Nkebiwe *et al. Chem. Biol. Technol. Agric. (2016) 3:15*
DOI 10.1186/s40538-016-0065-5

Chemical and Biological Technologies in Agriculture

RESEARCH Open Access

Improving fertilizer-depot exploitation and maize growth by inoculation with *plant growth-promoting bacteria*: from lab to field

Peteh M. Nkebiwe[1*], Markus Weinmann[2†] and Torsten Müller[1†]

Abstract

Background: Among other responses, plants tend to increase root growth to scavenge nutrients from more soil when soil nutrient concentrations are low. Placement of fertilizers near seeds or roots facilitates nutrient acquisition by target crop plants. Nevertheless, nutrient uptake from soil-placed fertilizer-depots depends on increased uptake rates and efficient spatial exploitation of the depot by roots. The aim of our study was to optimize exploitation of subsurface fertilizer-depots by inoculating the depot zone with promising plant growth-promoting microorganisms (PGPMs) as bio-effectors. If included in depots, root-attracting NH_4^+ or $HPO_4^{2-}/H_2PO_4^-$ ions may also enhance rooting within the depot, which in turn improves survival and root colonization by inoculated PGPMs; a consequence of high levels of microbial nutrients exuded in densely rooted soil.

Methods: We tested maize (*Zea mays* L.) in two greenhouse (pot and rhizobox) and two field experiments (2014 and 2015). A core treatment was NH_4^+-fertilizer placed as a subsurface depot (Depot). In the field, there was also NH_4^+-fertilizer broadcasted and incorporated in soil (Broad). Depot and Broad were each with PGPM as bio-effector (BE) or without (NoBE). Bio-effectors included: *Pseudomonas* sp. DSMZ 13134 (BE1) and *Bacillus amyloliquefaciens* FZB42 (BE2, only in field trials).

Results and discussion: In pots, Depot with BE1 led to 59 % higher shoot dry matter, 50 % higher shoot N content, and 64 % higher shoot P content than without PGPM. In rhizoboxes, higher root length density (RLD), lower rhizosphere pH, and higher BE1-colonization rate were measured in the fertilizer depot compared to the corresponding zone for controls with homogenous NO_3^- supply. Depot led to higher shoot N and P concentrations (+26.6 % *N*; +20.6 % *P*) and contents (+11.1 % *N*; +17.6 % *P*) than control. BE1 led to higher shoot N concentration (+13.5 %) than NoBE. In the field, fertilizer-depot soil had higher RLD than corresponding non-depot soil. BE1 led to doubled fertilizer-depot RLD in comparison to without (2014). In 2014, Depot led to 7.4 % higher grain yield than Broad (not statistically significant), whereas BE broadcast had no effect. In 2015, Depot led to 5.8 % higher fresh shoot biomass than Broad, below-seed placement of BE1 led to higher fresh (+7.1 %) and dry (+8.0 %) shoot biomass than NoBE.

Conclusion: Results show promising growth-effects of *Pseudomonas* sp. DSMZ 13134 on field-grown maize.

Keywords: Fertilizer placement, Localized root growth, Nutrient acquisition, PGPM

*Correspondence: Mehdi.Nkebiwe@uni-hohenheim.de
†Markus Weinmann and Torsten Müller authors contributed equally to this work
[1] Fertilisation and Soil Matter Dynamics (340 i), University of Hohenheim, 70593 Stuttgart, Germany
Full list of author information is available at the end of the article

Background

Of increasing importance in sustainable agriculture systems is the effective use of crop bio-stimulants [1, 2] and/or plant growth-promoting microorganisms (PGPMs). PGPMs have been shown to fix N [3], mineralize organic soil N [4], stimulate plant root growth [5–7] and mycorrhization [8–10], which enhances spatial nutrient acquisition from large soil volumes, and induce tolerance or resistance to biotic [11] and abiotic stresses [12, 13]. Nevertheless, plant growth-promoting effects of PGPMs realized in labs and greenhouses tend to be weak or even disappear when PGPMs are tested under field conditions. PGPM ineffectiveness in the field is likely due to a suboptimal or unfavorable interaction between field-inoculated PGPM and the biotic and abiotic environment [14, 15].

Central to the concept of sustainable agriculture is the reduction of environmental costs associated with farming. Among others, it requires responsible use of chemical fertilizers. This can be achieved through use of suitable fertilizer types and application rates timed to crop demand, seasons, and weather conditions with low risk of fertilizer loss to the environment. Responsible use of chemical fertilizers also requires utilization of effective fertilizer application techniques. In contrast to conventional fertilizer application by even broadcast on the soil surface (with or without incorporation), several innovative fertilizer placement techniques have been developed, through which fertilizer can be targeted to the seed, root, or canopy of young crop plants. Furthermore, fertilizer placement in soil improves fertilizer acquisition by target crop plants as opposed to weeds [16, 17] and reduces the risk of nutrient loss to the environment. Based on fertilizer composition, application technique and timing, effective fertilizer placement can lead to reduced leaching of nitrate to ground waters [18], low emission of nitrous oxide [19], methane [20], and ammonia [21] originating from fertilizer applied in soil. Fertilizer placement can also improve nutrient content in crop above-ground biomass as well as crop yield in comparison to conventional fertilizer broadcast (meta-analysis with 40 field studies, Nkebiwe et al. 2016, submitted unpublished observation).

Some of the earliest studies on fertilizer placement reported positive plant growth and yield effects [22, 23]. Today, "starter" fertilizers (e.g., di-ammonium phosphate for maize), are commonly applied close to plant roots to ensure optimal N and P supply during critical early growth stages especially in cold climate regions [24]. "Complete" fertilizer placement is also performed, in which the fertilizer need for the vegetative season is supplied as a single rich subsurface fertilizer-depot. Nevertheless, poor root growth in the fertilizer-depot zone often limits crop nutrient acquisition from the depot. Inclusion of root-attracting nutrients like ammonium and orthophosphate ions, or inoculation of root growth-stimulating PGPMs in the fertilizer depot zone, is a possible solution. There is evidence that fertilizer depots comprising ammonium and phosphates lead to higher N and P uptake and yield than fertilizer depots comprising either ammonium or phosphate and not both (meta-analysis, Nkebiwe et al. 2016, submitted unpublished observation). This phenomenon is primarily due to stronger localized root growth induced within the fertilizer depot by the presence of ammonium than by phosphates [25]. Secondly, NH_4^+-uptake from NH_4^+-rich subsurface fertilizer depots induces rhizosphere acidification around the depot zone, which enhances plant P-acquisition in neutral to alkaline soils [25]. Low rhizosphere pH may also modify proliferation and cell-wall mechanical properties of root cells [25]. [26] proposed the term controlled long-term ammonium nutrition (CULTAN) to describe a technique for "complete" N-fertilizer placement in which a subsurface fertilizer depot based on toxic concentrated NH_4^+ solution is placed at a rate to cover crop N demand during the vegetation season.

Although subsurface fertilizer placement and soil-inoculated plant growth-promoting microorganisms (PGPMs) have been separately studied considerably and somewhat also separately adopted, little is known about the combination of both. We propose that root colonization by PGPMs can be enhanced if PGPMs are inoculated in rhizosphere "hotspots," developing around NH_4^+-based fertilizer depots, due to NH_4^+-induced dense root growth and consequently, high levels of organic nutrients for microbes released as root exudates [15, 27].

Preliminary studies on fertilizer placement in combination with inoculation of PGPMs, such as subsurface banding of inorganic P fertilizer combined with seed-inoculated PGPM(s) [28] or subsurface banding of NPK-enriched bio-compost treated with PGPMs [29], have produced promising results.

Using maize (*Zea mays* L.) as a test crop, our objective was to investigate the effect of N fertilization by placement of an NH_4^+-depot and substrate-inoculation with the most promising PGPMs on root growth, rhizosphere modification, root colonization by PGPM, plant growth and development, shoot nutrient concentration and content, and yield. PGPMs were selected based on initial in vitro laboratory tests, from which promising candidates showed considerable tolerance to high levels of stabilized NH_4^+ and ability to solubilize insoluble $Ca_3(PO_4)_2$ (Nkebiwe et al. 2014 unpublished). We hypothesized that: (1) Placement of NH_4^+-fertilizer as a subsurface depot stimulates intense root growth around the depot, forming "rhizosphere hotspots." (2) Marked rhizosphere acidification occurs within and around the NH_4^+-depot zone. (3) Survival and colonization of inoculated PGPMs

is higher in the "rhizosphere hotspot" than in the comparable soil volume with respect to plant position that is supplied homogeneously with NO_3^- fertilizer. (4) Inoculated and established PGPMs further promote root development around the NH_4^+-depot zone. (5) Consequently, NH_4^+-depot fertilization combined with inoculated PGPMs leads to higher nutrient uptake and higher yields than NH_4^+-depot fertilization without PGPMs.

Methods

Greenhouse experiments

Choice of N-form for placement

A central theme in this study was N-fertilizer placement in subsurface soil to improve crop N acquisition. Effective N-fertilizer placement required application of a suitable N-source to form a relatively stable subsurface N-depot that is sufficiently close to seeds or plant roots for optimal N acquisition but distant enough not to impair seed germination and plant growth. Therefore, for main experimental treatments, NH_4^+ was selected over NO_3^- and $CO(NH_2)_2$ because of its low mobility in soil owing to a low effective diffusion coefficient and low mass flow [30–32] and also due to its ability to bind or be fixed to negatively charged sites on clay particles [33]. This property of NH_4^+ favorably inhibits N movement out of the depot zone to the surrounding unfertilized soil. For these reasons, it is not logical to locally place NO_3^- as a N-depot in soil because it will rapidly move out of the original spot into the surrounding soil by diffusion and mass flow [31]. NH_4^+ was also selected because it induces stronger localized root growth at the site of contact with roots than NO_3^- or $CO(NH_2)_2$ [31, 34–36]. This feature coupled with low mobility in soil makes NH_4^+ the ideal N-form to stimulate the formation of densely rooted soil zones, "rhizosphere hotspots." The NH_4^+-fertilizer chosen was further stabilized with a nitrification inhibitor (3, 4-dimethylpyrazole phosphate (DMPP) [37] to reduce N movement out of the depot as NO_3^-. Moreover, to minimize microbial nitrification, a highly concentrated toxic NH_4^+ solution was used to create a stable persisting NH_4^+- depot in which microbial-growth, root growth, and root N uptake is initially limited to the outer boarder areas with less toxic NH_4^+ levels [26]. Therefore, in these experiments on natural-soils or soil-based substrates, localized supply of N could only be realized as a subsoil NH_4^+-depot—with its associated effects on localized root growth stimulation and rhizosphere acidification—and not as NO_3^-.

If the experimental treatment is localized nutrient supply, the logical control should be uniform nutrient supply [38] given that the quantity of nutrient supplied per experimental unit (treatment or control) is the same.

For control treatments, NO_3^- (e.g., calcium ammonium nitrate, CAN) or $CO(NH_2)_2$ were suitable low-cost N-fertilizers commonly used by farmers and applied simply by broadcast and incorporation. In natural soil, NO_3^- or $CO(NH_2)_2$ is not normally and cannot be placed locally as a subsurface depot; not without the use of wax membranes [34] or other water-tight barriers [38]. This is because NO_3^- or $CO(NH_2)_2$ cannot to bind to clay particles and are very mobile soil [34]. For these reasons, NO_3^- homogenously mixed in the substrate was chosen as a suitable control. It was not considered necessary to include homogenously mixed NH_4^+ and locally placed NO_3^- for the sake of completeness because with NH_4^+ nitrification and NO_3^- diffusion, as discussed, these treatments will, within a few days, become essentially the same as homogeneously mixed NO_3^-.

