Acquisition of rock phosphate by combined application of ammonium fertilizers and *Bacillus amyloliquefaciens* FZB42 in maize as affected by soil pH

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**Keywords**  
ammonium fertilizers, *Bacillus amyloliquefaciens* FZB42, maize, rock phosphate, soil pH.

**Abstract**

**Aims:** The use of plant growth-promoting micro-organisms (PGPMs) to improve plant-nutrient acquisition has a long history but reproducibility remains a challenge. Recent findings suggest an important role of suitable inoculant-fertilizer combinations for the expression of PGPM-effects, particularly with respect to nitrogen (N) supply. In face of the well-documented N form effects on rhizosphere pH, this study addressed the impact of ammonium-assisted PGPM-interactions on the acquisition of sparingly soluble calcium-phosphates as affected by soil pH.

**Methods and Results:** The effects of stabilized ammonium fertilization combined with the PGPM inoculant *Bacillus amyloliquefaciens* FZB42 on the acquisition of rock phosphate in maize were examined on two soils (moderately acidic-pH 5.6 and alkaline-pH 7.8). On the two contrasting soils, FZB42 improved the P status and promoted plant growth by different mechanisms. On the acidic soil, a combination of ammonium-fertilization with FZB42 increased P-acquisition by Rock P solubilization via rhizosphere acidification but P-supply in the noninoculated control was already sufficient to meet the plant demands. By contrast, on the alkaline soil, plant growth-promotion was associated with FZB42-induced root growth stimulation.

**Conclusion:** The results suggest a significant impact of soil pH on performance and the mode of action of PGPM inoculants, to be considered for practical applications.

**Significance and Impact of the Study:** The study advanced existing knowledge on PGPM-assisted P solubilization as affected by different soil properties. The results suggest perspectives for management options to be considered for efficient use of PGPMs in terms of selecting application strategies with compatible PGPM-fertilizer combinations, depending on soil pH conditions.

**Introduction**

Phosphorus (P) is an important macronutrient in crop production but P deficiencies in the world’s arable lands are estimated to limit crop yield by 30–40% (Runge-Metzger 1995; Uexküll and Mutert 1995; Vance 2001; Vance et al. 2003), which makes application of P fertilizers obligatory to maintain yield stability. Moreover according to Russell (1973), only 20% or less of the applied P is removed by the first years of plant cultivation while the remaining 80% are rapidly sequestered in forms with limited plant availability (Kimble et al. 2000). This bears a high risk of P overfertilization in agricultural soils with subsequent losses by erosion and surface run-off into water bodies, contributing to eutrophication and hypoxia of the aquatic ecosystems (Runge-Metzger 1995; Bumb...
and Baanante 1996; Elena et al. 2001). Since mineral P fertilizers are produced via mining of rock phosphates (RP) as a limited natural resource, this raise concerns on sustainability aspects of mineral P fertilization. Despite variable prognoses on the longevity of RP reserves, ranging from several decades to several centuries (Van Kauwenbergh et al. 2013), improving the use efficiency of P fertilizers in agricultural production systems, remains a major challenge for the future. Proposed strategies comprise approaches of P-saving by use of P fertilizers based on organic and inorganic waste-recycling products (Kirchmann et al. 2005; Stoffella et al. 2014) as well as improved plant P acquisition by fertilizer placement strategies close to the roots (Nkebiwe et al. 2016a), exploiting the genetic potential for root-induced changes in rhizosphere chemistry and root growth (Bonser et al. 1996; Gahoonia and Nielsen 2004; Campos et al. 2018), and the assistance of microbial inoculants with plant growth-promoting properties (PGPMs) (Sharma et al. 2013). Root growth promotion by production of auxins or other signal compounds, down-regulation of excessive stress-induced ethylene production by ACC deaminase, soil pH reduction by release of protons, organic and mineral acids as well as increased P mineralization or enhanced recruitment of other beneficial microbes are discussed as potential modes of action of PGPM-assisted P acquisition (Illmer and Schinner 1995; Mardad et al. 2013; Sharma et al. 2013; Kröber et al. 2014; Thonar et al. 2017). However, recent findings suggest that the efficiency of PGPM-assisted fertilization strategies also depends on combination with suitable fertilizers (Abbasi et al. 2015; Nkebiwe et al. 2016b; Thonar et al. 2017; Mpanga et al. 2018; Vinci et al. 2018a,b; Bradačová et al. 2019). Own preliminary work indicates preferential performance of many PGPMs in combination with ammonium instead of nitrate-based fertilizers. Positive effects on utilization of sparingly soluble Ca-P sources by combination with stabilized ammonium fertilizers have been recorded in more than 16 pot and field experiments with 24 PGPM strains or strain combinations in three crops (Mpanga et al. 2018). However, so far, no studies are available with direct comparisons of soil pH effects on PGPM performance in combination with ammonium fertilization.

