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# Soil Phosphate Desorption Induced by a Phosphate-Solubilizing Fungus

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# Soil Phosphate Desorption Induced by a Phosphate-Solubilizing Fungus

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*A series of in vitro experiments were carried out to evaluate the effectiveness of a phosphorus-solubilizing fungus to desorb phosphate (Pi) from soil samples differing in their Pi-sorption capacity. The results indicate that the fungus* Mortierella *sp. was effective in desorbing Pi from all soil samples tested by producing oxalic acid, and its effectiveness varied among soil orders. The effectiveness of the fungus was influenced by the soil Pi-sorption capacity, which could be used as a predictor of the effectiveness of microbes to increase soluble Pi via desorption from soils. The Pi desorption was most pronounced in a Mollisol followed by an Oxisol, an Ultisol, and then by three Andisols. The quantity of Pi desorbed by the fungus was also greater when the amount of sorbed Pi was high.*

**Keywords** Andisol, Mollisol, *Mortierella*, phosphorus, Oxisol, Ultisol



## Introduction

Phosphate (Pi) fixation is a serious problem in agricultural soils, particularly in highly weathered soils and those derived from volcanic ash (Do Carmo Harta and Torrent 2007; Trolove et al. 2003; Shoji, Nanzyo, and Dahlgren 1993; Sanchez and Uehara 1980). It is estimated that 1018 million ha of soils in the tropics have a high Pi-fixation capacity (Sanchez and Logan 1992). The term *Pi fixation* is used to describe adsorption reactions that remove bioavailable Pi from the soil solution and incorporate it into the soil solid phase (Barber 1995). This is particularly strong on iron and aluminum hydrous oxides that predominate in the highly weathered soils of humid regions and acidic savannas (Onweremadu 2007; Jackman et al. 1997; Mattlingly 1975). Jones (1981) and Jackman et al. (1997) found that the surface area of goethite was a primary factor responsible for Pi sorption by Puerto Rican and Hawaiian soils. They also found that Pi sorption was significantly correlated with the sum of the surface areas of soil minerals and their potential Pi-sorption sites. Hence, Pi sorption was satisfactorily predicted by soil mineralogical composition.

In soils derived from volcanic parent materials, humus–aluminum (Al)/iron (Fe) complexes, allophanes, ferrihydrite, and goethite are responsible for the strong Pi sorption (Vistoso et al. 2012; Jackman et al. 1997; Shoji, Nanzyo, and Dahlgren 1993; Schwertmann and Herbillon 1992; Parfitt 1989). Fox and Kamprath (1970) showed that the degree of Pi

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fixation varies among soils, with those classified as Andisols (U.S. soil taxonomy) having a very high Pi fixation capacity (Boul et al. 1997), followed by Ultisols and Oxisols.

Many organic acids are effective in either reducing Pi sorption or desorbing Pi (Guppy et al. 2005; Hue 1991). These acids are produced by roots (Nwoke et al. 2008; Kirk, Santos, and Findenegg 1999) and soil microorganisms (Gyaneshwar et al. 2002; Reddy et al. 2002; Welch, Taunton, and Banfiled 2002; Pandey et al. 2006; Marschner 1997). Bolan et al. (1994) studied the influence of monocarboxylic (acetic, formic, and lactic), dicarboxylic (malic, tartaric, and oxalic), and tricarboxylic (citric) acids on the solubilization and sorption of Pi in an Andisol and an Alfisol of New Zealand and observed that the organic acids significantly decreased soil Pi sorption. The effectiveness of the acids in this regard followed the order tricarboxylic > dicarboxylic > monocarboxylic. These authors explained this phenomenon based on the constant of formation (log $K_{Al}$) of complexes between aluminum (Al) and the conjugate anions as proposed by Hue, Craddock, and Adams (1986). Hue (1991) found similar results on the availability of soil Pi when he added organic acids to two Andisols, an Oxisol, an Ultisol, and a Vertisol of Hawai'i. The effectiveness of the acids in reducing Pi sorption from a soluble source [monopotassium phosphate ($KH_2PO_4$)] was greater with malic acid (monohydroxy dicarboxylic), followed by protocatechuic acid (dihydroxy monocarboxylic) and acetic acid (monocarboxylic) (Hue 1991).

