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The effect of *Penicillium bilaii* on wheat growth and phosphorus uptake as affected by soil pH, soil P and application of sewage sludge

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Abstract

Background: *Penicillium bilaii* may enhance P availability to plants, since it has been shown to increase plant growth and P uptake. There is currently increasing interest in using microorganisms to promote P mobilisation from organic P sources. An investigation was conducted to determine the effects of *P. bilaii* on P uptake and growth of wheat in the presence and absence of sewage sludge. Two soils differing in P contents and pH were used, as it was hypothesised that these affect the efficiency of P mobilisation.

Methods: A pot experiment, in which wheat was grown for 35 days in a moderately acidic soil of low P status and a calcareous soil of moderate P status, was conducted. A full factorial design was used with two non-sterilised soils, three amendments [control, sewage sludge and triple superphosphate (TSP)] and two *P. bilaii* treatments (with/without). Shoot and root length, biomass and nutrient contents were analysed in plant, whereas soil samples were analysed for water-extractable P and soil pH.

Results: The shoot length and root biomass of wheat were significantly higher when sewage sludge was applied in combination with *P. bilaii* seed inoculation, in the moderately acidic soil. In contrast, shoot length and biomass and root biomass were higher with *P. bilaii* compared to the control, but no synergistic effects of *P. bilaii* and the organic P source were observed in the calcareous soil. A systematic, but not significant increase in total P uptake was found for all treatments inoculated with *P. bilaii* and for both soils, with the control of the low fertility moderately acidic soil being a notable exception.

Conclusions: Sewage sludge was seen to be an efficient P source, on par with TSP in the moderately acidic soil. In the calcareous soil, the *P. bilaii* treatments without added P fertilisers had the greatest effect, with both root and shoot biomass increasing significantly.

Keywords: *P. bilaii*, Sewage sludge, Phosphorus solubilisation, Wheat, Acidic soil, Calcareous soil

Background

Plant-available phosphorus (P) exists in soil solution only in small amounts due to various processes: (1) precipitation as secondary minerals, (2) sorption to mineral surfaces and (3) immobilisation in microbial biomass; all fix P to non-soluble forms. P forms insoluble complexes with cations, particularly aluminium and iron under acid conditions and calcium under neutral to basic conditions [1]. Soil pH is the most important single factor affecting P sorption and dissolution, where the optimum availability is around pH 6.5.

Therefore, in modern agricultural systems, mineral P fertilisers need to be regularly applied. Most mineral P fertilisers are derived from rock phosphate, which is a non-renewable resource becoming increasingly depleted

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and consequently will also eventually become scarce and expensive [2]. The most common mineral fertiliser is triple superphosphate (TSP) derived by acidification of rock P. An alternative to mineral P fertilisers is the waste product sewage sludge, which represents an important P source with around 2–4 % of P depending on the wastewater source and the treatment methods [3], around 70–90 % of the total P is inorganic P and considered potentially equivalent to inorganic fertilisers [4, 5]. Depending on the chemicals used to precipitate P and the amount used in the wastewater treatment facilities, the immediate availability of P to plants from sewage sludge can be very different [6]. Furthermore, specific soil properties, such as soil pH, soil P absorption capacity or P content, can also influence plant P availability in relation to sludge [7–9].

Plant P acquisition and uptake can be enhanced through rhizospheric activity, which is characterised by biological (microbial activity), chemical and spatial (root growth) features [10, 11]. Soil microorganisms can promote P uptake by plants through different mechanisms, such as organic P (P_o) mineralisation, enhanced root growth either by mycorrhizal associations or by hormonal stimulation, and finally by solubilisation of inorganic phosphorus (P_i) [12]. The potential mechanisms for phosphate solubilisation by microorganisms appear to be acidification of the soil, the release of organic acid anions (i.e. citrate, oxalate, gluconate) and the secretion of phosphatases and phytases [12, 13]. Several authors report a wide range of soil bacteria and fungi that are able to solubilise different precipitated P forms under limiting P conditions [14–16]. Wakelin et al. [17] have found that *Penicillium* spp. are commonly present on wheat roots grown in Australia and demonstrate a P-solubilising capacity. *Penicillium* spp. has been found to solubilise rock phosphate in liquid culture [17, 18] and calcium phosphate in an agar medium [19]. Among *Penicillium* species, *Penicillium bilaii (P. bilaii*) has been shown to produce oxalic and citric acid as its major metabolites [20]. Kucey [21] suggests that *P. bilaii* may enhance P availability to plants by releasing organic acids, which may acidify specific areas of the rhizosphere or act as a chelator of cationic partners of the phosphate anion [22]. Furthermore, *P. bilaii* has been identified to increase biomass production and P uptake and grain yield in wheat, canola, bean, pea and lentil in experiments conducted in growth chambers and in the field [14, 23–25]. Most of the studies in which *P. bilaii* has been shown to promote plant growth and P solubilisation have been conducted in calcareous soils in Canada with moderate or low P levels [18, 19, 23]. Wakelin [26] also demonstrated the potential use of *Penicillium* spp. inoculants to increase plant growth in alkaline soils in Australia. Studies on the effect of *P. bilaii* on acidic soils have not been found in the literature.

To reduce farmers' dependence on mineral P fertilisers and find efficient ways of using organic P sources, the potentially beneficial interaction between P-rich wastes and P-solubilising microorganisms requires further studies [27–29]. Consequently, the objective of the present work was to investigate P uptake and growth of wheat and the effects of *P. bilaii* on P mobilisation in the presence of sewage sludge using two soils that differ in P content and soil pH. Therefore, associated with an expected higher P solubilisation in the calcareous soil, it was hypothesised that: (1) *P. bilaii* inoculation leads to stronger plant growth in calcareous soil compared to moderately acidic soil. Secondly, since a better solubilisation and more adequate supply are expected from the calcareous soil, it is hypothesised that (2) the fertilisation with sewage sludge results in a higher immediate P fertilisation response, i.e. higher root and shoot growth in the moderately acidic low P soil compared to the calcareous soil. Finally, due to the higher expected responsiveness to P fertilisation in the moderately acidic soil, it is further hypothesised that (3) seed inoculation with *P. bilaii* will result in a higher plant response in the sewage sludge treatment of the moderately acidic soil compared to that in the calcareous soil, due to *P. bilaii* mobilisation of insoluble forms of P from the sewage sludge.