In this study, Bacillus amyloliquefaciens FZB42 characterized as one of the most efficient PGPMs in the previous reports (Mpanga et al. 2018, 2019; Vinci et al. 2018a) with abilities to solubilize organic and inorganic P (Nkebiwe et al. 2017) and root growth-promoting and stress-protective properties (Borris 2015; Mpanga et al. 2018, 2019) was selected as a representative PGPM strain. Maize was used as a model plant with a low inherent capacity for root-induced P solubilization (Gaume et al. 2000; Liu et al. 2016). For comparison, the plants were grown on two low P soils with moderately acidic (pH 5–6) or moderately alkaline pH (pH 7–8) supplied with RP fertilization and stabilized ammonium. Plants with full soluble P fertilization and nitrate supply were included as positive controls.

**Materials and methods**

**Soil properties**

**Location and culture conditions**

The experiment was carried out in a screen house (Schuch 2018) at the School of Agriculture and Technology, University of Energy and Natural Resources, Ghana. Average screen house temperature, dew point and relative humidity: 38, 20°C and 78% respectively. Plastic pots of 3-5 l were used with 3 kg soil detailed in Table 1 and watered up to 70% water holding capacity after sowing to reach a soil water-holding capacity of 70% the maize seeds. Watering was performed gravimetrically once a day for the first 2 weeks and then changed to twice a day from week 3 until harvested on 38 DAS.

**Treatments and fertilization**

The treatments comprised; (i) no fertilization (negative control); (ii) ammonium+RP; (iii) ammonium+RP+FZB42

| Table 1 Physical and chemical properties of the soils from Ghana (Mpanga et al. 2018) |
|--------------------------------|----------------|----------------|
| Soil properties                | Soil origin     | Atebubu        |
| Total nitrogen (%)             | 0.05            | 0.30           |
| NO₃-N (mg kg⁻¹ soil)           | 2.4             | 4.24           |
| Plant available P (mg kg⁻¹ soil) | 7.22 (P CAL) | 2.22 (P Olsen) |
| Total P (mg kg⁻¹ soil)         | 90              | 473            |
| K (mg kg⁻¹ soil)               | 33.2            | 357            |
| Mg (mg kg⁻¹ soil)              | 110             | 250            |
| Total Ca (mg kg⁻¹ soil)        | 632             | 10 523         |
| Fe (mg kg⁻¹ soil)              | 56.5            | 29.0           |
| Zn (mg kg⁻¹ soil)              | <1              | 4.0            |
| Mn (mg kg⁻¹ soil)              | 188.0           | 27.3           |
| Cu (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           |
| Soil origin                    | Atebubu Dormaa Ahenkro |

CAL, calcium acetate-lactate extract; CAT, calcium chloride/di-ethylamine pentaacetic acid extract; ICP-OES, inductively coupled plasma optical emission spectrometry.
and (iv) nitrate-soluble P (positive control). The P fertilizers were applied as: Rock P- (Granuphos 18% P₂O₅; Landor, Birsfelden Switzerland) or superphosphate (single superphosphate, 18% P₂O₅; Triferto, Gent, Belgium) at 100 mg P kg⁻¹ soil. Nitrogen was applied as ammonium sulphate stabilized with the nitrification inhibitor DMPP- (3,4-dimethylpyrazole-phosphate; Novatec solub; Compo Expert GmbH, Münster, Germany) at 100 mg N kg⁻¹ soil. Calcium nitrate was fertilized as N form to the positive control at 100 mg N kg⁻¹ soil. Potassium was applied at 100 mg K kg⁻¹ soil as K₂SO₄ for all treatments except the zero fertilization treatment without N, P and K. To compensate for low background nitrate levels on the acid soil N was applied as a mixture of 15% Ca (NO₃)₂ and 85% (NH₄)₂SO₄.

Test plant

Maize (Zea mays cv Wandataa-NBS/16/wan/wm) (Naa Bawa Seidu, Wa, Ghana) was used for the experiments. Three seeds were sown per pot and thinned to one plant of similar height and vigour at 7 days after sowing (DAS) and cultivated until 38 DAS.