Because many soil microorganisms have the ability to produce and release organic acids, they may desorb Pi from surfaces of soils, as suggested by Hoberg, Marschner, and Lieberei (2005) and He and Zhu (1998). However, the results obtained are not completely conclusive in this regard. The objective of this investigation was to determine the effectiveness of the rhizosphere fungus *Mortierella* sp. to increase soil solution Pi by desorbing Pi from the surfaces of soil samples differing in their Pi-sorption capacity.

## Materials and Methods

### *Preliminary Soil Pi Sorption Isotherms*

Samples of six Colombian soils (Neira, Carimagua, Caucasia, Naranjal, La Selva, and Guarne) (Table 1) differing in their Pi-sorption capacities were passed through a 2-mm aperture sieve and retained in a 0.5-mm aperture sieve. Soil Pi sorption isotherms were constructed in triplicates following the procedure developed by Fox and Kamprath (1970).

**Table 1**
Soil classification, location, pH, and $P_{0.2}$ value

| Soil | Soil classification | Latitude, longitude | Soil pH (w, 1:2) | $P_{0.2}$ value (mg $kg^{-1}$) | Soil P sorption category[a] |
|---|---|---|---|---|---|
| Guarne | Melanudand | 6° 15′ N, 75° 30′ W | 5.4 | 4000 | Very High |
| La Selva | Endoaquand | 6° 8′ N, 75° 25′ W | 5.8 | 2222 | Very High |
| Naranjal | Melanudand | 4° 58′ N, 75° 39′ W | 5.7 | 1429 | Very High |
| Caucasia | Paleoudult | 8° 3′ N, 75° 7′ W | 4.3 | 714 | High |
| Carimagua | Haplustox | 4° 34′ N, 71° 20′ W | 4.9 | 417 | Medium |
| Neira | Haplustoll | 5° 8′ N, 75° 35′ W | 5.4 | 45 | Low |

[a]As proposed by Juo and Fox (1977).

**Table 2**
Amount of P added (mg kg$^{-1}$) to soil minerals and soil to achieve four target solution P concentrations

| Target solution P (mg L$^{-1}$) | Guarne (0.005)[a] | La Selva (0.016) | Naranjal (0.009) | Caucasia (0.003) | Carimagua (0.003) | Neira (0.002) |
|---|---|---|---|---|---|---|
| Initial | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.05 | 2254 | 987 | 339 | 382 | 241 | 16 |
| 0.1 | 2944 | 1638 | 926 | 512 | 299 | 26 |
| 0.2 | 3635 | 2288 | 1512 | 642 | 358 | 45 |

[a]Initial solution P (mg/L) levels found in the soils without $KH_2PO_4$ addition are in parentheses.

To avoid microbial activity the 50-mL plastic centrifuge tubes containing 2 g of a soil, 20 mL of 0.01 *M* calcium chloride ($CaCl_2$) solution, with or without graded amounts of $KH_2PO_4$, were autoclaved (120 °C, 0.1 MPa, 30 min). Although autoclaving may result in changes to soil sorption capacity due to the aging of noncrystalline Fe and Al oxides, preliminary studies (unpublished) showed that there were not significant differences in soil Pi sorption between the use of toluene and autoclaving soil samples. The centrifuge tubes and their contents were shaken on a reciprocal shaker for 7 days, twice a day, for 30 min each time. The $P_{0.2}$ value (amount of P (mg kg$^{-1}$) required to achieve a solution P of 0.2 mg L$^{-1}$) was estimated. This allowed to readily quantifying the Pi-sorption capacity of each soil (Table 2).



### *Inoculum of* **Mortierella** *sp.*

*Mortierella* sp. was originally isolated from an Andisol of Hawaii (Osorio and Habte 2001) and has been multiplied and stored on yeast mannitol agar (YMA; 0.5 g of $KH_2PO_4$, 0.2 g of magnesium sulfate ($MgSO_4 \cdot 7H_2O$), 0.1 g sodium chloride (NaCl), 10.0 g of mannitol, 1.0 g of yeast extract, 15.0 g of agar per liter) slants at 4 °C. For this study, the fungus was multiplied in Petri dishes on YMA for 3 days at 28 °C. Then the mycelia of the fungus were removed with a sterile loop, suspended in sterile deionized water, and shaken by hand until the clumps were dispersed. The fungal suspension contained $4 \times 10^5$ colony-forming units of *Mortierella* sp. mL$^{-1}$.