Methods

Materials

Penicillium bilaii was used in a formulation as water-dissolvable concentrate containing spores of the fungus obtained from the commercial product Jumpstart [7.2×10^8 colony-forming units per g (CFU/g)] from Novozymes. Wheat plants (*Triticum aestivum* L. cv. Dacke) were grown in this study. Two different non-sterilised soils were used: a low fertility moderately acidic soil collected from the Nutrient Depletion Trial at the Experimental Research Farm in Taastrup (Denmark) and a moderately fertile calcareous soil from Moncada (Valencia, Spain). The moderately acidic soil was sandy loam (clay 17 %, silt 17 % and sand 66 %) with a pH of 5.5 (1:5; soil:distilled water). The total C content was 1.1, 0.13 % total N and 2.2 mg kg^{-1} water-soluble P (estimated in original sieved 4 mm soil). From 1964 to 1985, it was depleted of P and K by fertilising solely with N (60 kg N $ha^{-1}y^{-1}$). Since 1996, the fertiliser application was increased to a more normal dose of 120–20–120 kg $ha^{-1}y^{-1}$ of N–P–K, respectively. Between 1996 and the present day, crop rotation has largely consisted of cereals. The calcareous soil was sandy loam (clay 16 %, silt 24 % and sand 60 %) with a pH of 8.7 (1:5; soil:distilled water). The total C content was 1.2, 0.14 % total N and

4.86 mg kg^{-1} water-soluble P. The soil has been planted with citrus trees since the last 6 years and fertilised with 120–20–120 kg $ha^{-1}y^{-1}$ of N–P–K, respectively. Triple superphosphate (TSP) and sewage sludge (SS) were used as phosphorus fertilisers. SS was collected from the Bjergmarken Wastewater Treatment Plant (WWTP) (Roskilde, Denmark). The WWTP process included biological and chemical precipitation of P [including some addition of $FeCl(SO_4)$, $AlCl_3$ and $AlSO_4$]. The sludge was transferred to a thermophilic biogas digester at the plant and processed with 15 days' hydraulic retention time. The effluent was treated with flocculants (polymers) and dewatered in a screw press + decanting centrifuge to produce the raw SS (ca. 27 % DM). SS had a content of total N 44 g kg^{-1}, total P 36 g kg^{-1}, total K 2.8 g kg^{-1} and inorganic N 9.3 g kg^{-1} with a pH of 8.4.

Pot experiment

A pot experiment was set up with wheat plants. Soils were mixed with sand to facilitate root growth and root isolation at the end of the experiment. For each 0.8 kg pot, 0.6 kg of sieved moderately acidic soil (<4 mm) was mixed with quartz sand (0.4–0.9 mm) (3:1; soil:sand; w:w) and 0.56 kg of sieved calcareous soil (<4 mm) was mixed with sand (2.34:1; soil:sand; w:w). The two ratios were used to achieve the same total sand content and hence approximate water retention in the mixtures. All the pots were packed by hand to reach 1.4 g cm^{-3}. Then, a P-free modified Hoagland nutrient solution containing 120 mg N (as NH_4NO_3), 90 mg K, 24 mg Ca, 0.12 mg Cu, 12 mg Mg, 0.24 mg Zn, 0.01 mg Mo, 0.24 mg Fe, 0.17 mg B and 0.36 mg Mn was added. The moisture content was adjusted to 40 % of WHC (water-holding capacity) of the soil–sand mixtures. The mix was pre-incubated at 20 °C for 7 days. The treatments for the moderately acidic soil were: (1) control (moderately acidic soil) (C-a), (2) *P. bilaii*-inoculated seed (CPb-a), (3) sludge (SS-a), (4) sludge + *P. bilaii*-inoculated seed (SSPb-a), (5) triple superphosphate (TSP-a) as a positive control, and (6) triple superphosphate + *P. bilaii*-inoculated seed (TSPP-a). The treatments for the calcareous soil were the same as for the moderately acidic soil (C-c, CPb-c, SS-c, SSPb-c, TSP-c, and TSPPb-c). Four replicates were made for each treatment, giving a total of 48 pots.

At the day of sowing, the P fertilisers (SS and TSP) were applied to the mixed soil in an amount equivalent to 40 mg total P kg^{-1}, corresponding to 3.3 g sludge/pot and 0.18 g TSP/pot. Both P fertilisers were added to the soil bit by bit to ensure that the mixes were homogenously distributed. The rate of sludge application was 10.7 tonnes/ha (calculated for 20 cm soil depth) being lower than regular field rates; but P content (SS and TSP) was 100 kg P/ha, similar to fertilisation field rates. Two seeds of wheat were sown in the centre of each pot at 2 cm depth, and after germination one of the plants were removed. *P. bilaii* was inoculated on the seeds at the rate of 1.4×10^6 CFU/seed. The experiment was conducted in a growth chamber with day/night of 16/8 h, temperature levels of 20 °C and humidity of 65/72 % RH for 35 days. After 35 days, most of the plants reached the G30-39 Zadoks growth stage, corresponding to stem elongation. Only plants under C-a and CPb-a showed P deficiency with a slower growth rate and poor tillering (GS20-29 Zadoks growth stage). A moisture level of 40 % of WHC was maintained during the first week of growing by weighing each pot. After that, the level was increased to 50 % WHC and kept at that level for the remaining growing period.

Shoot and root analysis

Shoot length was measured every 2 days, starting 5 days after sowing until the end of the experiment. At harvest, fresh roots were cleaned of soil and washed with MiliQ water and stored in 96 % ethanol. Later, the fresh roots were scanned with an STD4800 Epson scanner and WinRHIZO software (V5.0, Regent Instruments, Quebec, Canada) and total, fine (≤0.3 mm) and coarse (>0.3 mm) root length were recorded. Sampled shoots and roots were freeze dried at −80 °C for 2 days and weighed. Shoots and roots were ground separately and analysed for macro- and micronutrient content. After digestion in a microwave oven (Multiwave 3000, software version 1.24, Anton Paar GmbH, Graz, Austria), all the samples and reference plant materials were analysed by inductively coupled plasma optical emission spectrometry (Agilent 5100, ICP-OES).

Soil analysis

At harvest, soil samples were taken from each pot to determine the water content, pH and water-extractable P. pH was measured in all pots in water (1:5 w/w). Water-extractable P was analysed by the method described by Van der Paauw [30]. Briefly, the soil P was extracted with milliQ water (1:60), shaken for 1 h and filtered at 0.45 µm. Ortho-P content was analysed on the resulting extracts by flow injection (FIA star 5000, Foss Analytical, Denmark).

Statistical analysis

All recorded data were analysed using descriptive statistics (mean ± standard error) and normality was checked in all cases. Differences between treatments in shoot and root length, biomass, nutrient uptake, soil water-extractable P and soil pH were subjected to two-way analysis of variance (ANOVA). Significant differences ($P < 0.05$) among treatments were assessed by the post hoc Fisher

LSD test. All statistical analysis was carried out using the STATISTICA program (StatSoft, 2001).

Results

Shoot and root growth

In general, shoot growth was significantly higher for wheat grown in the moderately acidic soil (Table 1), compared to the calcareous soil. Shoot growth was significantly affected by treatments during the growing period. For plants grown in moderately acidic soil, 10 days after sowing (DAS) the shoot length was significantly higher in the sewage sludge-amended soil with *P. bilaii* (SSPb-a) compared to all other treatments (Table 1). At the end of the experiment (35 DAS), although SSPb-a showed the highest shoot length (37.7 cm), it was no longer significantly higher than the non-inoculated (SS-a) (36 cm) or TSP-a treatments. *P. bilaii* application alone, CPb-a, resulted in a smaller shoot length (28.1 cm) than the control (33.4 cm).