PGPR inoculation

Rhizovital FZB42 fl (ABiTEP GmbH, Berlin, Germany), which is based on a liquid formulation of B. amyloliquefaciens FZB42 also termed as Bacillus velezensis (2.5 × 10¹⁰ CFU per gram) was applied three times from sowing in weekly intervals by drenching 20 ml of 10⁹ spores per kg soil.

Plant biomass and root length

The plants were harvested at the end of the culture period and oven-dried in an oven at 60°C until a constant weight is achieved. Rhizosphere soil was sampled by vigorous shaking root of the excavated root systems with adhering soil particles and air-dried in the screen house for later analysis of plant available rhizosphere soil P and pH. Total root length was estimated using the WinR-HIZO root analysis system (Reagent Instruments, Quebec, Canada) after separating the roots in a water film in transparent Perspex trays and digitalized with EPSON expression 1000 XL scanner (Seiko Epson Corp. Tokyo, Japan).

Shoot N, P, K, Ca, Mg, Mn and Zn concentration and content

Maize shoot N was measured with a Vario Max CN macro-elementar analyser (Elementar Analysensysteme, Hanau, Germany). For P, K, Ca, and Mg, Mn and Zn, 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. 14 mol l⁻¹ HNO₃ (1 : 3) and 2 ml 0.185 mol l⁻¹ 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 to obtain a clear assay based on the method by Upreti (1984), mixed 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 concentration was estimated spectrophotometrically (Hitachi Ltd, Tokyo, Japan) according to Gericke and Kurmis (1952). Magnesium, calcium, manganese and zinc concentrations 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). The shoot content of mineral nutrients was estimated by multiplying mineral concentrations with dry shoot biomass.

Plant-available rhizosphere soil phosphorus and soil pH

The air-dried soils were sieved with 2 mm mesh size and sub-sampled for P and pH analysis. For the slightly alkaline soil, the Olsen P method (Olsen et al. 1954) was used for soil extraction and measured with inductively coupled plasma optical emission spectrometry while on the slightly acid soil, calcium acetate-lactate (CAL) extraction developed by (VDLUFA 2012) was used for extraction of potentially plant-available P and measured spectrophotometrically (Hitachi Ltd) according to the method of Gericke and Kurmis (1952).

Soil pH was measured after 1 h shaking of soil suspension in 0.01 mol l⁻¹ CaCl₂ in a 1 : 1 ratio (Digital pH-Meter E532; Metrohm Harisau, Switzerland).

Statistical analysis

The experimental set up was arranged in a completely randomized design with weekly re-arrangements of the pots. After checking the normality and residuals of the data in SAS 9.4, one-way ANOVA was performed at alpha 0.05. Proc glimmix procedure was performed for significance testing while the t-test at alpha 0-05 was employed for pairwise comparisons between selected treatments and their controls. To examine the relationships between shoot P, total root length, rhizosphere pH and P, correlation analysis was carried out with the mean values obtained from the ANOVA test.
Results

Plant growth

Generally, better performance of maize plants was recorded on the pH 5·6 (Fig. 1a) as compared with the pH 7·9 soil (Fig. 1b). On the moderately acidic sandy soil, ammonium fertilization with Rock-P (RP) supply increased plant growth and shoot biomass production over the unfertilized control to a level not significantly different (alpha = 0·05) from the positive control with full soluble P fertilization. FZB42 inoculation had no additional growth effects (Fig. 1a,c). Root length development was increased by the N fertilization treatments without any additional FZB42 effects (Fig. 2).

By contrast, on the pH 7·8 soil, the ammonium fertilization increased plant growth and shoot biomass production over the unfertilized control exclusively in combination with FZB42 inoculation but reached only about 30% of the biomass compared with the positive control with soluble P supply (Fig. 1b,c). A similar trend was recorded for root length development of the plants (Fig. 2).

![Figure 1](attachment:figure1.jpg)  
**Figure 1** Habitus (a and b) and dry shoot weight (c) of maize supplied with DMPP-stabilized ammonium and Rock P (RP) fertilization, with and without *Bacillus amyloliquefaciens* (FZB42) inoculation, as compared with an unfertilized control (no fert) and soluble P fertilization with nitrate supply (Nitrate_Soluble P), on two soils with moderately acidic and alkaline pH. Means and SE of five replicates. For each soil, significant treatment differences are indicated by different characters (Tukey test, alpha = 0·05 compared to ammonium-RP) (no fert; ammonium_RP; ammonium_RP_FZB42; nitrate_soluble P).