Pot experiment

Maize (*Zea mays* L. var Colisee, KWS, Germany) was grown in 1.6 l pots (20 × 10 cm Ø) under controlled root-zone temperature of 20 ± 2 °C. The substrate was based on 66 % low-P soil from a long-term unfertilized grassland (0–20 cm depth; P_{CAL}, 30 mg kg^{-1}; P_{total}, 667 mg kg^{-1}; K_{CAL}, 233 mg kg^{-1}; Mg_{CaCl2}, 66 mg kg^{-1}; pH ($CaCl_2$), 7.1; C_{org}, 2.4 %; N_{total} 0.24 %) and 34 % quartz sand (0.6–1.2 mm Ø), on weight basis. There was a control treatment without any fertilizer (No P). The substrate for the treatment NH4-Depot was fertilized as follows (kg^{-1} soil DM): 100 mg N ($(NH_4)_2SO_4$); 150 mg K (K_2SO_4); 50 mg Mg ($MgSO_4$); and 22 % H_2O (75 % max water holding capacity). Apart from $(NH_4)_2SO_4$, which was applied in salt form as a concentrated depot (7 cm long band located 5 cm below and 5 cm to the side of the maize seed, 5 × 5 cm), all other nutrients where homogenously mixed in the substrate. There were two variants of the NH4-Depot treatment; one without PGPM and the other inoculated with *Pseudomonas* sp. DSMZ13134 as bio-effector (BE1). Bio-effectors (BEs) are viable plant growth-promoting microorganisms (PGPMs) and/or active natural compounds which directly or indirectly promote plant growth with a negligible direct input of nutrients and/or organic matter [39]. Additionally, there was a positive P control (+P), with its substrate fertilized similarly to that of NH4-Depot described above except that N (100 mg N as $CaNO_3$) and P [150 mg as $Ca(H_2PO_4)_2$] where homogenously mixed in the substrate.

The inoculum of *Pseudomonas* sp. DSMZ13134 (BE1) was prepared from the commercially available product Proradix, which is a powder formulation of viable cells and other additives (Sourcon Padena, Tübingen, Germany). For treatment of turf, the producer recommended

rate is 10 g Proradix suspended in 200–400 l water and applied on an area of 1000 m^2. This rate is commensurate with 4.4×10^6 CFUs kg^{-1} soil DM, assuming a soil bulk density of 1.5 g cm^{-3} and a treated soil depth of 10 cm. The producer also refers to higher application rates for different plant species: 1×10^{10} CFUs kg^{-1} soil for substrate-inoculation in pot-grown tomato or barley [40, 41] and 8×10^{10} CFUs kg^{-1} seed for seed-inoculation in pot- and field-grown barley [41]. In an initial screening test with *Pseudomonas* sp. DSMZ13134 among other bacterial and fungal PGPMs on maize, the inoculation rate of 2×10^8 CFUs kg^{-1} soil DM led to little or no effect on root or shoot growth in comparison to the non-inoculated controls. Therefore, in this pot experiment, we employed a high inoculation rate of 1×10^{11} CFUs kg^{-1} soil DM. This was to improve the chance for early root colonization in the immediate seeding zone as well as late root colonization in the fertilizer depot zone.

Each pot was filled with 1.9 kg of substrate. For each pot of treatment BE1, half of the total quantity of inoculum was drenched over the seeding hole at sowing to ensure early root colonization and the other half was placed directly over the NH_4^+-depot to promote root colonization in the developing rhizosphere "hotspot." To prepare the inoculum for the seed-hole, Proradix (6.6×10^{10} CFUs g^{-1}) was suspended in 2.5 mM $CaSO_4$ to a concentration of 5×10^9 CFU ml^{-1} and 10 ml of the suspension (5×10^{10} CFUs) was applied by drenching over the seed in the seed-hole. To maintain a concentrated NH_4^+-depot, the inoculum suspension applied over the NH_4^+-depot was more concentrated than the one drenched over the seed-hole. It had a concentration of 2.5×10^{10} CFU ml^{-1} and 2 ml of the suspension (5×10^{10} CFUs) was pipetted directly over the $(NH_4)_2SO_4$ depot. The total inoculation rate for BE1 pots was, therefore, 1×10^{11} CFUs kg^{-1} soil DM (1 kg Soil DM pot^{-1}). For other treatments, volumes of 2.5 mM $CaSO_4$ were applied accordingly. There were four replicates per treatment arranged in a completely randomized design. There was 16 h light and 8 h darkness. Average daily temperature was 20 ± 2 °C (max 26.9 °C and min 14.6 °C).

The diameter of the stem base and the maximum area of the youngest fully developed leaf were measured at 55 days after sowing (DAS). At 56 DAS, SPAD values were measured on the youngest fully developed leaf (average of 6 measurements $leaf^{-1}$) using SPAD 502 Plus (Konica Minolta, Chiyoda, Japan). SPAD values represent chlorophyll concentrations, which positively correlate with leaf N concentration.

Shoot and root biomass (65 DAS) were harvested and dried (60 °C 48 h). Shoot N and P concentrations were measured using CN elemental analyzer and molybdate–vanadate method [42] respectively.

Rhizobox experiment

Maize (*Zea mays* L. var Colisee) was grown in rhizoboxes (40 × 20 × 2 cm; H × W × D). The substrate was based on 80 % low-P, loess-based, C-horizon subsoil (P_{CAL}, 5 mg kg^{-1}; P_{total}, 332 mg kg^{-1}; pH ($CaCl_2$), 7.6; C_{org}, <0.3 %; N_{total} 0.02 %), and 20 % quartz sand (0.6–1.2 mm Ø), on weight basis. The substrate was adequately supplied with the following nutrients (kg^{-1} soil DM): N (100 mg, $CaNO_3$ or $(NH_4)_2(SO_4)$; P [150 mg, $Ca(H_2PO_4)_2$]; K (150 mg, K_2SO_4); Mg (50 mg, $MgSO_4$); micronutrients: 20 µmol Fe, Sequestrene; 2.6 mg Zn, $ZnSO_4$; 1 mg Cu, CuSO4); and H_2O (60 % max water holding capacity, 18 % moisture). Each rhizobox was filled with 2.4 kg of substrate.

Treatments included two N levels: (1) $CaNO_3$ homogenously mixed in the substrate (NO3-Mixed) and (2) Concentrated $(NH_4)_2SO_4$, fertilizer (64 mg N ml^{-1}) stabilized with the nitrification inhibitor 3, 4-dimethylpyrazole phosphate (DMPP) [37] (NovaTec® Solub 21, Compo Expert, Münster, Germany) placed as a depot 5 × 5 cm to the maize seed (NH4-Depot); in factorial combination with two BE levels: (1) no inoculation (NoBE) and (2) inoculation with (BE1) at the rate 1×10^9 CFUs kg^{-1} soil DM (×2 applications). The inoculation rate of 1×10^9 CFUs or Spores kg^{-1} soil DM for bacterial bio-effectors was later recommended by project management as consistent with producer suggested rates for different soil-inoculated microbial PGPMs.

To prepare the inoculum, Proradix (6.6×10^{10} CFUs g^{-1}) was suspended in 2.5 mM $CaSO_4$ to a concentration of 5×10^8 CFU ml^{-1}. Through the rhizobox window after sowing, 3.26 ml of the inoculum suspension was pipetted on the substrate 2.5 cm around the NH_4^+-depot zone or corresponding soil zone in NO3-mixed treatments. The second inoculation was performed 2 weeks after sowing. There were four replicates per treatment arranged in a completely randomized design. Greenhouse conditions were set at 16 h light at 25 °C and 8 h darkness at 18 °C.

At 32 DAS, SPAD values on the youngest fully developed leaf, plant height and stem diameter were measured. Plants were harvested at 55 DAS. pH on the root surface 0–8 cm from and >8 cm away from the NH_4^+ depot or corresponding soil zone in NO3-mixed treatments was assessed qualitatively for color changes with Bromocresol-purple pH-indicator agar [43, 44] and quantitatively by measurement of potential difference using antimony micro-electrodes [43, 45].

For qualitative pH assessment with Bromocresol-purple pH-indicator agar, 1 % bromocresol-purple solution was prepared 2 weeks before use as recommended. For it, 1 g bromocresol-purple was suspended in 80 ml dest water in a 100 ml Erlenmeyer flask. For dissolution to occur, 1 N NaOH was added dropwise under continuous

stirring, ensuring with a pH meter that the pH of the solution did not exceed 9. After about 30 min, the pH ceased to decrease indicating complete dissolution. At that point, the pH of the solution was lowered to 6 using 1 N H_2SO_4. The flask was filled up with dest water to the 100 ml mark. Under stirring, 5 g agar was suspended and cooked in 400 ml dest water in a 500 ml Erlenmeyer flask to completely dissolve. 5 ml of 1 % bromocresol-purple solution was added and then the flask was filled up with dest water to 500 ml. At about 40 °C, bromocresol-purple-agar solution was then poured on a Plexiglas tray to a layer about 3 mm thick. Once solidified, the layer of agar was carefully placed over the soil surface on the rhizobox window to cover the NH_4^+-depot zone or the corresponding zone in NO3-Mixed treatments. After a few minutes, color change along the root surface could be observed, yellow for acidification below pH 5.2 and purple for alkalinization above pH 6.8. In order to read pH changes, color standards were prepared by mixing 50 µl pH buffer solutions (4, 5, 6, 7, 8, 9, and 10) with 450 µl bromocresol-purple-agar solution in small transparent glass-vial caps and allowed to solidify.

For quantitative potentiometric pH measurements, antimony micro-electrodes were calibrated by measuring the potential difference (200–500 mV) of pH buffer solutions (4, 5, 6, 7, 8, 9, and 10) and generating the following best-fitting sigmoidal calibration curve with five parameters: $f = y0 + a/(1 + \exp(-(x-x0)/b))\text{^}c$; $r^2 = 0.99$, SEM = 0.2; f = pH; x = potential difference (mV); $a = 6.1419$; $b = 47.8570$; $c = 1.9364$; $x0 = 339.7333$; and $y0 = 4.0089$). For the regression, *SigmaPlot 12.0* was used [Systat Software Inc.(SSI), San Jose, California, USA]. The potential difference on the root surface below the bromocresol-purple-agar was measured with a pH meter (pH 320, WTW GmbH Weilheim, Germany) connected to an antimony micro-electrode and to a reference calomel-electrode. Measured potential differences were back-transformed to pH using the sigmoidal calibration curve.

Separately, roots located within 8 cm or more than 8 cm away from the NH4-Depot or corresponding zone were harvested, washed, scanned, and root length and architecture analyzed using *WinRhizo Pro V. 2009c* (Regent Instruments Inc., Canada). To measure the number of rhizoplane-dwelling fluorescent Pseudomonads per unit volume of substrate in the NH_4^+ depot or corresponding zone in NO3-Mixed treatments, 0.5–1.5 g of fresh root sample were thoroughly washed with sterile deionized water (autoclaved 121 °C for 20 min), shaken with 50 ml of sterile ice-cooled 0.1 % proteose peptone and 10 sterile glass beads at 250 rpm for 15 min using autoclaved 250 ml Erlenmeyer flasks. After shaking, flasks were cooled on ice, 5 ml extracts were serially 10-fold diluted with 0.1 % proteose peptone, plated on selective Kings B medium containing 45 mg Novobiocin l^{-1} and 45 mg Penicillin l^{-1} [46], and incubated 23 h at 30 °C. The number of colonies were counted and the colonization rate per gram fresh root was calculated. Using colonization rate and weight of fresh roots per unit substrate volume around the fertilizer depot zone (or corresponding zone for NO_3^- treatments), we calculated the colonization rate per unit substrate volume.