For plants grown in calcareous soil at 22 DAS the shoot length was significantly higher in wheat treated with *P. bilaii* (CPb-c) compared to the non-inoculated control (C-c), in the majority of sampling. Finally, after 35 DAS, wheat amended with TSP and SS had the longest shoots, but with no significant effect of *P. bilaii* inoculation, and only the TSP-c treatment was significantly higher than the C-c. Similar differences were observed for shoot biomass (Table 2). The addition of either SS or TSP significantly ($P < 0.05$) increased shoot biomass, while it did not significantly affect biomass when each was combined with *P. bilaii* inoculation, for plants grown in the moderately acidic soil. All treatments significantly increased shoot biomass compared to the control in the calcareous soil.

Root biomass measuring 35 DAS (Table 2) showed large differences among treatments and between soils. For plants grown in moderately acidic soil, root biomass was significantly higher in treatments amended with SS and TSP compared to the control (C-a). Inoculation with *P. bilaii* increased root biomass in both amended treatments, although only significantly in SSPb-a. In the non-fertilised soil, *P. bilaii* inoculation decreased root biomass. For plants grown in calcareous soil, root biomass was not influenced by substrate amendment. Inoculation with *P. bilaii* increased root biomass in all treatments, although not significantly for the SSPb-c.

Root length in the moderately acidic soil was generally increased by soil amendments. The highest total root length 35 DAS in the moderately acidic soil was obtained for TSPPb (4188 cm) and was only significantly different from CPb (1873 cm) (Fig. 1a). In the calcareous soil, *P. bilaii* inoculation generally increased total root length, with CPb-c and TSPPb-c being significantly higher than C-c and TSP-c. The highest total root length was for CPb-c and TSPPb-c at 3848 and 3789 cm, which were significantly different from the other treatments (Fig. 1b). Fine and coarse root lengths followed the same pattern as total root length in both soils.

Phosphorous uptake

P. bilaii inoculation did not significantly increase P uptake, except in the case of roots in the calcareous soil treated with TSP. However, there is a tendency for numerically higher uptake compared between inoculated and non-inoculated roots for all treatments in both soils, except in the non-fertilised control of the moderately acidic soil (Table 2).

SSPb-a showed significantly higher total P uptake in shoots than the control (TSP-a). Moreover, a significantly enhanced total P uptake in shoots and roots was obtained when plants were amended with sewage sludge and triple superphosphate (SS-a, SSPb-a, TSP-a and TSPPb-a), being more than fourfold higher than CPb-a and C-a (Table 2). Higher total P uptake in shoots and roots was obtained for all treatments with added *P. bilaii*, but was only statistically significant for the root from TSPPb-c

Table 1 Shoot length (cm) of wheat grown in acidic soil (-a) and calcareous soil (-c) measured on days 10, 22 and 35 after sowing

Treatment	Shoot length in acidic soil (cm)			Treatment	Shoot length in calcareous soil (cm)		
	10 DAS	22 DAS	35 DAS		10 DAS	22 DAS	35 DAS
C-a	11.4 ± 0.6^{bc}	24.5 ± 2.0^{c}	33.4 ± 1.2^{c}	C-c	8.9 ± 0.4^{bc}	21.8 ± 1.2^{b}	32.1 ± 0.4^{b}
CPb-a	11.1 ± 0.5^{bc}	22.5 ± 2.4^{c}	28.1 ± 1.0^{d}	CPb-c	11.2 ± 0.2^{a}	25.3 ± 1.0^{a}	32.5 ± 1.4^{b}
SS-a	10.5 ± 1.0^{c}	28.3 ± 1.0^{ab}	36.0 ± 0.7^{abc}	SS-c	9.2 ± 0.5^{ab}	23.4 ± 1.4^{ab}	32.7 ± 1.1^{ab}
SSPb-a	14.6 ± 0.2^{a}	31.6 ± 0.7^{a}	37.7 ± 1.0^{a}	SSPb-c	9.5 ± 0.4^{ab}	24.7 ± 1.1^{ab}	34.1 ± 0.5^{ab}
TSP-a	12.5 ± 0.4^{b}	28.5 ± 0.7^{ab}	36.9 ± 1.3^{ab}	TSP-c	8.0 ± 0.3^{bc}	24.1 ± 1.0^{ab}	35.4 ± 0.6^{a}
TSPPb-a	12.7 ± 0.4^{b}	27.9 ± 0.7^{ab}	34.4 ± 0.8^{bc}	TSPPb-c	6.8 ± 1.5^{c}	25.5 ± 0.9^{a}	32.6 ± 1.2^{ab}

Unamended control soil *C*, control soil with *P. bilaii CPb*, soil amended with sewage sludge *SS*, sewage sludge and *P. bilaii SSPb*, triple superphosphate *TSP* and triple superphosphate and *P. bilaii TSPPb*. Values are the mean of four replicates ± standard error. Different letters indicate significant differences between treatments ($P < 0.05$)

Table 2 Shoot and root biomass, P uptake and phosphorus concentration in the shoots and roots of wheat grown in acidic soil (-a) and calcareous (-c) soil measured 35 days after sowing

	Acidic soil				Calcareous soil			
	Treatment	Biomass (g)	P uptake (μg plant^{-1})	P concentration (%)	Treatment	Biomass (g)	P uptake (μg plant^{-1})	P concentration (%)
Shoot	C-a	0.25 ± 0.01^{b}	581 ± 41^{c}	0.229 ± 0.007^{d}	C-c	0.44 ± 0.00^{b}	1486 ± 35^{c}	0.34 ± 0.006^{c}
	CPb-a	0.19 ± 0.01^{b}	367 ± 28^{c}	0.196 ± 0.006^{e}	CPb-c	0.56 ± 0.03^{a}	1704 ± 122^{bc}	0.31 ± 0.008^{d}
	SS-a	0.64 ± 0.06^{a}	2433 ± 234^{ab}	0.383 ± 0.006^{a}	SS-c	0.53 ± 0.03^{a}	1876 ± 117^{ab}	0.35 ± 0.004^{bc}
	SSPb-a	0.73 ± 0.05^{a}	2547 ± 178^{a}	0.351 ± 0.003^{b}	SSPb-c	0.54 ± 0.04^{a}	1877 ± 157^{ab}	0.35 ± 0.007^{bc}
	TSP-a	0.67 ± 0.01^{a}	2166 ± 64^{b}	0.326 ± 0.007^{c}	TSP-c	0.50 ± 0.02^{ab}	1924 ± 81^{ab}	0.39 ± 0.004^{a}
	TSPPb-a	0.69 ± 0.02^{a}	2276 ± 69^{ab}	0.327 ± 0.002^{c}	TSPPb-c	0.56 ± 0.02^{a}	2122 ± 68^{a}	0.38 ± 0.024^{ab}
	Ref. values[a]			0.2–0.5				0.2–0.5
Root	C-a	0.08 ± 0.00^{c}	66 ± 2^{b}	0.080 ± 0.003^{b}	C-c	0.08 ± 0.00^{bc}	75 ± 4^{ab}	0.094 ± 0.007^{bc}
	CPb-a	0.04 ± 0.00^{d}	26 ± 2^{b}	0.070 ± 0.002^{b}	CPb-c	0.11 ± 0.01^{a}	100 ± 17^{a}	0.090 ± 0.006^{c}
	SS-a	0.13 ± 0.02^{b}	126 ± 27^{a}	0.097 ± 0.006^{a}	SS-c	0.08 ± 0.01^{bc}	84 ± 9^{ab}	0.112 ± 0.006^{a}
	SSPb-a	0.17 ± 0.02^{a}	159 ± 19^{a}	0.092 ± 0.002^{a}	SSPb-c	0.09 ± 0.01^{ab}	96 ± 8^{a}	0.105 ± 0.007^{abc}
	TSP-a	0.15 ± 0.01^{ab}	144 ± 22^{a}	0.094 ± 0.006^{a}	TSP-c	0.06 ± 0.01^{c}	64 ± 8^{b}	0.111 ± 0.004^{ab}
	TSPPb-a	0.18 ± 0.00^{a}	166 ± 04^{a}	0.094 ± 0.002^{a}	TSPPb-c	0.09 ± 0.01^{ab}	104 ± 7^{a}	0.119 ± 0.003^{a}