### *Microbial Pi Desorption Experiments*

Based on the soil Pi-sorption isotherms, graded amounts of $KH_2PO_4$ in 0.01 *M* $CaCl_2$ solution were mixed with 2-g portions of each soil sample in 50-mL centrifuge tubes to establish target solution Pi concentrations of 0.05, 0.1, and 0.2 mg L$^{-1}$ (Table 2). The initial solution Pi concentration of a soil sample was determined by adding to it 0.01 *M* $CaCl_2$ solution alone instead of 0.01 *M* $CaCl_2 + KH_2PO_4$. The suspension pH was adjusted to 6.0 with drops of 0.1 *M* NaOH. The tubes were horizontally shaken on a reciprocal shaker (at 100 cycles per minute) for 7 days at 25 °C. Then, the solution was discarded and the pellets were washed three times with deionized water. The soil samples were oven dried in the tubes at 60 °C for 2 days. The Pi remaining on the soils was considered as adsorbed; likely the drying process may result also in occlusion of Pi by oxide coating.

After the end of the drying treatment, the soil pellets were suspended with 19 mL of a nutrient solution that contained 1.0 g NaCl, 0.2 g $CaCl_2 \cdot 2H_2O$, 0.4 g $MgSO_4 \cdot 7H_2O$, 1.0 g ammonium nitrate ($NH_4NO_3$), and 10.0 g glucose $L^{-1}$. The sole source of Pi was Pi fixed on the surfaces of the soil samples. Then, the tubes and their contents were autoclaved (120 °C, 30 min, 0.1 MPa). One mL of a fungal suspension of *Mortierella* sp. was aseptically transferred into each tube. Tubes not inoculated with the fungus received 19 mL of the nutrient solution and 1 mL of deionized sterile water. The tubes were continuously shaken on a reciprocal shaker (at 100 cycles $min^{-1}$) for 6 days at 25 °C.

At the end of the incubation period, the suspensions were centrifuged at 5000*g* for 15 min and the supernatant fluid was filtered through a Whatman No. 42 filter paper (GE Healthcare, Buckinghamshire, UK) followed by filtration through a Millipore membrane filter (0.45 $\mu$m). Solution Pi concentration in the filtrates was determined using the molybdate-blue method (Murphy and Riley 1962). The presence of Pi in solution was considered as evidence of Pi desorption. The effectiveness of *Mortierella* sp. in desorbing Pi was calculated by subtracting soil solution Pi concentration measured in the absence of the fungus from that measured in its presence and was designated as microbially desorbed Pi.

Each soil was used in a separate experiment. The treatments consisted of a factorial combination of four levels of sorbed P (at four target solution Pi) and two levels of phosphate-solubilizing microorganism (PSM) inoculation (with or without *Mortierella* sp.). There were three replicates per treatment arranged in a completely randomized design. Data were subjected to analysis of variance (ANOVA), and the least significant difference (LSD) test (*P* value of 0.05) was used to separate means when the F statistics was significant. For this purpose, the software Statgraphics Plus version 4.0 (Statpoint, Inc.; Herdon, Va.) was used.



## Results

The Pi-sorption capacity of the soils ranged from very high with the three Andisols (Guarne > La Selva > Naranjal) to high for the Caucasia soil (a clayey and Al-rich Ultisol), medium for the Carimagua soil (Oxisol), and low for the Neira soil (Mollisol) (Table 1).

Inoculation with *Mortierella* sp. significantly increased soil solution Pi in the presence of all soils (Table 3, Figure 1), but the effect of *Mortierella* sp. was significantly affected by the concentration of sorbed Pi with the exception of the Neira soil. In this soil, *Mortierella* sp. increased soil solution Pi regardless of the concentration of Pi initially sorbed on the soil. The magnitude of Pi desorbed by the activity of *Mortierella* sp. was in excess of 0.25 mg $L^{-1}$.