Shoot and root biomass were harvested and dried (60 °C 48 h). Shoot N and P concentrations were measured.

Field experiments

2014

Maize (*Zea mays* L. var Colisee) was grown on soil with moderate N_{min} and available P levels at the research station of the University of Hohenheim, Ihinger Hof, Renningen, Germany (48°44′42.3″N 8°55′26.7″E; 475 m above sea level; 688 mm av. annual rainfall; 8.8 °C mean annual daily temperature). Soil properties were Haplic luvisol, 24–28 % clay, 67–72 % silt, 4–5 % sand, pH ($CaCl_2$) 6.9, C_{org}, 1 %, N_{min}, 38 kg ha^{-1}; P_{CAL}, 120 mg kg^{-1}. There were 8 treatments (Table 1) arranged in a Latin rectangle

Table 1 Treatments (field experiments 2014 and 2015)

		Starter N and P	Additional P	Additional N	BE
Treatments 2014					
1	Zero	–	–	–	–
2	+P	MAP	TSP	NH4-Broad	–
3	NH4-Broad	MAP	–	NH4-Broad	–
4	NH4-Broad*BE1	MAP	–	NH4-Broad	BE1
5	NH4-Broad*BE2	MAP	–	NH4-Broad	BE2
6	NH4-Depot	MAP	–	NH4-Depot	–
7	NH4-Depot*BE1	MAP	–	NH4-Depot	BE1
8	NH4-Depot*BE2	MAP	–	NH4-Depot	BE2
Treatments 2015					
1	Zero	–	–	–	–
2	+P	DAP	TSP	NH4-Broad	–
3	NH4-Broad	DAP	–	NH4-Broad	–
4	NH4-Broad*BE1	DAP	–	NH4-Broad	BE1
5	NH4-Broad*BE2	DAP	–	NH4-Broad	BE2
6	NH4-Depot	DAP	–	NH4-Depot	–
7	NH4-Depot*BE1	DAP	–	NH4-Depot	BE1
8	NH4-Depot*BE2	DAP	–	NH4-Depot	BE2

Starter (starter fertilizers placed 5 × 5 cm to seeds) *MAP* mono-ammonium phosphate, 17 kg N and 35 kg P ha^{-1}, *DAP* di-ammonium phosphate, 28.8 kg N and 32 kg P ha^{-1}, *TSP* triple superphosphate broadcasted and incorporated at 10 cm depth before sowing, 2014, 133 kg P; 2015, 130 kg P ha^{-1}, *NH4-Broad* stabilized $(NH_4)_2SO_4$ broadcasted over the crop canopy 2014, 135 kg N; broadcasted and incorporated before sowing 2015, 100 kg N ha^{-1}, *NH4-Depot* stabilized concentrated $(NH_4)_2SO_4$ solution in water placed as a depot at 10 cm soil depth, 2014, 135 kg N; 2015, 100 kg N ha^{-1}, *BE1 Pseudomonas* sp. DSMZ 13134, *BE2 Bacillus amyloliquefaciens* FZB42; BE application: 2014, broadcast/incorporation 1 × 10^6 CFU g^{-1} soil DM; 2015, placement of a band of BE-treated pumice stones in the sowing row; 0.13 × 10^6 CFU g^{-1} soil DM

design with 5 columns and 5 rows (there were 17 other treatments as part of another study). After sugar beet harvest, the soil was ploughed with a moldboard plough to 20 cm depth in autumn 2013. Plot area was 45 m^2 (4.5 × 10 m) and contained 6 maize rows (75 cm inter-row distance). Data were collected only from the central four core rows (2–5). The first and last 1 m length of each plot and rows 1 and 6 were excluded as plot boarders.

Fertilizer type, application methods and rates were as follows (Table 1): (1) MAP: Mono-ammonium phosphate (12 % NH4-N, 22 % P) (Krista™ MAP, Yara GmbH, Germany); 17 kg N and 35 kg P ha^{-1}. MAP, was placed as "starter" fertilizer on 21 May. "Starter" fertilizer placement was performed at 5 cm to both sides of and 5 cm below the seeding zone with the assistance of GPS and additional on-site positioning tools; (2) TSP: Triple superphosphate (20 % P) hand-broadcasted (20 May 2014) and incorporated at 10 cm depth the following day before sowing; 133 kg P ha^{-1}; (3) NH4-Broad: Stabilized $(NH_4)_2SO_4$ (21 % NH4-N, 24 % S) broadcasted over the canopy at 5–6 leaf stage (24–25 June), (NovaTec® Solub 21, Compo Expert, Münster, Germany); 135 kg N ha^{-1}; (4) NH4-Depot: Concentrated stabilized $(NH_4)_2SO_4$ (21 % NH4-N, 24 % S) in water (64 g N l^{-1}) placed at 10 cm depth midway between rows 1–2, 3–4, and 5–6 at 5–6 leaf stage (24–25 June); 135 kg N ha^{-1}.

The bio-effectors applied included BE1 (already described) and BE2, *Bacillus amyloliquefaciens* FZB42 (2.5×10^{10} spores g^{-1}), a commercially available product in liquid formulation containing spores and other additives (RhizoVital FZB42, ABiTEP GmbH, Berlin Germany). The producer recommended application rates are 100–500 ml ha^{-1} for seed treatment and 1000–2000 ml ha^{-1} for soil application by drenching or spraying. These rates are commensurate with 1.7–8.3 × 10^6 spores kg^{-1} soil DM (for seed treatment) and 1.7–3.4 × 10^7 spores kg^{-1} soil DM (for soil treatment), assuming a treated soil depth of 10 cm and a bulk density of 1.5 g cm^{-3}.

Like BE1, BE2 was also applied at a rate of 1×10^9 Spores kg^{-1} soil DM as recommended by project management. To apply bio-effectors, stock suspensions were freshly prepared, diluted on field site, and applied on the soil surface on the same day. Bio-effectors were applied one day before sowing (20 May) and again at 2–4 leaf stage (17 June). For the first application of BE1, 1 kg of Proradix (6.6×10^{10} CFUs g^{-1}) was suspended in about 18 l Cl-free water to produce 20 l BE1 stock suspension with a concentration of 6.75×10^{12} CFU l^{-1}. 2 l of stock were diluted with Cl-free water to 24 l, applied using a watering can over the soil surface and incorporated to 10 cm depth (Soil bulk density 1.5 g cm^{-3}) just before sowing on 21 May. The total quantity of BE1 inoculum used in the second application (2–4 leaf stage, 17 June) was reduced as a means to reduce costs while maintaining the CFU density of 1×10^9 CFU kg^{-1} soil DM in the crop row. In order to achieve this, the inoculum was sprayed only over the maize row to drench the soil beneath (about 10 cm width) instead of over the entire plot area. For this, a dilute BE1 stock was prepared (9.0×10^{11} CFU l^{-1}) from which 2 l were diluted with Cl-free water to 24 l and applied using a watering can.

For the first application of BE2, 5.4 kg of RhizoVital FZB42 (2.5×10^{10} spores g^{-1}) were suspended in about 14.6 l Cl-free water to produce 20 l of BE2 stock suspension with a concentration of 6.75×10^{12} spores l^{-1}. Like BE1, BE2 was applied at a rate of 1×10^9 CFU kg^{-1} soil DM. The second application of BE2 was also performed at 2–4 leaf stage (17 June). 2 l of BE2 stock (9.0×10^{11} CFU l^{-1}) was further diluted with Cl-free water to 24 l and applied over the maize row.

Using a pneumatic plot drill and positioning tools (GPS and on-site correction devices), plots were seeded at the pre-defined rows with untreated maize at the rate of 9–10 seeds m^{-2} on 21 May. Top dressing of $(NH_4)_2SO_4$ at 5–6 leaf stage (24–25 June) resulted in leaf injury if fertilizer was trapped on the leaf surface. Plants later fully recovered. Concentrated stabilized $(NH_4)_2SO_4$ solution placed at 10 cm depth as a depot showed no signs of injury to the plants.

Plant emergence 16 days after sowing (DAS; number of emerged plants along 2 m row length × 4), number of 2-leaf stage plants 16 DAS, BBCH 12 (measured similarly to emergence) and plant height (23 and 78 DAS; 10 successive plants row^{-1} × 4) were measured for the controls: Zero, NH4-Broad, and +P. At 35 and 53 DAS (1 and 19 days after placement of NH4-fertilizer depot respectively), soil samples 0–30 cm depth were collected from the midway point between rows 1–2, 3–4, and 5–6 (NH4-Depot zone or corresponding soil zone for non-NH4-Depot treatments). N_{min} concentrations in samples were measured. SPAD (43 and 79 DAS; average of 4 measurements $leaf^{-1}$ × 5 successive plants row^{-1} × 4), ear-leaf N and P concentrations (79 DAS; 4 ear-leaf samples row^{-1} × 4) were measured. For treatments, NH4-Broad*BE1 and NH4-Depot*BE1 at 81 DAS (47 days after placement of depot), soil core samples (30 cm L, 5 cm Ø) were collected; four samples were collected from the NH4-Depot zone (or corresponding soil zone for NH4-Broad treatment) at midway point between rows 1–2, 3–4, or 5–6 and four from the non-Depot zone, between rows 2–3 or 4–5. Soil samples were washed, roots were collected, scanned, and analyzed (*WinRhizo Pro*). Grain was harvested on 8 Nov. (172 DAS).

2015

Maize (*Zea mays* L. var Colisee) was grown on soil at another site in Ihinger Hof research station. Like 2014, this site had moderate N_{min} and available P levels. Soil properties included: Haplic luvisol, clay loam, silty loam, pH 7.0, N_{min}, 61 kg ha^{-1}, P_{CAL}, 110 mg kg^{-1}. There were 8 treatments (Table 1) arranged in a completely randomized block design with 5 blocks (10 additional treatments were part of another study). Plot area was 58.5 m^2 (4.5 × 13 m) with 6 maize rows (75 cm inter-row distance). Like in 2014, plot boarders were excluded during data collection.

Fertilizer types, application methods and rates included (Table 1): (1) DAP: starter fertilizer as di-ammonium phosphate placed 5 × 5 cm to seeds at sowing (13 May); 28.8 kg N and 32 kg P ha^{-1}; (2) TSP: Triple superphosphate broadcasted by hand and incorporated at 10 cm depth before sowing (11 May); 130 kg P ha^{-1}; (3) NH4-Broad: Stabilized $(NH_4)_2SO_4$ broadcasted and incorporated 10 cm deep before sowing (11 May); 100 kg N ha^{-1}; (4) NH4-Depot: Concentrated solution of stabilized $(NH_4)_2SO_4$ in water (62.7 g N l^{-1}) placed as a depot at 10 cm depth midway between rows 1–2, 3–4, and 4–5 (4–5 leaf stage, 18 June); 100 kg N ha^{-1}.