![Figure 2](attachment:figure2.jpg)  
**Figure 2** Total root length of maize supplied with DMPP-stabilized ammonium and Rock P (RP) fertilization, with and without *Bacillus amyloliquefaciens* (FZB42) inoculation, as compared with an unfertilized control (no fert) and soluble P fertilization with nitrate supply (Nitrate_Soluble P), on two soils with moderately acidic and alkaline pH. Means and SE of five replicates. For each soil, significant treatment differences are indicated by different characters (*t*-test, alpha = 0·05 compared to ammonium-RP) (no fert; ammonium_RP; ammonium_RP_FZB42; nitrate_soluble P).
Rhizosphere pH, available rhizosphere P and plant nutritional status

Compared with the moderately acidic bulk soil pH (5.6), the pH of the root-adhering rhizosphere soil declined by 0.4; 0.9 and 1.4 units in the unfertilized control, ammonium-RP and ammonium-RP-FZB24 treatment respectively with significant lower values in the ammonium-RP and ammonium-RP-FZB24 variants as compared to the unfertilized control. By contrast, the plants with nitrate and soluble P fertilization significantly increased the rhizosphere pH by 0.5 units. On the pH 7.8 soil, only non-significant, marginal pH changes ≤0.3 were detectable (Fig. 3).

Potentially plant available CAL-P levels in the rhizosphere of the plants grown on the pH 5.6 soil showed an increasing trend in the order unfertilized control ≤ ammonium-RP < ammonium-RP-FZB42 ≤ nitrate and soluble P. No significant differences (alpha = 0.05) were recorded between the ammonium-RP-FZB42 variant and the positive control with nitrate supply and soluble P fertilization (Fig. 4). In the alkaline soil with nitrate and soluble P fertilization, the potentially plant-available Olsen P of the rhizosphere soil reached a level of approximately 80 mg kg⁻¹ (positive control), which was significantly higher than P in the remaining variants, reaching only 18–20 mg kg⁻¹ without significant treatment differences (alpha = 0.05) (Fig. 4c).

The P-nutritional status of the plants grown on the moderately acidic soil reached the sufficiency threshold of approximately 3 mg P g⁻¹ shoot DM (Campbell 2000) in the variants with soluble P supply and in the ammonium-RP variants, both with and without FZB42 inoculation (Fig. 4a). However, only the ammonium-RP-FZB42 treatment reached a P shoot accumulation that was not significantly different (alpha = 0.05) from the positive control with soluble P fertilization and nitrate supply (Fig. 4b). For the remaining nutrients K, Mg, Mn and Zn concentrations were in the sufficiency range although ammonium supply had a negative effect on the Mg status and a positive effect on Zn and Mn shoot accumulation and tissue concentrations, without any additional effect by the microbial inoculant.

The N status was below the deficiency threshold of 30 mg g⁻¹ shoot DM even in the variants with nitrogen supply. The highest N levels of 26 mg g⁻¹ were recorded in the FZB42 inoculated variant. Ammonium fertilization induced Ca deficiency (~25 mg g⁻¹ shoot DM) and reduced Ca shoot accumulation independent of FZB42 inoculation (Table 2A,B).

On the alkaline soil, the P-nutritional status remained in the deficiency range without treatment differences (Fig. 4a). Only the shoot P accumulation of the positive control with soluble P fertilization was significantly increased (alpha = 0.05) in comparison with the remaining variants (Fig. 4b). All the remaining nutrients remained in the sufficiency range with the exception of the positive control supplied with nitrate and soluble P fertilization where the N and Mn status was close to the deficiency threshold (Table 2A,B).

Discussion

On the moderately acidic, sandy loam soil with pH 5.6, the combination of RP with stabilized ammonium fertilization was equivalent to the soluble P fertilization with nitrate supply, in terms of biomass production and the P sufficiency status (Campbell 2000). The FZB42 inoculant had no additional effects on plant growth and on the shoot P concentration. Nevertheless, the data suggest an additional contribution of FZB42 to P acquisition since the potentially, plant-available CAL-P concentrations in the rhizosphere soil and shoot P accumulation were significantly increased over the noninoculated control (alpha = 0.05). Both reached levels equivalent to the positive control with soluble P fertilization (Fig. 4b,c).