Unamended control soil *C*, control soil with *P. bilaii CPb*, soil amended with sewage sludge *SS*, sewage sludge and *P. bilaii SSPb*, triple superphosphate *TSP* and triple superphosphate and *P. bilaii TSPPb*. Values are mean of four replicates ± standard error. Different letters indicate significant differences between treatments ($P < 0.05$)

[a] Sufficient range nutrient concentrations proposed by Mills and Jones [31]

(104 μg) compared to TSP-c (64 μg) (Table 2). Generally, plants amended with P did not show higher total P uptake in shoots and roots compared with non-amended plants.

P concentrations in the shoots ranged from 0.23 to 0.38 % for plants grown in moderately acidic and from 0.31 to 0.39 % (Table 2) for plants grown in calcareous soil. All treatments had P concentrations within the sufficiency ranges of spring wheat (0.2–0.5 %) proposed by Mills and Jones [31]; however, the C-a and CPB-a were at the lower end of the sufficiency range (0.2), which together with the low yield indicated P stress. P concentration in the root for both soils was significantly higher when SS and TSP were added. *P. bilaii* inoculation did not affect root P concentrations.

Uptake of other macro- and micronutrients

Shoot nutrient concentrations of Mg, S and Cu in wheat grown in both soils were within the sufficiency ranges described by Mills and Jones [31] (Table 3). However, K, Ca, B and Mn concentrations were higher than the sufficient ranges. In both soils, the addition of SS increased Al, Cu and Fe concentrations in shoots. In contrast, in the control of the acidic soil, the concentrations of Ca, Mg and Mn in shoot were markedly higher than those of the fertilised treatments. Cu concentrations appeared lower in shoots from the Pb-control treatment in acidic soil than in the untreated control. *P. bilaii* inoculation showed a tendency to decrease Fe and Al uptake in shoots in both soils.

Total nutrient uptake in shoots grown in moderately acidic soils were in all cases significantly lower in the control than in the SS and TSP treatments (Table 4). In the same soil, the K, Mg, S, B and Cu uptake was lower in roots in the CPb-a compared with C-a. In the calcareous soil, *P. bilaii* tended to increase the Mg and S shoot concentration in all the treatments, being only significantly higher in CPb-c compared to C-c. Similar effects were also observed in root total content of K, Ca, B, Fe, Mn and Al, which was significantly higher in non-amended plants inoculated with *P. bilaii* compared to non-inoculated plants.

Changes in soil pH and water-soluble P

The initial pH in the moderately acidic soil was 5.5, but after the growing period it decreased to 4.8 on average irrespective of the treatment. For the calcareous soil, the pH decreased from 8.7 to 8.4. There were no significant differences in pH among treatments for the moderately acidic soil or the calcareous soil (data not shown).

Soil water-soluble P concentrations at harvest differed between soils. Water-soluble P ranged from 2.21 to 4.63 μg g^{-1} in the treatments in the moderately acidic soil. For the calcareous soil, water-soluble P showed higher results ranging from 4.36 to 9.30 μg g^{-1}. *Penicillium bilaii* effects were not observed in any of the treatments, and a high variability among replicates was found in most of the treatments (data not shown). Since the information on water-extractable P does not indicate

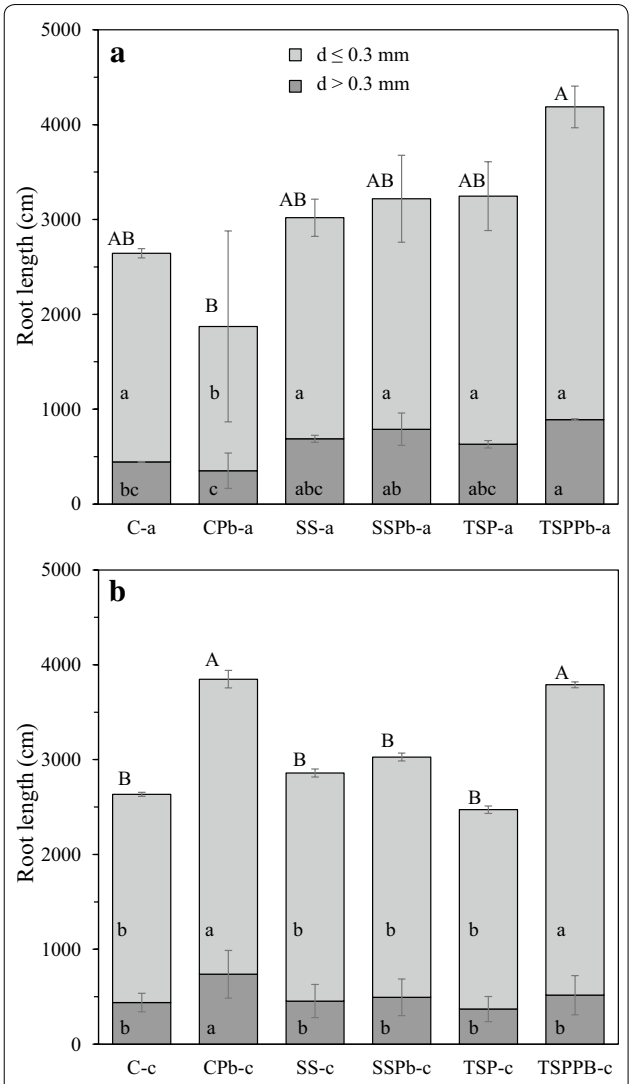

Fig. 1 Length of fine (d ≤ 0.3 mm) and coarse (d > 0.3 mm) roots of wheat grown in **a** the acidic soil and **b** in the calcareous soil. Legend: unamended control soil (C), soil and *P. bilaii* (CPb), soil amended with sewage sludge (SS), sewage sludge and *P. bilaii* (SSPb), triple super phosphate (TSP) and triple super phosphate and *P. bilaii* (TSPPb) in acidic soil (denoted "-a") and calcareous soil (denoted "-c"). The total height of the *bars* represents the total root length. *Error bars* represent the standard error of the mean in (-a) ($n = 2$) and in (-c) ($n = 4$). *Capital letters* indicate significant differences of total root length between treatments and *small letters* indicate differences in fine and coarse root length for all the treatments

treatment-related differences, apart from the completely obvious effect of fertilisation on WEP, we abstain from further discussion.