Bio-effectors included BE1 and BE2, each placed as a band of BE-treated pumice stones (Table 1). To treat pumice stones (Rotocell 0.3–1.5, density 320 kg m^{-3}, ROTEC GmbH & Co. KG, Mülheim-Kärlich, Germany) with BE, Cl-free water suspensions of Proradix (6.6 × 10^{10} CFUs g^{-1}) and RhizoVital FZB42 (2.5 × 10^{10} spores g^{-1}) were each prepared to a concentration of 2 × 10^{12} CFUs l^{-1} or spores l^{-1}. Each suspension was evenly applied using a pressurized hand pump sprayer at the rate of 0.23 l kg^{-1} pumice stones, which were spread on a plastic sheet (0.47 × 10^{12} CFUs or spores kg^{-1} pumice stones). Pumice stones were then turned over several times to homogenize inoculum absorption, air-dried at room temperature and applied on the field on the same day. Application was done by placement in 5–10 cm deep furrows cut in the sowing row. The application rate was 32 g pumice stones m^{-1} furrow (100 ml pumice stones m^{-1} furrow). Furrows were covered with soil and the entire plot was tilled with a rototiller to 10 cm depth. The final inoculum density in soil within the sowing row was 1 × 10^9 CFU kg^{-1} soil DM (15 kg soil DM m^{-1} furrow; 10 cm row width; and 10 cm row depth, soil bulk density 1.5 g cm^{-3}), which was about ten times higher the inoculum density (10 cm depth) if the inoculum was evenly applied over the entire plot area (0.13 × 10^9 CFU kg^{-1}).

On 12 May, plots were sown at the rate of 9–10 seeds m^{-2} as in 2014. For treatment NH4-Depot only, soil samples 0–30 cm depth were collected from the midway point between rows 1–2, 3–4, and 5–6 (NH4-Depot side) and 2–3 and 4–5 (Non-NH4-Depot side) on 30 Jun. (48 DAS) and N_{min} concentration was measured. For treatments Zero, +P, NH4-Broad, NH4-Depot, NH4-Depot*BE1, and NH4-Depot*BE2, plant height 48 DAS were recorded. For all treatments, plant height (71 DAS), SPAD (68 DAS), stem diameter (68 DAS, max diameter between nodes 2 and 3, sampling was done as for plant height 2014) were collected. To measure root length density in the fertilizer depot zone for treatments NH4-Broad and NH4-Depot at 99 DAS (63 days after placement of depot), soil core samples (30 cm L, 5.5 cm Ø) were collected, four from the NH4-Depot zone (or corresponding soil zone for NH4-Broad treatment), midway point between rows 1–2, 3–4, or 5–6 and four from the non-Depot zone, midway point between rows 2–3 or 4–5. Soil samples were washed, roots were collected, scanned, and analyzed (*WinRhizo Pro*). On 21 Sep. (132 DAS), aboveground biomass was harvested for maize silage.

Statistics

For the pot and rhizobox experiments, One and Two-Way ANOVA with pair-wise comparisons (Tukey test, $\alpha = 0.05$) were performed [*SigmaPlot 12.0*, Systat Software Inc. (SSI), San Jose, California, USA]. For field experiments, One and Two-Way ANOVA with pair-wise comparisons (Tukey test, $\alpha = 0.05$) or ANOVA on Ranks for not-normally distributed data were performed (*SAS 9.4*, SAS Institute Inc., Cary, NC, USA).

Results

Pot experiment

Stem base diameter increased in the following order: NoP (8.5 mm) = NH4-Depot (8.8 mm) <NH4-Depot + BE1 (10.8 mm) = +P (12.0 mm); and it strongly correlated with shoot P content ($r^2 = 0.83$, $P < 0.00001$). Maximum leaf area of the youngest fully developed leaf also strongly correlated with shoot P content ($r^2 = 0.71$, $P < 0.00001$). SPAD increased in the following order: NoP (27.6) = +P (29.2) < NH4-Depot (36.3) = NH4-Depot + BE1 (37.4).

After harvest, NoP plants showed the lowest shoot dry weight (4.7 g plant^{-1}) and shoot P content (6.1 mg P plant^{-1}). +P plants showed the highest shoot dry weight (11.0 g plant^{-1}) and shoot P content (13.9 mg P plant^{-1}). For NH4-Depot plants, inoculation with BE1 (8.9 g plant^{-1}) led to 59 % more shoot dry weight than without (5.6 g plant^{-1}) (Fig. 1a). With BE1, NH4-Depot plants (11.3 mg P plant^{-1}) had 64 % higher shoot P content than without (6.9 mg P plant^{-1}) (Fig. 1b). Similarly, with BE1 (149.9 mg N plant^{-1}), there was 50 % higher shoot N content in plants than without (99.8 mg N plant^{-1}).

There was no difference in the shoot P concentration between treatment pairs. Shoot N concentration

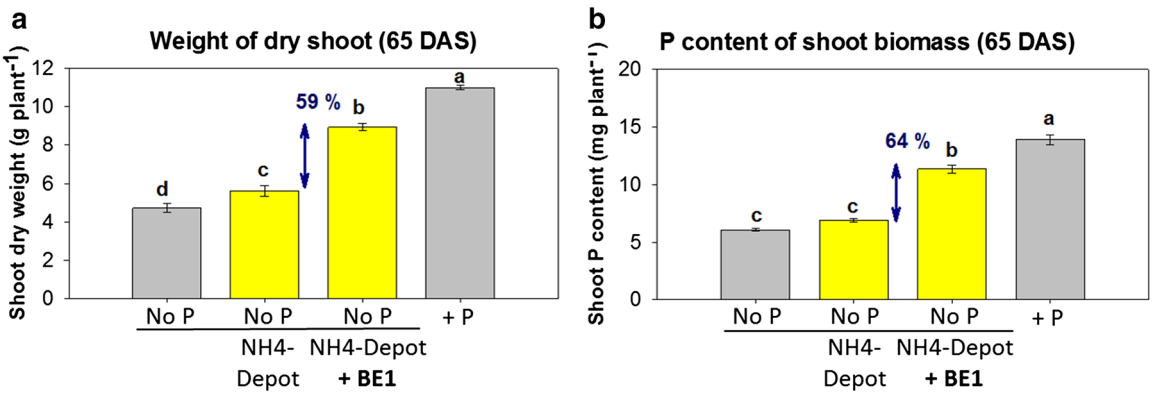

Fig. 1 Shoot dry weight (**a**) and P content (**b**) (Pot experiment). *No P* No P fertilizer, *+P* 100 mg NO_3-N and 150 mg soluble-P kg^{-1} soil, *NH4-Depot* 100 mg NH_4-N kg^{-1} soil, *BE1 Pseudomonas* sp. DSMZ13134

increased in the following order: NoP (7.7 mg g^{-1}) < +P (10.1 mg g^{-1}) < NH4-Depot + BE1(16.8 mg g^{-1}) = NH4-Depot (17.9 mg g^{-1}).

Rhizobox experiment

At 32 DAS, there was no difference in the SPAD value of the youngest fully developed leaf between pairs of treatments. BE (NoBE or BE1) had an effect on SPAD (BE1, 46.1 > NoBE, 43.6; $P = 0.040$), whereas N (NO3-Mixed or NH4-Depot) did not. BE had an effect on plant height (NoBE, 86.6 cm > BE1, 82.3 cm; $P = 0.012$), whereas N did not. Furthermore, N had an effect on stem diameter (NO3-Mixed, 11.6 mm > NH4-Depot, 9.8 mm; $P = 0.008$), whereas BE did not. Stem diameter was not statistically different between treatment pairs.

At 55 DAS, there was higher root length density (RLD) in the fertilizer depot zone in treatment NH4-Depot compared to the corresponding soil zone in treatment NO3-Mixed (Fig. 2a). N had a strong effect on RLD within these zones (NH4-Depot, 10.2 cm cm^{-3} > NO3-Mixed, 6.3 cm cm^{-3}; $P < 0.001$) and BE did not. RLD in the remaining substrate volume of the rhizobox was affected by N (NO3-Mixed, 4.85 cm cm^{-3} > NH4-Depot, 4.28 cm cm^{-3}; $P = 0.04$) and by BE (NoBE, 4.88 cm cm^{-3} > BE1, 4.26 cm cm^{-3}; $P = 0.03$), without any N * BE interaction.

Rhizosphere pH was lower in the NH_4^+ depot zone (NH4-Depot) than in the corresponding soil zone with homogenous NO_3^- supply (NO3-Mixed) (Fig. 3a) and only slightly lower for measurements in outer zones (Fig. 3b). Rhizosphere acidification in the fertilizer depot

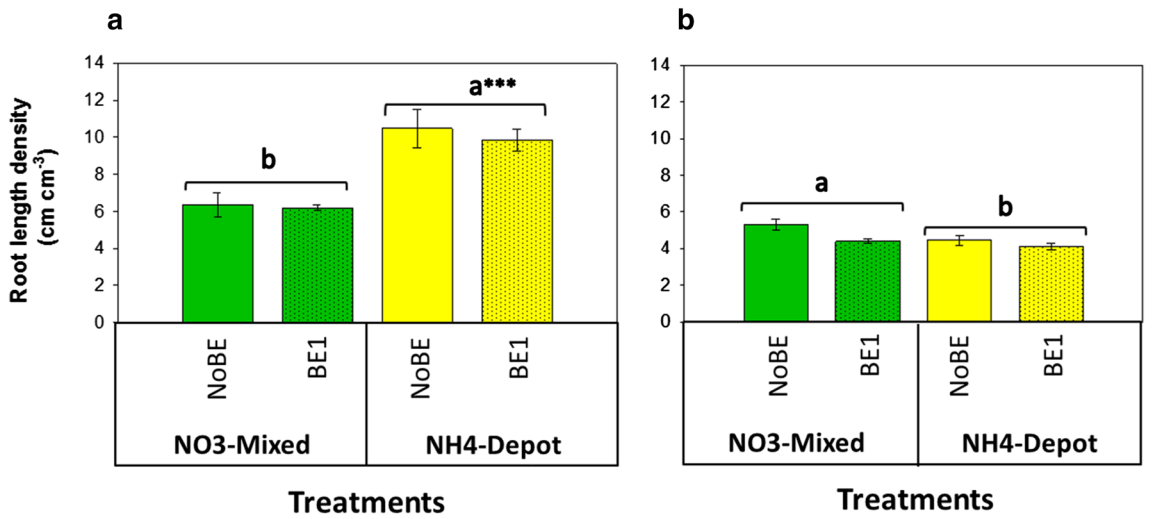

Fig. 2 Root length density within (**a**) or outside (**b**) the NH4-Depot or corresponding soil zone (Rhizobox). *NO3-Mixed* $Ca(NO_3)_2$ homogenously mixed in substrate; *NH4-Depot* stabilized $(NH_4)_2SO_4$ placed in substrate as a depot, *BE* bio-effector, *NoBE* no bio-effector, *BE1 Pseudomonas* sp. DSMZ 13134. *Different letters* between N levels show a significant N-effect, ***$P < 0.001$ (Two-way ANOVA, Tukey test, $\alpha = 0.05$). There was no BE-effect within the NH4-Depot zone. For RLD outside the zone, NO3-Mixed was higher than NH4-Depot ($P = 0.04$) and NoBE was higher than BE1 ($P = 0.03$)