This was associated with a declining rhizosphere pH (Fig. 3), suggesting improved solubilization of RP due to FZB42-induced rhizosphere acidification down to pH 4.2. To the best of our knowledge, this is the first report suggesting a direct contribution of FZB42 to RP solubilization in the rhizosphere, which may work at least on light sandy soils with a low pH buffering capacity. Accordingly, a low pH buffering capacity can be expected for
the investigated loamy sand pH 5.6 indicated by extremely low total Ca concentrations (0.06%) and low organic matter (content 0.7%). Rock P-solubilizing potential of FZB42 has been reported already on artificial growth media by Nkebiwe et al. (2016b). In face of the extremely low organic matter content of the investigated soil (0.7%), a significant contribution of FZB42 to P mineralization via the release of secretory phosphatases seems to be less likely. However, the results need to be interpreted with some caution since also indirect effects of the inoculant cannot be excluded: that is, Ögüt et al. (2011) reported stimulation of proton extrusion by the roots of the host plant after inoculation with certain strains of Bacillus sp. Also, stimulation of root hair development induced by the inoculants (Dobbelare et al. 1999) would increase the root surface area that is available for ammonium-induced rhizosphere acidification (Neumann and Römheld 2002) and thereby might intensify the acidification potential of the roots. Moreover improved recruitment of other plant beneficial micro-

Figure 4 Shoot P concentration and P deficiency threshold (horizontal line) based on Campbell (2000) (a) shoot P content (b) and plant-available soil Phosphorus in the rhizosphere (c) of maize supplied with DMPP-stabilized ammonium and Rock P (RP) fertilization, with and without Bacillus amyloliquefaciens (FZB42) inoculation, as compared with an unfertilized control (No fert) and soluble P fertilization with nitrate supply (Nitrate_Soluble P), on two soils with moderately acidic and alkaline pH. Means and SE of five replicates. For each soil, significant treatment differences are indicated by different characters (* = t-test, alpha = 0.05 compared to no fertilization) □ no fert.; □ ammonium_RP; □ ammonium_RP_FZB42; □ nitrate_solute P). [Colour figure can be viewed at wileyonlinelibrary.com]
organisms after FZB42 inoculation has been repeatedly reported in other studies (Eltlbany et al. 2019; Kröber et al. 2014; Thonar et al. 2016; Yusran et al. 2007). Under similar soil conditions, increased P solubilization from supplemented wood ash, improving the P status of maize, has been recently reported also by Mercl et al. (2018, 2019), using bacterial (Paenibacillus mucilaginosus) and fungal inoculants (Penicillium sp. PK112 and Trichoderma harzianum OMG08).

Interestingly, the significantly increased concentrations of potentially plant available CAL-P in the rhizosphere and the increased P shoot accumulation in the FZB42 inoculated variant and in the positive control with soluble P supply, did not translate into any additional positive effects on shoot biomass production (Fig. 1a, c). Although the P status was sufficient, obviously N limitation emerged as a growth limiting factor at the end of the culture period due to the limited pot volume, indicated by N concentrations below the deficiency threshold of 30 mg g⁻¹ shoot FM in all treatments (Table 2A; Campbell 2000). Consequently, a surplus of P supply could not be transformed into biomass production due to the lack of Nitrogen. However, on this slightly acidic soil, the well-documented stimulatory effect of FZB42 on root length development in maize (Mpanga et al. 2018, 2019a, 2019b) was not detectable. One explanation could be the strong reduction of the rhizosphere pH in the ammonium-RP and particularly in the ammonium-RP-FZB42 variants down to pH 4.2–4.7, which obviously caused Ca deficiency in the respective treatments (Table 2A) and may also be associated with a risk of induced Al toxicity on the weakly buffered sandy soil, both, with detrimental effects on root growth (Emanuelsson 1984; Njoku et al. 1987; Kochian et al. 2005). Accordingly, the highest root length was recorded in the positive control with soluble P supply and nitrate fertilization, which is possibly due to the rhizosphere alkalization to pH 6.0 (Fig. 3), triggered by preferential nitrate uptake (Neumann and Römheld 2002).

Lambers et al. (2015) suggested a positive relationship between Mn shoot concentrations and carboxylate-mediated Mn solubilization in the rhizosphere. However, although the Mn concentrations increased in the variants with ammonium fertilization on the moderately acidic soil, no further increase was induced by FZB42 inoculation. This finding suggests an increased Mn status because of the well-documented Mn solubilization mediated by ammonium-induced rhizosphere acidification (Marschner 1995) whereas additional carboxylate production of the inoculated bacteria, reported for many PSMs (Sharma et al. 2013) appears unlikely.