Discussion

Fertiliser effects

In both soils, a decrease in pH was observed, which was independent of the treatments. It is most likely a result of the initial fertigation with the modified Hoagland nutrient solution containing NH_4NO_3, in which the ammonium can expect to lead to a decrease in pH when it is oxidised by microbes, or taken up and subsequently metabolised in the plant [32]. This decrease may be expected to be all the more strongly expressed in a mixture of soil and sand (as was the case under ambient conditions) than in pure soil, due to weaker buffering capacity of the mixture.

Wheat growth in the moderately acidic soil responded more than the calcareous soil when amended with P fertilisers. Fertilising with SS increased shoot and root growth compared to the control (C-a), resulting in similar values to those observed for TSP-a, suggesting that SS was as effective at releasing plant-available P as TSP. Several authors have reported increases in biomass and growth after SS application, suggesting that sewage sludge has a P fertilisation potential equivalent to inorganic fertilisers [4, 5]. Antolín et al. [33] found an increase in barley crop yield associated with an improvement in the early seedling establishment after application of sewage sludge. Azam and Lhodi [34] found positive effects of sewage sludge on the aboveground biomass of wheat plants, which increased by 41 % compared to the control plants. However, different fertilisation effects were observed between soils with the addition of P amendments. Sewage sludge-amended plants grown in moderately acidic soil (SS-a) had a shoot biomass 2.5 times and significantly higher than the control plants (C-a), while plants from SS-c showed a shoot biomass 1.2 times higher than C-c. The greater effect of SS in the moderately acidic soil compared to the calcareous soil is most probably caused by the difference in the initial content of plant-available P. Thus, the initial concentration of water-extractable P was more than twofold higher in the calcareous soil compared to the moderately acidic soil. In a similar vein, Frossard et al. [5] found a reduction in the effect of sludge because of a high soil-available P content of 6 mg water-extractable P kg^{-1} soil. Therefore, despite the moderate P content in calcareous soil, in the present experiment this was not substantially limiting plant growth. Thus, we could confirm our second hypothesis that fertilisation with SS results in a higher immediate P fertilisation response, i.e. higher root and shoot growth in moderately acidic low P soil compared to calcareous soil.

Similar to plant growth responses, P uptake in plants grown in moderately acidic soil presented significantly higher P shoot and root concentrations for treatments with added P fertilisers. Thus, the fertiliser treatments were within the sufficiency concentration range [31], while the unfertilised treatments had P scarcity. Similar differences were not seen in plants grown in calcareous soil, where all treatments were within the sufficiency

Table 3 Nutrient concentration in the shoots and roots of wheat grown in the acidic soil (-a) and calcareous (-c) control soils (C), soil and *P. bilaii* (Pb), soil amended with sewage sludge (SS), sewage sludge and *P. bilaii* (SSPb), triple superphosphate (TSP) and triple superphosphate and *P. bilaii* (TSPPb)