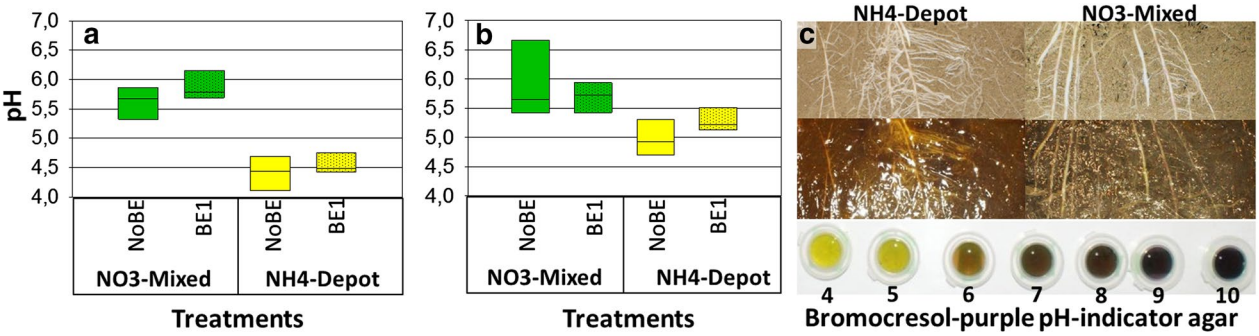

Fig. 3 Root surface pH within (**a**, **c**) or outside (**b**) the NH4-Depot or corresponding soil zone (Rhizobox). *NO3-Mixed* $Ca(NO_3)_2$ homogenously mixed in substrate, *NH4-Depot* stabilized $(NH_4)_2SO_4$ placed in substrate as a depot, *BE* bio-effector, *NoBE* no bio-effector, *BE1 Pseudomonas* sp. DSMZ 13134

zone could be qualitatively confirmed by yellow coloration in Bromocresol-purple pH-indicator agar along roots growing in the NH4-Depot zone (Fig. 3c). Root colonization by fluorescent *Pseudomonas* sp. around the fertilizer depot zone in treatment NH4-Depot was higher than that of the corresponding soil zone in treatment NO3-Mixed (Fig. 4).

Maize shoots from NH4-Depot treatments had higher shoot concentrations and contents of N and P than those from NO3-Mixed treatments (Table 2). There was no difference in shoot DM between pairs of treatments (Table 2).

Field experiment 2014

There was no difference in seed emergence (16 DAS), number of 2-leaf stage plants (16DAS), and plant height

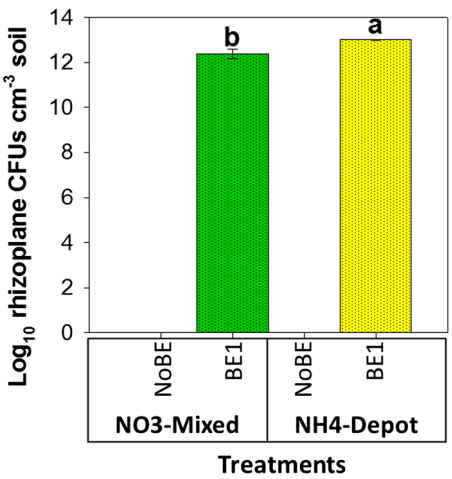

Fig. 4 Root colonization by fluorescent *Pseudomonas* sp. within the NH4-Depot or corresponding soil zone (Rhizobox). *NO3-Mixed* $Ca(NO_3)_2$ homogenously mixed in substrate, *NH4-Depot* stabilized $(NH_4)_2SO_4$ placed in substrate as a depot, *BE* bio-effector, *NoBE* no bio-effector, *BE1 Pseudomonas* sp. DSMZ 13134, (*t* test, $P = 0.045$)

(23 and 78 DAS) between pairs of control treatments: Zero, NH4-Broad, and +P.

One day after main N fertilization (35 DAS), soil NH_4-N concentration at 0–30 cm depth (fertilizer depot zone or corresponding zone) for treatment NH4-Depot (304 kg NH_4-N ha^{-1}) was higher than that for NH4-Broad (36 kg NH_4-N ha^{-1}) and that for Zero (2.5 kg NH_4-N ha^{-1}). After 19 days (53 DAS), NH_4-N concentrations had reduced: NH4-Depot (204 kg NH_4-N ha^{-1}), NH4-Broad (14.9 kg NH_4-N ha^{-1}), and Zero (2.15 kg NH_4-N ha^{-1}).

At 43 DAS, there was no difference in the SPAD value of the youngest fully developed leaf of plants between pairs of treatments: Zero (31.0), NH4-Broad (39.5), and NH4-Depot (38.2). By 79 DAS, ear-leaf SPAD value for treatment Zero (50.8) was less than that for treatments NH4-Depot (56.9), NH4-Depot*BE1 (56.3), NH4-Depot*BE2 (57.7), NH4-Broad (58.3), and +P (57.3) ($P < 0.012$). N-fertilizer application method had an effect on ear-leaf P concentration (79 DAS) (Broad > Depot; $P < 0.0001$, Table 3), whereas BE did not. Ear-leaf P concentration for treatment +P (4.90 mg P g^{-1} DM) was higher than that for other treatments. Zero had the lowest concentration (2.37 mg P g^{-1} DM), which was not different from that of NH4-Depot (3.31 mg P g^{-1} DM), NH4-Depot*BE1 (3.40 mg P g^{-1} DM), or NH4-Depot*BE2 (3.17 mg P g^{-1} DM). Similarly, N-fertilizer application method had an effect on ear-leaf N concentration (79 DAS) (Broad > Depot; $P = 0.0029$, Table 3), whereas BE did not. The concentration for Zero (2.52 %) was lower than that for each of the other treatments ($P \leq 0.0485$), among which ear-leaf N concentrations were not different between pairs.

At 81 DAS, N-fertilizer application method ($P < 0.001$) and BE ($P = 0.005$) positively affected root length density (RLD) with a significant interaction between both factors ($P = 0.003$). RLD was higher in soil on the sides of maize rows with a fertilizer depot (midway point between rows

Table 2 Shoot N and P concentration and content, and shoot dry matter, 55 days after sowing, (Rhizobox experiment)

	N conc. (% DM)	N content (mg N plant^{-1})	P conc. (mg P g^{-1} DM)	P content (mg P plant^{-1})	Shoot DM (g plant^{-1})
LS means N*BE					
NO3-mixed*NoBE	1.83	114.0	2.18	13.7	6.35
NO3-mixed*BE1	2.22	130.1	2.32	13.6	5.91
NH4-Depot*NoBE	2.47	137.1	2.93	16.2	5.60
NH4-Depot*BE1	2.66	133.9	3.15	15.9	5.09
Standard error	0.134	4.69	0.146	0.62	0.420
Two-way ANOVA					
N	***0.002*** **	***0.014*** *	***<0.001*** ***	***0.002*** **	*NS*
NO3	2.03 b	122.0 b	2.52 b	13.6 b	6.13
NH4	2.57 a	135.5 a	3.04 a	16.0 a	5.35
BE	***0.051***	*NS*	*NS*	*NS*	*NS*
NoBE	2.15	125.7	2.56	15.0	5.97
BE1	2.44	132.0	2.73	14.7	5.50
N*BE	*NS*	*NS*	*NS*	*NS*	*NS*

P values are in italics; *NS* no significant difference when $P \geq 0.1$; $P < 0.1$ is bold italics; * $P < 0.5$; ** $P < 0.01$; *** $P < 0.001$; Means not sharing the same letters are significantly different from each other, Tukey test $\alpha = 0.05$; factors and interaction is bold italics; *NO3* $Ca(NO_3)_2$ homogenously mixed in substrate

NH4 stabilized $(NH_4)_2SO_4$ placed in substrate as a depot, *BE* bio-effector, *NoBE* no bio-effector, *BE1 Pseudomonas* sp. DSMZ 13134

1–2, 3–4, and 5–6) in comparison to those sides without (midway point between rows 2–3 and 4–5) (Fig. 5a). RLD in the fertilizer-depot zone was higher with BE1 than without (Fig. 5b).

N-fertilizer application method (despite NH4-Depot—7.69 Mg ha^{-1} being 7.4 % higher than that of NH4-Broad—7.16 Mg ha^{-1}) and BE had no statistically significant effect on grain yield (Table 3). As expected, Zero produced the lowest grain yield (6.31 Mg ha^{-1}). Only NH4-Depot*NoBE (8.45 Mg ha^{-1}) led to higher grain yields than Zero ($P = 0.0086$).

Field experiment 2015

There was severe soil compaction (>15 cm depth) in many parts of the field site that led to placement of starter fertilizer and the NH_4^+-fertilizer depot often at a shallower depth than intended. Soil compaction also coincided with extreme drought and high temperatures in the summer months which caused NH_4^+-fertilizer depots to be pulled up to the soil surface forming a salt crust at several areas.

12 days after placement of fertilizer depots in treatment NH4-Depot (48 DAS), soil NH_4-N concentration at 0–30 cm depth in fertilizer depot zones (midway point between rows 1–2, 3–4 and 5–6; 337 kg NH_4-N ha^{-1}) was higher than that for zones without fertilizer depot (midway point between rows 2–3 and 4–5; 1.8 kg NH_4-N ha^{-1}, $P < 0.001$).

At 48 DAS, plant heights were statistically similar for NH4-Depot with or without BE (NH4-Depot, 93 cm; NH4-Depot*BE1, 95 cm; NH4-Depot*BE2, 93 cm). Only +P (107 cm) and NH4-Broad (101 cm) plants were taller than Zero plants (83 cm, $P \leq 0.0476$). At 71 DAS, N-fertilizer application method affected plant height (Broad > Depot, $P = 0.0071$), whereas BE did not (Table 4). Only +P (235 cm), NH4-Broad (229 cm), and NH4-Broad* BE2 (236 cm) plants were taller than Zero plants (210 cm, $P \leq 0.0485$).

At 68 DAS, N-fertilizer application method had an effect on SPAD for the youngest fully developed leaf (Broad > Depot, $P = 0.0087$), whereas BE did not (Table 4). SPAD values for all other treatments were higher than that for Zero (49, $P \leq 0.0336$). At 68 DAS similarly, N-fertilizer application method had an effect on stem diameter (Broad > Depot; $P = 0.0011$), whereas BE did not (Table 4). Stem diameter for +P (24.4 mm), NH4-Broad (24.6 mm), NH4-Broad*BE1 (24.8 mm), and NH4-Broad*BE2 (24.7 mm) only, were higher than that of Zero (22.4 mm, $P \leq 0.0207$).

Like in 2014 furthermore, N-fertilizer depot positively affected root length density (RLD) ($P < 0.001$). However, unlike in 2014, BE1 had no effect. RLD doubled in soil in the sides of maize rows with fertilizer depot (midway point between rows 1–2, 3–4, and 5–6) in comparison to sides of maize rows without fertilizer depot (midway point between rows 2–3 and 4–5) (Fig. 5b).

