenge for the use of microbial biostimulants in agricultural practice.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2073-4395/8/10/213/s1>. Figure S1: Tomato rhizosphere pH for alkaline soil-Experiment 1, Table S1: Type III error tables.

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