	K (%)	Ca	Mg	S	B (ppm)	Cu	Fe	Mn	Zn	Al
Shoot										
C-a	3.65 ± 0.16^{a}	2.33 ± 0.28^{a}	0.24 ± 0.02^{a}	0.32 ± 0.01^{a}	16.0 ± 2.4^{a}	7.0 ± 0.4^{a}	122 ± 21^{ab}	942 ± 117^{a}	55 ± 1.4^{a}	91 ± 28^{ab}
CPb-a	3.26 ± 0.13^{b}	2.58 ± 0.45^{a}	0.24 ± 0.03^{a}	0.31 ± 0.007^{a}	15.6 ± 1.6^{a}	6.1 ± 0.4^{a}	134 ± 27^{ab}	999 ± 188^{a}	50 ± 2.4^{ab}	118 ± 37^{ab}
SS-a	3.73 ± 0.06^{a}	1.36 ± 0.05^{b}	0.16 ± 0.01^{b}	0.32 ± 0.009^{a}	14.2 ± 0.9^{a}	8.0 ± 0.2^{a}	154 ± 14^{a}	454 ± 8.1^{b}	54 ± 2.7^{b}	126 ± 16^{b}
SSPb-a	3.73 ± 0.08^{a}	1.47 ± 0.13^{b}	0.17 ± 0.01^{b}	0.32 ± 0.006^{a}	14.5 ± 0.8^{a}	7.4 ± 0.2^{a}	102 ± 4.9^{b}	503 ± 38^{b}	52 ± 4.2^{ab}	60 ± 6.8^{bc}
TSP-a	3.74 ± 0.05^{a}	1.43 ± 0.07^{b}	0.17 ± 0.01^{b}	0.31 ± 0.002^{a}	14.0 ± 0.9^{a}	7.2 ± 0.1^{a}	93 ± 6.7^{b}	516 ± 28^{b}	46 ± 0.7^{b}	51 ± 9.4^{c}
TSPPb-a	3.72 ± 0.04^{a}	1.36 ± 0.09^{b}	0.16 ± 0.01^{b}	0.32 ± 0.004^{a}	15.5 ± 0.7^{a}	7.2 ± 0.1^{a}	89 ± 6.2^{b}	493 ± 22^{b}	50 ± 2.7^{ab}	49 ± 6.7^{c}
C-c	4.19 ± 0.05^{a}	1.24 ± 0.04^{ab}	0.24 ± 0.003^{ab}	0.33 ± 0.01^{ab}	9.7 ± 0.7^{ab}	9.7 ± 0.2^{ab}	88 ± 9.5^{ab}	46 ± 2.7^{a}	73 ± 2.4^{b}	56 ± 16^{ab}
CPb-c	3.92 ± 0.09^{b}	1.22 ± 0.03^{ab}	0.23 ± 0.004^{b}	$0.32 \pm .004^{b}$	9.4 ± 0.4^{ab}	9.2 ± 0.1^{ab}	76 ± 3.1^{b}	47 ± 1.5^{a}	69 ± 3.9^{b}	38 ± 2.4^{ab}
SS-c	3.99 ± 0.07^{b}	1.26 ± 0.08^{a}	0.24 ± 0.009^{ab}	$0.33 \pm .004^{ab}$	8.5 ± 0.4^{b}	9.5 ± 0.1^{b}	103 ± 13^{a}	44 ± 1.2^{a}	74 ± 1.8^{b}	82 ± 21^{b}
SSPb-c	3.90 ± 0.05^{b}	1.24 ± 0.09^{ab}	0.25 ± 0.007^{ab}	$0.33 \pm .003^{ab}$	10.0 ± 0.4^{a}	9.7 ± 0.1^{a}	81 ± 3.8^{b}	45 ± 1.3^{a}	76 ± 2.7^{b}	38 ± 3.5^{bc}
TSP-c	4.30 ± 0.07^{a}	1.11 ± 0.01^{b}	0.25 ± 0.006^{a}	$0.33 \pm .002^{ab}$	8.9 ± 0.3^{ab}	9.9 ± 0.1^{ab}	92 ± 5.4^{ab}	46 ± 2.0^{a}	89 ± 4.6^{a}	57 ± 8.0^{c}
TSPPb-c	4.27 ± 0.09^{a}	1.14 ± 0.07^{ab}	0.25 ± 0.006^{a}	$0.34 \pm .003^{a}$	9.5 ± 0.3^{ab}	9.9 ± 0.2^{ab}	78 ± 1.3^{b}	38 ± 1.6^{b}	77 ± 4.8^{a}	38 ± 3.7^{c}
Ref. *	1.5–3	0.2–0.5	0.15–0.5	0.15–0.4	6–10	5–25	25–100	25–100	15–70	
Root										
C-a	0.13 ± 0.01^{a}	0.19 ± 0.009^{c}	0.11 ± 0.009^{a}	0.12 ± 0.002^{bc}	5 ± 0.3^{a}	59 ± 3.9^{a}	4217 ± 443^{a}	425 ± 42^{b}	68 ± 5.6^{ab}	5040 ± 534^{a}
CPb-a	0.13 ± 0.03^{a}	0.19 ± 0.006^{c}	0.11 ± 0.016^{ab}	0.11 ± 0.003^{c}	4 ± 0.8^{ab}	14 ± 1.3^{ab}	4421 ± 840^{a}	412 ± 15^{b}	67 ± 9.9^{ab}	5227 ± 1001^{a}
SS-a	0.08 ± 0.01^{b}	0.24 ± 0.02^{a}	0.09 ± 0.006^{abc}	0.13 ± 0.0008^{a}	4 ± 0.3^{ab}	43 ± 1.1^{ab}	2881 ± 291^{abc}	801 ± 95^{a}	76 ± 6.7^{a}	3324 ± 327^{b}
SSPb-a	0.06 ± 0.004^{b}	0.23 ± 0.008^{ab}	0.07 ± 0.003^{c}	0.12 ± 0.003^{ab}	3 ± 0.2^{b}	16 ± 1.4^{b}	2192 ± 141^{c}	817 ± 49^{a}	62 ± 1.9^{abc}	2488 ± 170^{b}
TSP-a	0.07 ± 0.012^{b}	0.21 ± 0.01^{abc}	0.08 ± 0.007^{bc}	0.12 ± 0.003^{ab}	3 ± 0.5^{b}	13 ± 0.3^{b}	2511 ± 329^{bc}	717 ± 57^{a}	48 ± 2.8^{c}	3032 ± 429^{b}
TSPPb-a	0.07 ± 0.008^{b}	0.21 ± 0.01^{bc}	0.08 ± 0.006^{abc}	0.12 ± 0.002^{ab}	3 ± 0.3^{b}	15 ± 1.6^{b}	2418 ± 303^{c}	730 ± 113^{a}	53 ± 1.7^{bc}	2886 ± 368^{b}
C-c	0.06 ± 0.01^{ab}	0.39 ± 0.005^{c}	0.06 ± 0.005^{a}	0.12 ± 0.002^{bc}	4 ± 0.5^{ab}	15 ± 1.1^{ab}	1075 ± 191^{a}	44 ± 6.6^{a}	60 ± 10.8^{a}	1584 ± 293^{a}
CPb-c	0.07 ± 0.004^{a}	0.43 ± 0.002^{c}	0.07 ± 0.002^{a}	0.11 ± 0.004^{c}	5 ± 0.1^{a}	13 ± 1.6^{a}	1294 ± 4.5^{a}	48 ± 2.9^{a}	36 ± 6.7^{b}	1919 ± 88^{a}
SS-c	0.05 ± 0.002^{b}	0.40 ± 0.003^{a}	0.07 ± 0.001^{a}	0.12 ± 0.001^{ab}	3 ± 0.2^{b}	14 ± 2.1^{b}	981 ± 59^{a}	40 ± 2.0^{a}	36 ± 3.2^{b}	1423 ± 91^{a}
SSPb-c	0.05 ± 0.006^{b}	0.38 ± 0.003^{ab}	0.07 ± 0.003^{a}	0.12 ± 0.004^{ab}	3 ± 0.3^{b}	10 ± 0.6^{b}	943 ± 111^{a}	39 ± 3.5^{a}	24 ± 2.1^{b}	1351 ± 156^{a}
TSP-c	0.06 ± 0.009^{ab}	0.39 ± 0.002^{abc}	0.07 ± 0.004^{a}	0.13 ± 0.002^{ab}	3 ± 0.2^{b}	11 ± 0.9^{b}	1057 ± 157^{a}	42 ± 2.8^{a}	35 ± 5.4^{b}	1555 ± 244^{a}
TSPPb-c	0.05 ± 0.009^{ab}	0.45 ± 0.004^{bc}	0.06 ± 0.003^{a}	0.13 ± 0.002^{a}	4 ± 0.5^{ab}	13 ± 1.9^{ab}	962 ± 241^{a}	40 ± 6.1^{a}	34 ± 2.0^{b}	1375 ± 253^{a}

Values are mean of four replicates ± standard errors. Different letters indicate significant differences between treatments ($P < 0.05$)

[a] Sufficient range of nutrient concentrations proposed by Mills and Jones [31]

Table 4 Total nutrient uptake in shoots and roots of wheat grown in acidic soil (-a) and calcareous (-c) control soils (C), soil and *P. bilaii* (Pb), soil amended with sewage sludge (SS), sewage sludge and *P. bilaii* (SSPb), triple superphosphate (TSP) and triple superphosphate and *P. bilaii* (TSPPb)