N-fertilizer application method affected fresh above-ground biomass yield (Broadcast > Depot, $P = 0.03$), whereas BE had only a marginal effect (BE1 > NoBE, $P = 0.06$) (Table 4). Inoculation of BE1 showed a tendency to produce about 4.5 % higher fresh biomass than BE2 ($P = 0.15$). Fresh above-ground biomass of Zero

Table 3 NH_4^+-application and bio-effector effects on ear-leaf N and P, and grain yield (field experiment 2014)

	Ear-leaf N conc. (%)	Ear-leaf P conc. (mg g^{-1})	Grain (Mg ha^{-1})
LS means NH4*BE			
NH4-Broad	3.28	4.17	7.23
NH4-Broad*BE1	3.25	4.16	7.23
NH4-Broad*BE2	3.15	4.06	7.05
NH4-Depot	3.02	3.31	8.35
NH4-Depot*BE1	3.02	3.40	7.41
NH4-Depot*BE2	2.91	3.17	7.36
Standard error	0.09	0.17	0.45
Two-Way ANOVA			
NH4 application method	***0.0029*** **	***<0.0001*** ***	*NS*
NH4-Broad	3.22 ± 0.05 a	4.13 ± 0.09 a	7.16 ± 0.28
NH4-Depot	2.99 ± 0.05 b	3.29 ± 0.09 b	7.69 ± 0.29
BE	*NS*	*NS*	*NS*
NoBE	3.15 ± 0.06	3.74 ± 0.12	7.74 ± 0.34
BE1	3.14 ± 0.06	3.78 ± 0.12	7.35 ± 0.34
BE2	3.03 ± 0.06	3.61 ± 0.12	7.19 ± 0.34

P values are in italics, *NS* no significant difference when $P \geq 0.1$; $P < 0.1$ is bold italics; * $P < 0.5$; ** $P < 0.01$; *** $P < 0.001$; Means ± standard errors not sharing the same letters are significantly different from each other, Tukey test $\alpha = 0.05$; *NH4-Broad* starter fertilizer as mono-ammonium phosphate (MAP) followed by broadcasting and incorporation stabilized $(NH_4)_2SO_4$ over the canopy

NH4-Depot starter MAP and subsurface placement of concentrated stabilized $(NH_4)_2SO_4$ solution in water at 10 cm soil depth; *BE* bio-effector, *NoBE* no bio-effector, *BE1 Pseudomonas* sp. DSMZ 13134, *BE2 Bacillus amyloliquefaciens* FZB42, *Ear-leaf N, and P* (79 DAS, BBCH 61-75) and grain yield (172 DAS, BBCH 89-99)

(46.0 Mg ha^{-1}) was less than that for +P (55.0 Mg ha^{-1}), NH4-Broad*BE1 (56.6 Mg ha^{-1}), NH4-Broad*BE2 (56.5 Mg ha^{-1}), and NH4-Depot*BE1 (54.6 Mg ha^{-1}) ($P < 0.0293$).

N-fertilizer application method had no effect on dry above-ground biomass, whereas BE had an effect ($P = 0.0364$). Banding of BE1 below the seed row led to higher dry shoot biomass than banding of BE2 or without BE inoculation (Table 4). BE1 led 10.9 % ($P = 0.035$) higher dry biomass than BE2. Dry above-ground biomass for treatments +P (20.2 Mg ha^{-1}), NH4-Broad*BE1 (20.1 Mg ha^{-1}), and NH4-Depot*BE1 (20.5 Mg ha^{-1}) were higher than that for Zero (16.8 Mg ha^{-1}, $P < 0.0245$).

Discussion

In the pot experiment, inoculation of *Pseudomonas* sp. DSMZ 13134 strongly improved shoot P content. This was likely a result of improved plant P-acquisition from soil P pools that were previously not plant-available. Strong response of maize growth to inoculated *Pseudomonas* may have been possible due to high root colonization by *Pseudomonas* which could result from high inoculation rates and inoculation directly on the seed, seeding hole and fertilizer depot. If N is not limiting, optimal P supply enables plants to establish large leaf areas, which increases photosynthesis and growth rate, thus, resulting in more dry-biomass production than under P limitation [24].

In the rhizobox experiment, higher root length density (RLD) in soil around the fertilizer depot in comparison to that in soil distant from the depot or in soil with homogenous supply of NO_3^-, was due to high concentrations of root growth stimulating NH_4^+ present within the depot. NH_4^+ is known to strongly stimulate lateral root initiation and elongation at the site of contact with roots [25, 31, 34, 36]. However, the set-up of the rhizobox experiment did not make it possible to attribute the increase in RLD around the localized N-depot between localized N supply by placement and N supply as NH_4^+ differentially. Increased N-depot RLD could only be attributed to both. In our natural soil-based substrate without any water-tight barriers against mass flow and diffusion of N-sources like NO_3^- or $CO(NH_2)_2$, localized N supply could only be possible by localized placement of stabilized NH_4^+. NH_4^+ was stabilized with the nitrification inhibitor DMPP and further, by using a highly concentrated and toxic NH_4^+ solution, which also inhibits oxidation of NH_4^+ by soil microorganisms [47]. Improved establishment of *Pseudomonas* in the fertilizer depot zone was due to increased root density in the depot zone, which was likely associated with high levels of nutrients for rhizobacteria released as organic compounds in root exudates [15]. Shoot P and N content were mainly influenced by N-fertilizer form. Inoculation of *Pseudomonas* led only to a marginal increase in shoot N concentration. Rhizosphere acidification induced by NH_4^+-nutrition

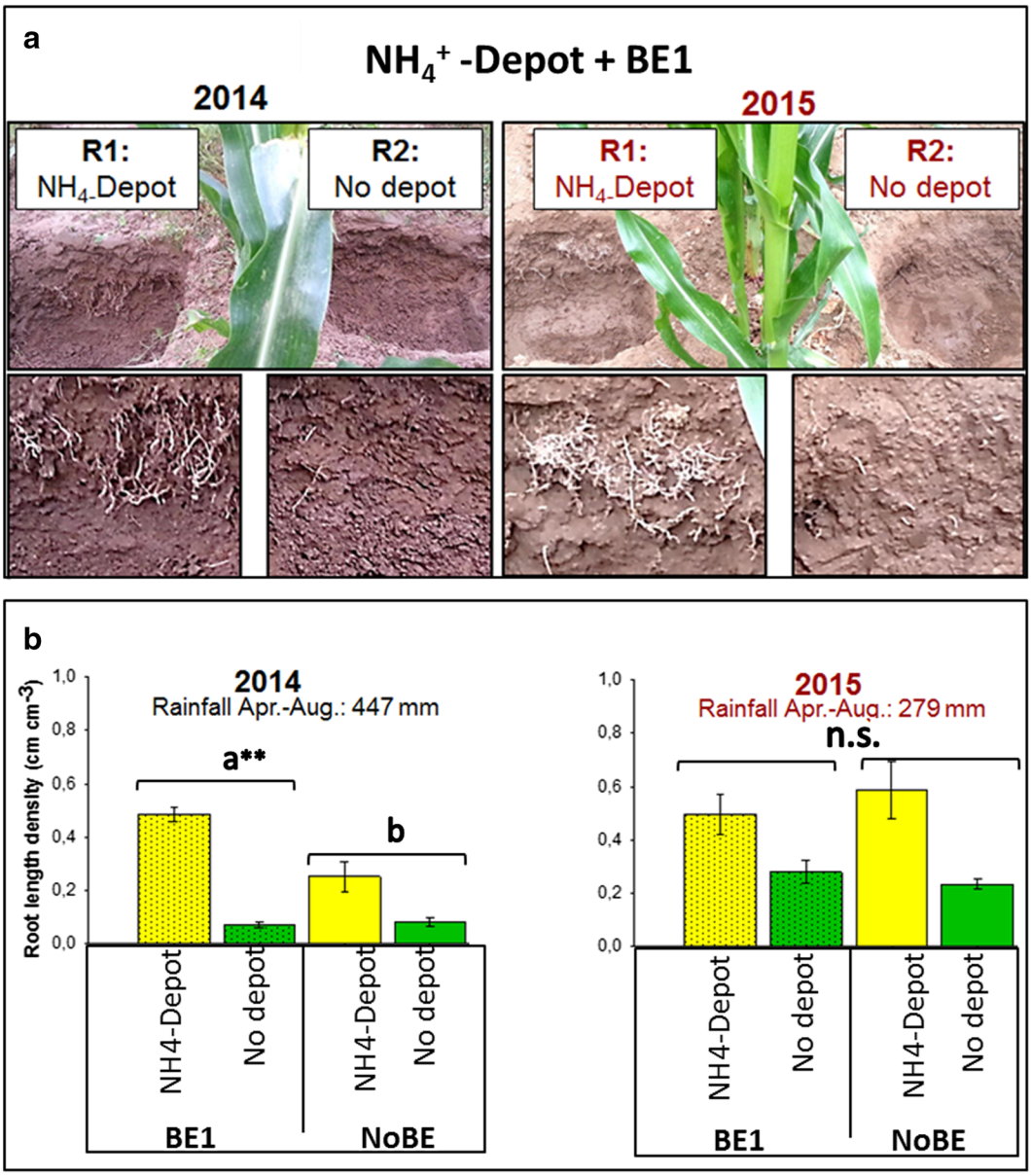

Fig. 5 Root growth (**a**) and density (**b**) in NH4-Depot and non-Depot row-sides (Field 2014 and 2015). *NH4-Depot* side of maize row with concentrated stabilized $(NH_4)_2SO_4$ solution placed as a depot at 10 cm depth; *No depot* other side of maize row without an NH_4^+-fertilizer depot; *BE* bio-effector, *NoBE* no bio-effector, *BE1 Pseudomonas* sp. DSMZ 13134. *Different letters* between BE levels show a significant BE-effect, $^{**}P < 0.01$ (Two-Way ANOVA, Tukey test, $\alpha = 0.05$). There was no BE-effect in 2015. There was strong NH4-Depot-effect on RLD in both 2014 ($P < 0.001$) and 2015 ($P < 0.001$)

[25] is known to enhance solubility of sparingly soluble calcium phosphates in soil, which also build up after application of water-soluble phosphates on neutral to alkaline soils [48, 49]. Furthermore, soil acidification inhibits NH_3 volatilization from urea or NH_4^+ fertilizers placed in soil [50].

In the pot and rhizobox experiments, improved plant growth was associated with marked increase in shoot P content without change in shoot P concentration. Shoot P concentration stayed the same or increased marginally. An explanation could be that on the low-P soils, plant P status was already in the critical range for deficiency with a threshold concentration of 0.25–0.4 % [51]. Under these conditions, any surplus in P supply and P uptake is immediately utilized for biomass production leading to dilution of P concentrations, which then restores the

Table 4 Sources of variation (two-way ANOVA, field experiment 2015)

	SPAD 68 DAS	Stem Ø 68 DAS (mm)	Height 71 DAS (cm)	Biomass F. M. (Mg ha^{-1})	Biomass D. M. (Mg ha^{-1})
NH4	***0.0087***	***0.0011***	***0.0071***	***0.0264***	*NS*
Broad	53.5 a	24.7 a	230.8 a	55.1 a	19.6
Depot	52.1 b	23.4 b	222.7 b	52.1 b	18.6
BE_Band	*NS*	*NS*	*NS*	***0.0635***	***0.0364***
NoBE	52.8	24.0	224.3	51.9	18.8 b
BE1	52.4	23.9	226.4	55.6	20.3 a
BE2	53.2	24.2	229.5	53.2	18.3 b
NH4*BE_Band	*NS*	*NS*	*NS*	*NS*	*NS*

DAS days after sowing, *SPAD* estimate of leaf N concentration, *Stem Ø* stem diameter, *Height* plant height, *P* values are in italics, *NS* no significant difference when $P \geq 0.1$; $P < 0.1$ is bold italics; Means not sharing the same letters are significantly different from each other, Tukey test $\alpha = 0.05$; *NH4-Broad* starter fertilizer as di-ammonium phosphate (DAP) followed by broadcast and incorporation of stabilized $(NH_4)_2SO_4$ before sowing, *NH4-Depot* starter DAP, and subsurface placement of concentrated stabilized $(NH_4)_2SO_4$ solution in water at 10 cm soil depth at 4–5 leaf stage; *BE* bio-effector, *BE1 Pseudomonas* sp. DSMZ 13134; *BE2 Bacillus amyloliquefaciens* FZB42, *Biomass* above-ground biomass (BBCH 85-87)

initial critical P concentrations. This suggests that positive PGPM effects on plant growth may be more achievable on soils with moderate fertility than on very poor or highly fertile soils.