*Mortierella* sp. was also effective in desorbing sorbed Pi from the Carimagua soil; however, solution Pi did not increase in soil unless the quantity of adsorbed Pi was increased via fertilization (Figure 1). Soil solution Pi in the inoculated soil reached values

**Table 3**
Significant *P* values of ANOVA tests for solution Pi concentration as a function of initial target P level in the presence of a PSF and six soils

| Source | Guarne | La Selva | Naranjal | Caucasia | Carimagua | Neira |
|---|---|---|---|---|---|---|
| Sorbed Pi (A) | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 |
| Inoculation (B) | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 |
| A × B | 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | 0.0008 | 0.1484 |
| CV (%) | 8.2 | 13.2 | 16.8 | 12.7 | 13.1 | 16.5 |

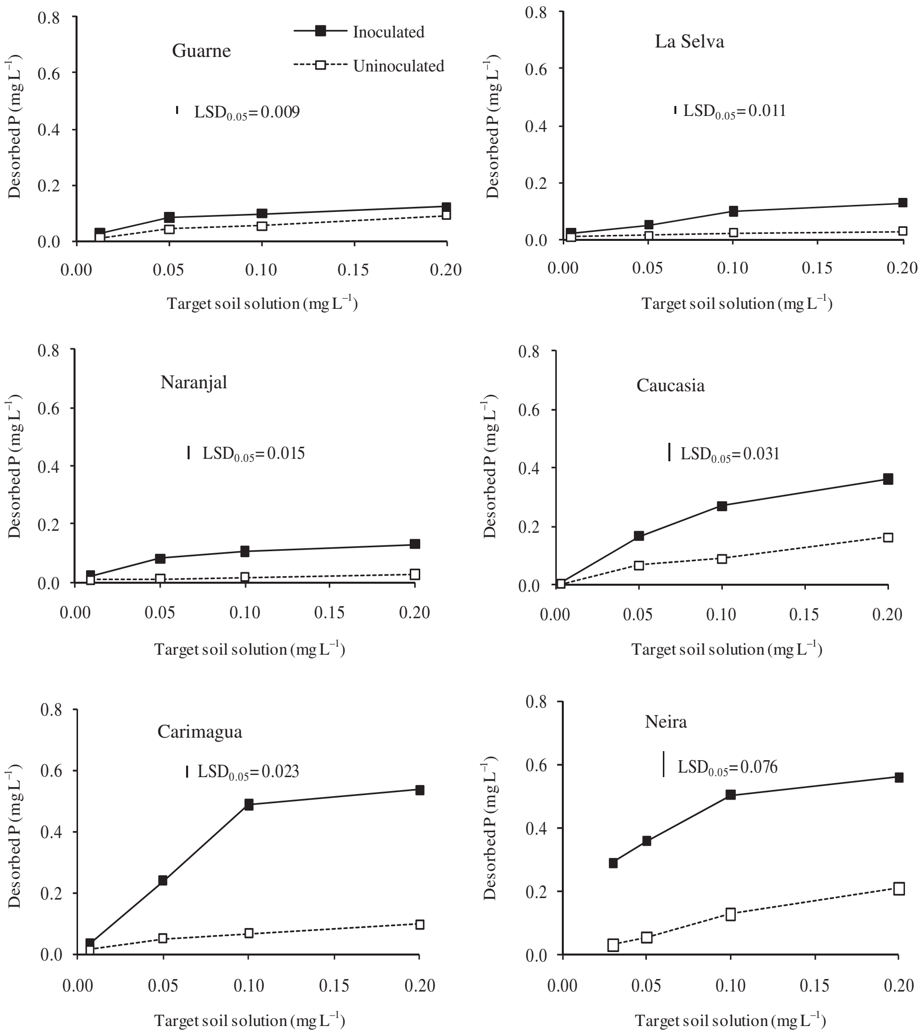


**Figure 1.** Concentrations of soluble P (mg $L^{-1}$) desorbed by *Mortierella* sp. from six soils as functions of four initial target solution P concentrations. The LSD ($P \leq 0.05$) value for each soil is used to compare uninoculated and inoculated treatments.

in excess of 0.2 mg $L^{-1}$. Similar results were obtained when the Caucasia soil was inoculated with *Mortierella* sp. (Figure 1). Although *Mortierella* sp. also desorbed Pi from all of the three Andisols studied, the magnitude of its effectiveness was less due certainly to the very high Pi-sorbing capacity (Figure 1). Phosphate desorption was particularly restricted in the most Pi-fixing soil (Guarne) (Figure 1). Despite this fact, the effect of the fungus was enhanced by increasing the level of sorbed Pi. Concentration of soil solution Pi in the inoculated and fertilized Andisols ranged from 0.057 to 0.131 mg $L^{-1}$.