	K (mg plant^{-1})	Ca	Mg	S	B (μg plant^{-1})	Cu	Fe	Mn	Zn	Al
Shoot										
C-a	9.2 ± 0.7^{b}	5.9 ± 0.8^{b}	0.61 ± 0.07^{b}	0.8 ± 0.1^{b}	4.10 ± 0.7^{b}	1.77 ± 0.16^{b}	31 ± 5.6^{c}	239 ± 33^{bc}	14 ± 0.8^{b}	23 ± 7.3^{c}
CPb-a	6.1 ± 0.5^{b}	4.7 ± 0.7^{b}	0.45 ± 0.04^{b}	0.6 ± 0.0^{b}	2.90 ± 0.3^{b}	1.15 ± 0.1^{b}	24 ± 4^{c}	183 ± 28^{c}	9.4 ± 0.7^{b}	21 ± 5.9^{c}
SS-a	24 ± 2.2^{a}	8.7 ± 1.0^{a}	1.02 ± 0.10^{a}	2.0 ± 0.2^{a}	9.08 ± 2.3^{a}	5.12 ± 1.1^{a}	96 ± 13^{a}	289 ± 29^{ab}	34 ± 4.3^{a}	78 ± 5.7^{a}
SSPb-a	27 ± 2.3^{a}	11 ± 0.4^{a}	1.19 ± 0.02^{a}	2.3 ± 0.1^{a}	10.6 ± 2.2^{a}	5.38 ± 1^{a}	75 ± 15^{b}	359 ± 8^{a}	38 ± 5.2^{a}	44 ± 15^{b}
TSP-a	25 ± 0.6^{a}	9.5 ± 0.4^{a}	1.10 ± 0.04^{a}	2.1 ± 0.0^{a}	9.32 ± 1.1^{a}	4.78 ± 0.2^{a}	62 ± 5.1^{b}	343 ± 17^{a}	30 ± 0.6^{a}	34 ± 13^{bc}
TSPPb-a	26 ± 0.5^{a}	9.5 ± 0.7^{a}	1.12 ± 0.07^{a}	2.2 ± 0.1^{a}	10.7 ± 0.5^{a}	5.02 ± 0.3^{a}	62 ± 4.5^{b}	343 ± 21^{a}	34 ± 1.1^{a}	34 ± 9.4^{bc}
C-c	18 ± 0.4^{b}	5.4 ± 0.2^{a}	1.05 ± 0.02^{b}	1.4 ± 0.1^{b}	4.26 ± 0.3^{b}	4.25 ± 0.1^{b}	38 ± 4.2^{b}	20 ± 1.1^{b}	32 ± 1^{b}	24 ± 6.9^{b}
CPb-c	22 ± 1.5^{ab}	6.7 ± 0.3^{a}	1.29 ± 0.07^{a}	1.8 ± 0.1^{a}	5.21 ± 0.4^{ab}	5.07 ± 0.2^{ab}	42 ± 3.1^{b}	26 ± 2.1^{a}	39 ± 3.8^{ab}	21 ± 1.9^{b}
SS-c	21 ± 1.3^{ab}	6.7 ± 0.3^{a}	1.26 ± 0.04^{a}	1.7 ± 0.1^{ab}	4.51 ± 0.4^{ab}	5.06 ± 0.3^{ab}	54 ± 4^{a}	23 ± 1.3^{ab}	39 ± 2.3^{a}	42 ± 8.9^{a}
SSPb-c	21 ± 1.8^{ab}	6.7 ± 0.7^{a}	1.33 ± 0.11^{a}	1.8 ± 0.2^{a}	5.40 ± 0.6^{a}	5.25 ± 0.5^{a}	44 ± 3.1^{b}	25 ± 2.5^{ab}	41 ± 3.2^{a}	21 ± 2.2^{b}
TSP-c	21 ± 1.1^{ab}	5.5 ± 0.2^{a}	1.24 ± 0.06^{ab}	1.6 ± 0.1^{ab}	4.43 ± 0.3^{ab}	4.88 ± 0.2^{ab}	45 ± 1.2^{ab}	23 ± 1.2^{ab}	44 ± 2^{a}	28 ± 3^{a}
TSPPb-c	24 ± 0.6^{a}	6.5 ± 0.7^{a}	1.42 ± 0.08^{a}	1.9 ± 0.1^{a}	5.30 ± 0.1^{ab}	5.57 ± 0.3^{a}	44 ± 2^{b}	21 ± 1.3^{ab}	43 ± 1.7^{a}	22 ± 3.1^{b}
Root										
C-a	0.11 ± 0.01^{a}	0.16 ± 0.01^{b}	0.09 ± 0.00^{bc}	0.10 ± 0.01^{b}	0.39 ± 0.01^{a}	4.86 ± 0.5^{a}	342 ± 20^{ab}	35 ± 4.2^{b}	5.7 ± 0.7^{bc}	409 ± 25^{ab}
CPb-a	0.05 ± 0.01^{b}	0.07 ± 0.01^{b}	0.04 ± 0.01^{c}	0.04 ± 0.00^{c}	0.15 ± 0.04^{b}	$0.52 \pm .04^{c}$	166 ± 39^{b}	15 ± 1.2^{b}	2.5 ± 0.5^{c}	197 ± 47^{b}
SS-a	0.10 ± 0.03^{ab}	0.32 ± 0.08^{a}	0.11 ± 0.03^{ab}	0.17 ± 0.03^{a}	0.49 ± 0.12^{a}	5.68 ± 1.1^{a}	378 ± 93^{a}	109 ± 31^{a}	10.3 ± 5.3^{a}	434 ± 104^{a}
SSPb-a	0.10 ± 0.01^{ab}	0.41 ± 0.05^{a}	0.13 ± 0.02^{ab}	0.21 ± 0.02^{a}	0.51 ± 0.08^{a}	2.78 ± 0.5^{b}	384 ± 158^{a}	143 ± 22^{a}	10.8 ± 1.3^{a}	436 ± 66^{a}
TSP-a	0.11 ± 0.03^{a}	0.32 ± 0.05^{a}	0.13 ± 0.02^{ab}	0.19 ± 0.02^{a}	0.53 ± 0.13^{a}	1.92 ± 0.2^{bc}	391 ± 92^{a}	109 ± 19^{a}	7.4 ± 1.1^{ab}	474 ± 116^{a}
TSPPb-a	0.13 ± 0.01^{a}	0.37 ± 0.02^{a}	0.15 ± 0.01^{a}	0.22 ± 0.00^{a}	055 ± 0.05^{a}	2.68 ± 0.3^{b}	429 ± 53^{a}	129 ± 18^{a}	9.5 ± 0.3^{ab}	512 ± 65^{a}
C-c	0.05 ± 0.01^{bc}	0.31 ± 0.03^{bc}	0.05 ± 0.00^{a}	0.10 ± 0.01^{abc}	0.29 ± 0.02^{b}	1.16 ± 0.1^{ab}	84 ± 11^{b}	3.46 ± 0.34^{b}	4.9 ± 1.04^{a}	121 ± 16^{b}
CPb-c	0.08 ± 0.01^{a}	0.48 ± 0.09^{a}	0.08 ± 0.01^{a}	0.12 ± 0.02^{a}	0.50 ± 0.08^{a}	1.46 ± 0.3^{a}	145 ± 24^{a}	5.37 ± 1.02^{a}	4.1 ± 0.97^{ab}	214 ± 35^{a}
SS-c	0.04 ± 0.00^{bc}	0.30 ± 0.04^{bc}	0.05 ± 0.01^{a}	0.09 ± 0.01^{bc}	0.26 ± 0.03^{b}	1.04 ± 0.1^{ab}	72 ± 5^{b}	$2,99 \pm 0.32^{b}$	2.7 ± 0.37^{bc}	105 ± 7.2^{b}
SSPb-c	0.05 ± 0.01^{ab}	0.35 ± 0.06^{abc}	0.06 ± 0.01^{a}	0.11 ± 0.01^{ab}	0.31 ± 0.03^{b}	0.92 ± 0.1^{b}	87 ± 14^{b}	3.6 ± 0.48^{b}	2.2 ± 0.36^{bc}	125 ± 19^{b}
TSP-c	0.03 ± 0.00^{c}	0.23 ± 0.04^{c}	0.04 ± 0.00^{a}	0.07 ± 0.01^{c}	0.19 ± 0.02^{b}	0.67 ± 0.1^{ab}	58 ± 4.5^{b}	2.38 ± 0.15^{b}	2.0 ± 0.39^{c}	85 ± 6.0^{b}
TSPPb-c	0.05 ± 0.01^{bc}	0.40 ± 0.06^{ab}	0.06 ± 0.01^{a}	0.11 ± 0.01^{ab}	0.32 ± 0.06^{b}	1.17 ± 0.2^{b}	87 ± 21^{b}	3.51 ± 0.62^{b}	3.0 ± 0.4^{abc}	123 ± 29^{b}