Under field conditions, placement of NH_4^+ fertilizer as a subsurface depot sustained high NH_4^+ concentrations within the depot that stimulated intense depot root growth. Sustained high concentrations of NH_4^+ within the depot was attributable on the one hand, to NH_4^+-stabilization effect of the nitrification inhibitor [37] and on the other, to high, toxic NH_4^+ concentrations in the depot. In 2014, root density in the fertilizer depot zone was higher with inoculation of *Pseudomonas* than without, indicating a potential for inoculated PGPMs to enhance root exploitation of subsurface N-fertilizer depots.

Unlike in the rhizobox experiment, N fertilization by subsurface placement of NH_4^+ as a depot did not improve shoot N and P status under field conditions. A reason could be that the fertilizer depot was closer to the maize seed in the rhizobox and pot experiments (5 × 5 cm) than in the field experiments (38 × 5 cm). Therefore, despite root growth within the fertilizer depot under field conditions at later growth stages, the distance between the depot and maize plants may have limited N acquisition from the depot. Therefore, it may be recommended that subsurface fertilizer depots should be placed as close to seeds as possible (5 × 5 cm) as long as fertilizer toxicity effects on seeds or young plants can be avoided. For this purpose, fertilizer placement in subsurface soil should be done at sowing or soon after to avoid mechanical damage to plant roots at later growth stages.

Although field soil had moderate levels of plant-available P, additional P fertilization led to improved shoot P status (2014). However, this did not lead to improved grain yield (2014) or improved yield of above-ground biomass (2015), suggesting that P was not the most limiting nutrient in the field sites. In 2014, placement of NH_4^+ as a depot led marginally to higher grain yield than broadcast of NH_4^+, whereas application of PGPM did not affect grain yield. A reason for the weak effect of the fertilizer depot could be the moderate initial N_{min} level of the field soil. Furthermore, soil N_{min} likely increased later in season as soil organic matter mineralization by soil microorganisms probably increased in the warm summer months. This explanation is supported by the high yield recorded for the unfertilized control treatment.

In the field in 2015, NH_4^+-fertilizer application by broadcast and incorporation led to higher yield in fresh above-ground biomass than by placement as a subsurface depot. One reason could be that plants supplied with N by broadcast and incorporation before sowing were able to acquire more N during critical early growth stages than those supplied with N by placement of a subsurface N-depot at 5–6 leaf stage, more than 1 month after sowing. Another reason could be that severe drought that followed placement of fertilizer as a subsurface depot inhibited N acquisition. Firstly, there was insufficient moisture for optimal N uptake from the fertilizer depot, and secondly, rapid water loss from the soil caused fertilizer depot salts to be pulled up from the soil to the surface forming unavailable salt crusts.

In 2014, inoculation of *Pseudomonas* did not increase maize grain yield. It may be attributed to PGPM application technique as well as to the absence of severe environmental stress factors. Application of a large quantity of inoculum (on hectare basis) as a suspension of viable cells in water by broadcast and incorporation may have been unfavorable for inoculum survival and propagation due to exposure to the biotic and abiotic environment.

In 2015, inoculation of *Pseudomonas* as a below-seed band led to higher dry above-ground biomass than

inoculation with *Bacillus amyloliquefaciens* or without inoculation of PGPM. This yield increase associated with *Pseudomonas* was not linked to improved root density in the fertilizer depot or improved leaf N status. Maize growth-promotion effect of inoculated *Pseudomonas* seemed to have depended on the one hand, on high *Pseudomonas* concentrations present in the immediate surroundings of maize seeds due to placement of inoculum as a below-seed band, producing a high critical PGPM density at the root-zone required for optimal PGPM effects on plant growth [14, 15]. On the other hand, it may have depended on favorable protective micro-environments for inoculum survival and propagation provided by pore spaces in pumice stones used as carrier [14]. Given extended drought and high temperatures on the field in 2015, plant growth-promotion by *Pseudomonas* may have occurred via induction of resistance to the prevalent abiotic stress factors. *Pseudomonas* are producers of the enzyme 1-aminocyclopropane-1-carboxylate (ACC)-deaminase, which utilizes ACC, the precursor of ethylene thereby lowering plant ethylene levels and stimulating resistance to heat and drought stress [52–55].

Protective pore spaces in the pumice stone carrier employed in 2015 may have also functioned at the same time as a slow-release tool for viable cells to be progressively supplied to plant roots. Additionally, nutrients for PGPM provided in the inoculum product (skimmed milk for *Pseudomonas*) may have been protected within the pore spaces from utilization by other non-target soil microorganisms. Therefore, with pumice stones as carrier, *Pseudomonas* cells may have been able to safely multiply within the protected niche of pore spaces. It is important to note that, with respect to low inoculation rates in the field experiment, the amount skimmed milk powder present in the *Pseudomonas* inoculum formulation had no direct plant fertilization significance. Due to smaller amounts of inocula required for PGPM application as a below-seed band, high quantities of inoculum and associated high costs for application by broadcast and incorporation could be avoided.

Because PGPM effects on plant growth largely depends on viability of inoculated cells in soil [14, 15], it may be worthwhile to also test non-microbial bio-effectors with root growth-promoting properties in combination with placement of subsurface fertilizer depots. In this context, such non-microbial bio-effectors may be particularly effective under conditions where PGPM activity is inhibited by unfavorable environmental conditions. Seaweed extracts with proven protective activity against abiotic stresses [56] could be promising candidates.

Growth-promotion effects of tested inoculated *Pseudomonas* on maize seemed also to have been determined by soil type and soil fertility level (especially for P). PGPM growth-promotion effect on maize was higher on low-P grassland soil (Pot) or low-P loess subsoil (Rhizobox) than on silty loam field soil with moderate levels of plant-available P.

Similarly to PGPM plant growth-promotion effects, growth-promotion effects of subsurface placed fertilizer depend on plant nutrient status, which in turn depends on initial soil fertility level or initial plant nutrient supply [24, 57, 58].

Conclusions

We hypothesized the following: (1) Marked rhizosphere acidification occurs within and around a "rhizosphere hotspot" formed by placement of an NH_4^+-depot in soil. (2) Survival and colonization of inoculated PGPMs is higher in the "rhizosphere hotspot" than in comparable soil zones with respect to plant position supplied homogeneously with NO_3^- fertilizer. (3) Inoculated and established PGPMs further promote root development around the NH_4^+-depot zone. (4) Consequently, NH_4^+-depot fertilization combined with inoculated PGPMs will result in higher nutrient uptake and higher yields than NH_4^+-depot fertilization without PGPMs.

Placement NH_4^+-fertilizer as a subsurface depot stimulated the formation of "rhizosphere hotspots" with intense root growth. Marked rhizosphere acidification within and around NH_4^+-induced "rhizosphere hotspots" led to improved plant P and N uptake. Combination of fertilizer placement in subsurface soil with inoculation of the PGPM *Pseudomonas* sp. DSMZ 13134 in soil led to improved plant growth-promotion effects under greenhouse and field conditions, however, with low reproducibility. Reproducible results may be achieved through optimization of PGPM inoculation techniques to enhance their survival in often hostile environmental conditions in field soil and through improvement of subsurface fertilizer placement to ensure optimal nutrient availability to target crop plants. PGPM application techniques involving stable dry spore formulations or viable cells in drought-resistant protective capsules or alginate may be promising options.

Abbreviations

ACC: 1-aminocyclopropane-1-carboxylate; BE: bio-effector or PGPM; BE1: *Pseudomonas* sp. DSMZ 13134; BE2: *Bacillus amyloliquefaciens* FZB42; Broad: fertilizer broadcasted and incorporated in soil (field); CAN: calcium ammonium nitrate; CULTAN: controlled long-term ammonium nutrition; DAP: di-ammonium phosphate; DAS: days after sowing; Depot: fertilizer placed as a subsurface localized depot; DMPP: 3, 4-dimethylpyrazole phosphate; MAP: mono-ammonium phosphate; Mixed: fertilizer evenly mixed in soil (greenhouse); NoBE: no inoculated BE or PGPM; PGPMs: plant growth-promoting microorganisms; RLD: root length density; TSP: triple superphosphate.

Authors' contributions
PMN participated in conceiving and designing all studies, ran and managed all experiments, collected plant-growth data (emergence, growth stage, SPAD, height, stem diameter etc.), collected soil, plant and root samples, performed laboratory analyses (rhizosphere pH, root growth characteristics, nutrient content in plant samples root colonization by *Pseudomonas* sp. DSMZ 13134 or Rifampicin-resistant *Bacillus amyloliquefaciens* FZB42), performed statistical analyses and interpretation results, drafted and participated in reviewing the manuscript, agrees to accountability. MW participated in conceiving and designing field studies, assisted in running and managing field experiments, collected soil, plant and root samples, participated in interpretation of results, participated in reviewing the manuscript, agrees to accountability. TM participated in conceiving and designing all studies, supervised progress of study, participated in interpretation of results, participated in reviewing the manuscript, agrees to accountability. All authors read and approved the final manuscript.

Author details
[1] Fertilisation and Soil Matter Dynamics (340 i), University of Hohenheim, 70593 Stuttgart, Germany. [2] Nutritional Crop Physiology (340 h), University of Hohenheim, 70593 Stuttgart, Germany.

Acknowledgements
This study was part of the BIOFECTOR project funded by the European Commission within the 7th Framework Program | Grant Agmt. No. 312117.
We would like to express our sincere thanks for the reliable support provided by the technical staff and student assistants of Fertilization and Soil Matter Dynamics (340 i) and the technical staff of Ihinger Hof Research station (301), University of Hohenheim, Stuttgart, Germany.

Competing interests
The authors declare that they have no competing interests.