## Discussion

The capacity of *Mortierella* sp. to desorb sorbed Pi was demonstrated in six soils representing a wide range of Pi-adsorbing capacities (Figure 1). The effectiveness with which the fungus desorbed Pi from soils and minerals is in general governed by the Pi-adsorbing capacity of these materials. While the capacity of *Mortierella* sp. to augment soil solution Pi concentration is directly correlated with the quantity of Pi adsorbed on the surface of soil, the magnitude of Pi desorbed is inversely related to the Pi-sorption capacity of soil.

Hence, the fungus was highly effective in desorbing Pi from the Neira soil, a Mollisol whose mineralogy is dominated by Montmorillonite (Buol et al. 1997) and therefore is one of the soils in which *Mortierella* sp. exhibited very high capacity to desorb Pi. The fungus was also very effective in desorbing Pi from the kaolinite-dominated Carimagua soil (IGAC 1991).

The fungus was limited in its effectiveness in desorbing Pi from the Andisols (Guarne, Naranjal, and La Selva) (Figure 1). The limited effectiveness of *Mortierella* sp. in the presence of these soils is largely due to the strength with which Pi is bound to them. Allophanes are the likely dominant minerals in the three Andisols (Guarne, La Selva, Naranjal).

The effectiveness of *Mortierella* in desorbing Pi from the Caucasia soil was intermediate between the two extremes (the Andisols on the one hand and the Carimagua and Neira soils on the other hand) considered previously. The most probable mineral composition of the Caucasia soil is a mixture of kaolinite and goethite (Jaramillo 1996).

The effectiveness of *Mortierella* sp. to increase soil solution Pi ensures the availability of sufficient Pi for itself and perhaps for other organisms, including plants and mycorrhizal fungi. This phenomenon may in part explain the synergistic effect of this fungus and mycorrhizal fungus to stimulate plant Pi uptake and growth of *Leucaena leucocephala* in a highly weathered Oxisol of Hawai'i with moderate Pi-sorbing capacity (Osorio and Habte 2001) and in the Oxisol of Carimagua (Osorio and Habte 2013).

The tendency of *Mortierella* sp. to desorb a greater concentration of Pi when more Pi is sorbed on the soil surface is not surprising (Figure 1). It is well known that the strength with which Pi is sorbed tends to decrease with increase in the degree to which the adsorptive surfaces are saturated with Pi (Do Carmo Harta and Torrent 2007; Bohn, McNeal, and O'Connor 1985). This is true for five of the six soils we tested, the Neira soil being the sole exception in which microbial desorption of native Pi occurred. This occurrence is consistent with the low Pi-sorption capacity of the soil. Our findings offer a possible explanation for the positive growth response observed in several studies in which the effect of PSM on plant performance was tested using soils having low Pi-sorbing capacity as the Neira soil (Peix et al. 2001; Omar 1998; Gleddie 1993; Kucey and Legget 1989; Asea, Kucey, and Stewart 1988; Kucey 1988). On the other hand, the magnitude of the microbial Pi desorption and the further increase in soil solution Pi with an increase in the level of adsorbed Pi in the Carimagua and Caucasia soils suggest that these soils are conducive for the Pi-desorbing activity of PSM. The effectiveness of *Mortierella* in the Caucasia soil is very interesting, because this Ultisol contained very high amounts of 1 *M* potassium chloride (KCl)–extractable Al (13.7 $cmol_c\ kg^{-1}$) and an Al saturation of 96.5% (soil organic-matter content 0.9%).