Values are mean of four replicates ± standard errors. Different letters indicate significant differences between treatments ($P < 0.05$)

range. In both soils, the addition of SS increased Al and Fe concentrations in shoots, which could be due to the amount of Al and Fe used to precipitate the sludge in WWTP. In the moderately acidic soil, the unfertilised treatments (C-a, CPb-a) developed quite poorly, which is in agreement with its low P status. Mn concentrations in acidic soil were higher than the sufficiency ranges. This effect was not observed in Fe and Al concentrations, so the high Mn concentration cannot be attributed to the low pH in this soil. However, a high content of MnO_2 in soil together with high water content could reduce the redox potential and furthermore release a high amount of M^{2+} plant available in soil. Whereas the SS and TSP applications resulted in a decrease in Ca, Mg and Mn concentrations in shoots, the Ca concentration in roots increased, which could be explained by the high levels of Ca in the sludge. Singh and Agrawal [35] reviewed the potential effects of several sewage sludges in soil, and Ca was found to increase significantly in all soils after sewage sludge application. The total nutrient uptake in shoots in moderately acidic soils in all cases was significantly lower in non-amended plants than that in SS and TSP treatments, clearly demonstrating that they were nutrient limited in their growth in the unamended soil.

Effects of *P. bilaii* inoculation

P. bilaii inoculation had the greatest effect in non-amended calcareous soil (CPb-c), increasing the shoot biomass to the same levels as P fertiliser treatments. Furthermore, *P. bilaii* inoculation in this calcareous soil increased root biomass and total root lengths for non-amended and TSP-treated soils. The same trend was observed for fine and coarse roots, suggesting that *P. bilaii* inoculation promoted root growth. These results confirm our first hypothesis that *P. bilaii* inoculation leads to stronger plant growth in calcareous soil compared to moderately acidic soil. These findings have previously been reported in several studies in which *P. bilaii* was shown to increase root length, root hair abundance and average root diameter under different environmental conditions [10, 22, 36]. It is generally assumed that *P. bilaii* inoculants are more effective for P mobilisation on neutral to alkaline soils, where the fungus is capable of solubilising Ca–P [16–19]. However, it would be difficult to conclude that the solubility of the calcium compounds was the factor influencing root promotion. The physical and chemical characteristics of calcareous soil might have triggered root growth promotion from *P. bilaii*. Predictions about how soil characteristics (e.g. soil pH, total and available P, P sorption capacity, clay composition) and seasonal determinants (e.g. soil moisture, temperature) interact with phosphorus-solubilising microorganisms remain poorly defined [12].

P. bilaii inoculation of non-amended moderately acidic soil further showed a decrease in root biomass and root length, coupled with a slightly lower shoot growth and a significantly lower shoot P concentration. This result might be related to very low P levels of moderately acidic soil, potentially leading to P competition between plant roots and the inoculated *P. bilaii*, immobilising P during its growth. Marschner et al. [37] stated that mutual demand for P can result in competition between plants and microorganisms, with the latter being more competitive. In fact, the combined application of *P. bilaii* and SS as P source in moderately acidic soil increased the shoot length and root biomass, and similar but slightly smaller differences were observed when *P. bilaii* was combined with TSP, suggesting the need for a minimum P level to satisfy the requirements of the fungi and the plant at the same time. These results confirm our third hypothesis, demonstrating that seed inoculation with *P. bilaii* will result in a higher plant response in the sewage sludge treatment of the moderately acidic soil compared to that in the calcareous soil, due to *P. bilaii* mobilisation of insoluble forms of P from the sewage sludge. On the other hand, the combined application of SS and *P. bilaii* might be a good strategy for improving the efficiency of SS as P fertiliser and increasing available P content in depleted soils.

While P concentration in the shoots and roots were generally not affected by *P. bilaii* inoculation, most of the treatments with SS or TSP in both soils showed a tendency to increase in total P uptake in shoots and roots when *P. bilaii* was added. In this study, it was not possible to relate a higher plant growth with a higher P solubilisation with the combination of SS and *P. bilaii*. Promotion of plant growth by *P. bilaii* inoculation without a concomitant increase in the P content of these plants has been found in several studies [22, 24, 38–41], showing that the *P. bilaii* root growth increase may be related to mechanisms other than P uptake, such as phytohormone production or other possible mechanisms against abiotic stresses [42–44]. *P. bilaii* effect for the first 10–18 DAS was gradually decreased. This could be explained by a decline of the fungal colony. The colonisation and successful establishment of artificially introduced P-solubilising fungi in soils have been greatly affected by (1) moisture content [45], (2) physical and chemical properties of soils (e.g. soil pH, total and available P, P sorption capacity, clay composition) [12], (3) presence of environmental pollutants [45], (4) competition with microbial communities and (5) availability of inadequate nutrients in the rhizosphere to produce enough organic acids among others [46].

Penicillium bilaii inoculation showed a tendency to decrease Fe and Al uptake in shoots in both soils;

however, this trend has not previously been reported in other studies. The inoculation of the control soil with *P. bilaii* decreased K, Mg, S, B and Cu uptake in roots compared to C-a in the moderately acidic soil, which could be associated with the significantly lower root biomass, whereas there was an increase in K, Ca, B, Fe, Mn and Al uptake in roots when the control soil was inoculated with *P. bilaii* in calcareous soil.

Conclusions

Sewage sludge was an efficient P source on par with TSP, in moderately acidic soil. *P. bilaii* treatment without added P fertilisers had a greater effect on calcareous soil, where both root and shoot biomass increased significantly. In contrast, in moderately acidic soil, there was a reduction in plant growth when *P. bilaii* was added, which could be related to the low soil P level resulting in plant–*P. bilaii* competition. A systematic, but not significant, increase of total P uptake was found for all treatments inoculated with *P. bilaii* and for both soils, with the unfertilised control of the low fertility moderately acidic soil being a notable exception.

Authors' contributions

SE developed the pot experiment and most of the laboratory analysis, interpretation of the results and manuscript writing. GM offered advice during the development of the experiment, interpretation of the results and manuscript writing. JM provided general conception and interpretation of results and manuscript writing. LSJ and de N helped with the interpretation of results and manuscript writing. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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