By contrast, on the alkaline pH 7.8 soil, FZB42 inoculation had a significant (alpha = 0.05) root growth promoting effect (Fig. 2), which translated into a moderately increased shoot biomass production (Fig. 1). However, the P nutritional status remained in the deficiency range, even in the variant with soluble P supply and nitrate fertilization. Obviously, this soil had a high P fixation potential (total Ca = 10 523 mg kg⁻¹ soil) (Table 1) that counteracted the effects of soluble P fertilization. This

Table 2 Shoot mineral (A) concentration (conc.) and (B) content (cont.) of maize supplied with DMPP-stabilized ammonium and Rock P (RP) fertilization, with and without Bacillus amyloidiquefaciens (FZB42) inoculation, as compared with an unfertilized control (no fert) and soluble P fertilization with nitrate supply (nitrate soluble P), on two soils with moderately acidic and alkaline pH. Means and SE of five replicates. For each soil, significant treatment differences are indicated by different characters (alpha = 0.05)

| Soil pH | N conc. (mg g⁻¹) | K conc. (mg g⁻¹) | Mg conc. (mg g⁻¹) | Ca conc. (mg g⁻¹) | Zn conc. (mg g⁻¹) | Mn conc. (mg g⁻¹) |
|---------|-----------------|-----------------|------------------|------------------|------------------|------------------|
| (A)     |                 |                 |                  |                  |                  |                  |
| Soil pH | N conc. (mg plant⁻¹) | K conc. (mg plant⁻¹) | Mg conc. (mg plant⁻¹) | Ca conc. (mg plant⁻¹) | Zn conc. (mg plant⁻¹) | Mn conc. (mg plant⁻¹) |
|---------|-----------------|-----------------|------------------|------------------|------------------|------------------|
| (B)     |                 |                 |                  |                  |                  |                  |
was reflected by a 30% reduced biomass even after supply of soluble P fertilization, as compared with the P adequate plants on the pH 5.6 soil. The high soil pH also reduced the bioavailability of micronutrients (Neumann and Römheld 2002) and the Mn status was critical in the variant with soluble P supply (Table 1). Furthermore, no changes in rhizosphere pH were recorded in the variants with ammonium fertilization, probably due to a high pH buffering capacity of the respective soil, excluding chemical P solubilization via rhizosphere acidification. Consequently, FZB42-induced root growth promotion only contributed to improved spatial acquisition of the low concentrations of soluble P but had no effects on P solubilization, resulting in a limited stimulation of plant growth. Accordingly, on both soils, P shoot accumulation was positively correlated with root length, whereas a correlation with declining rhizosphere pH was detectable exclusively on the pH 5.6 soil (Fig. 5).

Recent studies demonstrated that limitations of ammonium-induced P solubilization by high pH buffering on alkaline soils might be overcome by ammonium-, and P-placement strategies (Jing et al. 2010; Nkebiwe et al. 2016a). Local root growth proliferation in response to the locally placed N and P supply (Drew 1975) leads to a local intensification of rhizosphere acidification, which could be enough to mediate Ca-P solubilization even at soil pH >8 (Jing et al. 2010). Nkebiwe et al. (2016b) demonstrated that the local root proliferation can be further intensified by PGPM inoculation. Most recently Bradáčová et al. (2019) found improved PGPM-mediated P acquisition in open field tomato production on a low P, pH 7.9 soil with stabilized ammonium sulphate placement without additional P supply, associated with increased biomass production and fruit yield.

Although the potential effects of other soil properties independent of soil pH cannot be excluded, the presented results suggest a strong impact of soil pH on the performance PGPM inoculants in maize. On the two investigated soils, the mode of action of a single PGPM inoculant was distinctly different, promoting P

![Figure 5 Correlations between: rhizosphere soil pH and rhizosphere available P (a and b); rhizosphere soil pH and shoot P content (c and d); shoot P content and total root length (e and f) of maize in different pH soils (left = pH 5.6 soil and right = pH 7.8 soil). Rhizosphere soil P in the slightly acid and alkaline soils were extracted by CAL and Olsen methods respectively.](image-url)
solubilization on the acidic soil and spatial nutrient acquisition under moderately alkaline soil conditions. This aspect needs to be considered for practical applications in terms of selection and application strategies of compatible PGPM-fertilizer combinations.

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Conflict of Interest

The authors have no conflict of interest regarding this manuscript.

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