Received: 5 January 2016 Accepted: 14 April 2016
Published online: 09 May 2016

References

1. Sharma SHS, Lyons G, McRoberts C, McCall D, Carmichael E, Andrews F, Swan R, McCormack R, Mellon R. Biostimulant activity of brown seaweed species from Strangford Lough: compositional analyses of polysaccharides and bioassay of extracts using mung bean (*Vigna mungo L.*) and pak choi (*Brassica rapa chinensis L.).* J Appl Phycol. 2012;24(5):1081–91.
2. Halpern M, Bar-Tal A, Ofek M, Minz D, Muller T, Yermiyahu U. Chapter Two—the use of biostimulants for enhancing nutrient uptake. Adv Agron. 2015;130:141–74.
3. Barea JM, Toro M, Orozco MO, Campos E, Azcon R. The application of isotopic (32P and 15 N) dilution techniques to evaluate the interactive effect of phosphate-solubilizing rhizobacteria, mycorrhizal fungi and Rhizobium to improve the agronomic efficiency of rock phosphate for legume crops. Nutr Cycl Agroecosys. 2002;63:35–42.
4. Ferris H, Venette RC, van der Meulen H, Lau SS. Nitrogen mineralization by bacterial-feeding nematodes: verification and measurement. Plant Soil. 1998;203:159–71.
5. Mao X, Hu F, Griffiths B, Li H. Bacterial-feeding nematodes enhance root growth of tomato seedlings. Soil Biol Biochem. 2006;38(7):1615–22.
6. Jiang Y, Wu Y, Xu W, Cheng Y, Chen J, Xu L, Hu F, Li H. IAA-producing bacteria and bacterial-feeding nematodes promote Arabidopsis thaliana root growth in natural soil. Eur J Soil Biol. 2012;52:20–6.
7. Mohite B. Isolation and characterization of indole acetic acid (IAA) producing bacteria from rhizospheric soil and its effect on plant growth. J Soil Sci Plant Nutr. 2013;13:638–49.
8. Mauricio R. Mycorrhiza helper bacteria: a promising model for the genomic analysis of fungal–bacterial interactions. New Phytol. 2005;168:1–4.
9. Frey-Klett P, Garbaye J, Tarkka M. The mycorrhiza helper bacteria revisited. New Phytol. 2007;176(1):22–36.
10. Richardson A, Barea J, McNeill A, Prigent-Combaret C. Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. Plant Soil. 2009;321(1–2):305–39.
11. Ghorbani R, Wilcockson S, Koocheki A, Leifert C. Soil management for sustainable crop disease control: a review. Environ Chem Lett. 2008;6(3):149–62.
12. Yang J, Kloepper JW, Ryu C. Rhizosphere bacteria help plants tolerate abiotic stress. Trends Plant Sci. 2009;14(1):1–4.
13. Shakiri MA, Bano A, Arshad M. PGPR with ACC deaminase confer drought tolerance wheat. Soil Environ. 2012;31(1):108–12.
14. van Veen JA, van Overbeek LS, van Elsas JD. Fate and activity of microorganisms introduced into soil. Microbiol Mol Biol R. 1997;61(2):121–35.
15. Lugtenberg B, Kamilova F. Plant-growth-promoting rhizobacteria. Annu Rev Microbiol. 2009;63(1):541–56.
16. Blackshaw RE, Semach G, Janzen HH. Weed science society of America—fertilizer application method affects nitrogen uptake in weeds and wheat. Weed Sci. 2002;50(5):634–41.
17. Petersen J. Competition between weeds and spring wheat for 15 N-labelled nitrogen applied in pig slurry. Weed Res. 2005;45:103–13.
18. Ruidisch M, Bartsch S, Kettering J, Huwe B, Frei S. The effect of fertilizer best management practices on nitrate leaching in a plastic mulched ridge cultivation system. Agr Ecosyst Environ. 2013;169:21–32.
19. Nash PR, Motavalli PP, Nelson KA. Nitrous oxide emissions from claypan soils due to nitrogen fertilizer source and tillage/fertilizer placement practices. Soil Sci Soc Am J. 2012;76(3):983–93.
20. Linquist BA, Adviento-Borbe MA, Pittelkow CM, van Kessel C, van Groenigen KJ. Fertilizer management practices and greenhouse gas emissions from rice systems: a quantitative review and analysis. Field Crop Res. 2012;135:10–21.
21. Ma BL, Wu TY, Tremblay N, Deen W, McLaughlin NB, Morrison MJ, Stewart G. On-farm assessment of the amount and timing of nitrogen fertilizer on ammonia volatilization. Agron J. 2010;102(1):134–44.
22. Reith JWS. Recent advances in fertilizer placement. I. Fertilizer placement for swedes and turnips in Scotland. J Sci Food Agric. 1954;5(9):421–8.
23. Cooke GW. Recent advances in fertilizer placement. II.—Fertilizer placement in england. J Sci Food Agric. 1954;5(9):429–40.
24. Grant CA, Flaten DN, Tomasiewicz DJ, Sheppard SC. The importance of early season phosphorus nutrition. Can J Plant Sci. 2001;81(2):211–24.
25. Jing J, Rui Y, Zhang F, Rengel Z, Shen J. Localized application of phosphorus and ammonium improves growth of maize seedlings by stimulating root proliferation and rhizosphere acidification. Field Crop Res. 2010;119(2–3):355–64.
26. Sommer K. CULTAN-Düngung: physiologisch, ökologisch, ökonomisch optimiertes Düngungsverfahren für Ackerkulturen, Grünland, Gemüse. Gelsenkirchen-Buer: Zierpflanzen und Obstgehölze. Verlag Th. Mann; 2005.
27. Bonkowski M, Cheng W, Griffiths BS, Alphei J, Scheu S. Microbial-faunal interactions in the rhizosphere and effects on plant growth. Eur J Soil Biol. 2000;36:135–47.
28. Dutta D, Bandyopadhyay P. Performance of chickpea (*Cicer arietinum L.*) to application of phosphorus and bio-fertilizer in laterite soil. Arch Agron. Soil Sci. 2009;55(2):147–55.
29. Arshad M, Waqas R. Novel bacteria possessing ACC-deaminase enzyme enhances the water use efficiency in field crops. Int Post J Sci Technol. 2011;1(1):2.
30. Barber SA. Soil nutrient bioavailability: a mechanistic approach. New York: Wiley; 1984.
31. Anghinoni I, Barber SA. Predicting the effect of ammonium placement on nitrogen uptake by corn. Agron J. 1990;82(1):135–8.
32. Neumann G, Römheld V. Rhizosphere chemistry in relation to plant nutrition. In: Marschner P, editor. Marschner's mineral nutrition of higher plants. 3rd ed. London: Academic Press; 2012. p. 347–60.
33. Nieder R, Benbi D, Scherer H. Fixation and defixation of ammonium in soils: a review. Biol Fert Soils. 2011;47(1):1–14.
34. Drew MC. Comparison of the effects of a localised supply of phosphate, nitrate, ammonium and potassium on the growth of the seminal root system, and the shoot, in barley. New Phytol. 1975;75(3):479–90.
35. Hawkesford M, Horst W, Kichey T, Lambers H, Schjoerring J, Møller IS, White P. Functions of macronutrients. In: Marschner P, editor. Marschner's mineral nutrition of higher plants. 3rd ed. London: Academic Press; 2012. p. 135–78.
36. Jing J, Zhang F, Rengel Z, Shen J. Localized fertilization with P plus N elicits an ammonium-dependent enhancement of maize root growth and nutrient uptake. Field Crop Res. 2012;133:176–85.

37. Zerulla W, Barth T, Dressel J, Erhardt K, von Horchler Locquenghien K, Pasda G, Rädle M, Wissemeier A. 3,4-Dimethylpyrazole phosphate (DMPP)—a new nitrification inhibitor for agriculture and horticulture. Biol Fert Soils. 2001;34(2):79–84.
38. Robinson D. The responses of plants to non-uniform supplies of nutrients. New Phytol. 1994;127(4):635–74.
39. Weinmann M, Römheld V. Resource preservation by application of bioeffectors in European crop production (research project proposal): European community's seventh framework programme (FP7/2007-2013) under grant agreement n 312117 (BIOFECTOR). University of Hohenheim, 2012. http://www.biofector.org, 10.02.2016.
40. Yusran Y, Safrizal S, Weinmann M, Neumann G, Müller T, Römheld V. Improved mycorrhisation in tomato by soil inoculation with pseudomonas sp. proradix®. In: Tielkes E, editor. Utilisation of diversity in land use systems: sustainable and organic approaches to meet human needs. Book of abstracts. Göttingen: Cuvillier Verlag; 2007. p. 513–4.
41. Fröhlich A, Buddrus-Schiemann K, Durner J, Hartmann A, Rad U. Response of barley to root colonization by Pseudomonas sp. DSMZ 13134 under laboratory, greenhouse, and field conditions. J Plant Interac. 2012;7(1):1–9.
42. Gericke S, Kurmies B. Colorimetrische Bestimmung der Phosphorsäure mit Vanadat-Molybdat. Z Anal Chem. 1952;137(1):15–22.
43. Marschner H, Römheld V. In vivo measurement of root-induced ph changes at the soil-root interface: effect of plant species and nitrogen source. Z Pflanzenphysiol. 1983;111(3):241–51.
44. Häussling M, Leisen E, Marschner H, Römheld V. An improved method for non-destructive measurements of the ph at the root-soil interface (Rhizosphere). J Plant Physiol. 1985;117(4):371–5.
45. Schaller G, Fischer WR. pH-Änderungen in der Rhizosphäre von Mais- und Erdnußwurzeln. Z Pflanzenernaehr Bodenk. 1985;148(3):306–20.
46. Sugimoto EE, Hoitink HAJ, Tuovinen OH. Enumeration of oligotrophic rhizosphere pseudomonads with diluted and selective media formulations. Biol Fertil Soils. 1990;9(3):226–30.
47. Shaviv A. Control of nitrification rate by increasing ammonium concentration. Fert Res. 1988;17(2):177–88.
48. Lu DQ, Chien HS, Henao J, Sompongse D. Evaluation of short-term efficiency of diammonium phosphate versus urea plus single superphosphate on a calcareous soil. Agron J. 1987;79(5):896–900.
49. Moody PW, Edwards DG, Bell LC. Effect of banded fertilizers on soil solution composition and short-term root-growth. 2 Mono-ammonium and di-ammonium phosphates. Soil Res. 1995;33(4):689–707.
50. Ma Q, Zhang F, Rengel Z, Shen J. Localized application of NH4 +-N plus P at the seedling and later growth stages enhances nutrient uptake and maize yield by inducing lateral root proliferation. Plant Soil. 2013;372(1–2):65–80.
51. Barry DAJ, Miller MH. Phosphorus nutritional requirement of maize seedlings for maximum yield. Agron J. 1989;81(1):95–9.
52. Glick BR. Modulation of plant ethylene levels by the bacterial enzyme ACC deaminase. FEMS Microbiol Lett. 2005;251(1):1–7.
53. Shaharoona B, Arshad M, Zahir ZA, Khalid A. Performance of *Pseudomonas spp.* containing ACC-deaminase for improving growth and yield of maize (*Zea mays L.)* in the presence of nitrogenous fertilizer. Soil Biol Biochem. 2006;38(9):2971–5.
54. Naveed M, Khalid M, Jones DL, Ahmad R, Zahir ZA. Relative efficacy of *Pseudomonas spp.,* containing ACC-Deaminase for improving growth and yield of maize (*Zea mays L.)* in the presence of organic fertilizer. Pak J Bot. 2008;40:1243–51.
55. Zahir ZA, Ghani U, Naveed M, Nadeem SM, Asghar HN. Comparative effectiveness of *Pseudomonas* and *Serratia sp.* containing ACC-deaminase for improving growth and yield of wheat (*Triticum aestivum L.)* under salt-stressed conditions. Arch Microbiol. 2009;191(5):415–24.
56. Sangha JS, Kelloway S, Critchley AT, Prithiviraj B. Seaweeds (macroalgae) and their extracts as contributors of plant productivity and quality: the current status of our understanding. Sea Plants. 2014;71:189–219.
57. Buah S, Polito TA, Killorn R. No-tillage corn response to placement of fertilizer nitrogen, phosphorus, and potassium. Commun Soil Sci Plant Anal. 2000;31(19–20):3121–33.
58. Borges R, Mallarino AP. Deep banding phosphorus and potassium fertilizers for corn managed with ridge tillage. Soil Sci Soc Am J. 2001;65:376–84.