Although the Pi desorption induced by *Mortierella* sp. in the three Andisols was very limited, its effect in raising soil solution Pi concentration when sorbed Pi was increased is important. It is evident from the data that Pi can be raised to a level sufficient for mycorrhizal plants (Habte and Manjunath 1987, 1991). However, relatively large amounts of Pi fertilizers will be required to enhance the Pi desorption activity of *Mortierella* sp., and the cost–benefit ratio of the practice ought to be considered carefully. In Andisols with a long history of Pi fertilization, it is probable that PSM will increase the supply of Pi in the soil solution and hence will increase plant Pi uptake.

He and Zhu (1998) presumed that soil microorganisms were able to use sorbed Pi from soil minerals on the basis of a study in which soil was used as an inoculum. Unfortunately, their study did not take into account the possible involvement of other soil components (soil organic matter, humic and fulvic acids, low-molecular-weight organic acids/anions, inorganic anions), which can also desorb Pi from the surfaces of soil minerals (Jara et al. 2006; Guppy et al. 2005; Iyamuremye and Dick 1996). Therefore, the results of He and Zhu (1998) cannot be completely attributed to microorganisms present in the soil used as inoculum. More recently, Hoberg, Marschner, and Lieberei (2005) found that two microorganisms were able to take up Pi adsorbed on goethite. However, in that experiment soluble Pi decreased over time, due apparently to immediate absorption of all Pi desorbed by the microorganisms. A major characteristic required of effective PSM is that they must increase soluble Pi, which can be used by other organisms (e.g., plants and/or mycorrhizal fungi). The most conclusive reports about Pi desorption induced by microbes from soil minerals were published by He, Bian, and Zhu (2002) with the bacterium *Moraxella* sp. and Osorio and Habte (2012) with the fungus *Mortierella* sp. In both cases the microbes were capable of desorbing Pi from the surface of goethite, montmorillonite, and kaolinite.

The mechanism by which *Mortierella* sp. desorbed Pi is the release of oxalic acid/oxalate during its normal metabolic activity as detected by Osorio (2008) using capillary electrophoresis. (Hewlettt Packard 3D-CE; Hewlett Packard, Palo Alto, Calif.). It is very well known that organic acids/anions can displace Pi from sorbing sites (Jara et al. 2006; Sato and Comerford 2006; Jones et al. 2003; Trolove et al. 2003; Welch, Taunton, and Banfiled 2002; Ramirez and Osorio 2005). The effects of these acids on Pi sorption/desorption were studied by Sato and Comerford (2006), Bolan et al. (1994), and Hue (1991) in highly weathered soils and in volcanic ash soils similar to those used in the current study. The ability of PSM to produce organic acids/anions is most prominent in the rhizosphere whereby carbonaceous compounds are released by roots (Corrales et al. 2007; Amos and Walters 2006; Le Bayon et al. 2006; Marschner, Solaiman, and Rengel 2006; Marschner 1997). Once the root exudates are released they are metabolized by rhizosphere microorganisms, leading to the production of organic acids/anions (Gyaneshwar et al. 2002). Although this excretion appears to be genetically determined (Rodriguez et al. 2006, 2000), environmental conditions such as Pi deficiency or high ammonium concentration could trigger or enhance the production of organic acid/anions (Vyas et al. 2007; Chen et al. 2006; Whitelaw 2000).

The Pi desorption by organic acids is a mechanism that applies to the Pi adsorbed on the surface of minerals and not to Pi occluded. In this study, likely part of the Pi was occluded by oxide coating in the course of the drying process. Then, organic acids released by the fungus may not have access to this Pi pool, and thus the effects of Pi desorption may be greater. This situation should be considered in further studies. Results of the current study suggest that the value of $P_{0.2}$ obtained from the initial soil Pi sorption isotherm can be used for predicting the degree to which a PSM is effective in desorbing Pi from a given soil. This could save a lot of time in research and in making decisions on whether or not to inoculate with a PSM. The effectiveness of *Mortierella* sp. in desorbing Pi might have some practical implications such as lowering soil Pi fertilizer requirement and enhancing the residual effect of soluble Pi fertilizers.

The effectiveness of this fungus in increasing soil solution Pi by dissolving rock phosphate and desorbing sorbed Pi may play an important role in the alleviation of Pi deficiency in soils, particularly in the tropics where the high Pi sorption capacity of soils constrains plant productivity. Moreover, the potential role PSM play in soil Pi availability ought to be an important consideration in the development of biotechnological approaches to the management of soils.

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