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Biofertilization with Mycorrhizal Fungi and Phosphate Solubilizing Microorganisms Enhance Effectiveness of Phosphate Fertilizers in Tropical Soils

WALTER OSORIO* AND LAURA OSORNO

ABSTRACT

The low soil phosphate availability is one of the most serious constraints in tropical agriculture. This is due to either adsorption *of soluble Pi ions from the soil solution (where it is bioavailable) onto the surface of soil minerals (clays and oxides) or* precipitation *of Pi ions with iron and aluminum ions. This problem is particularly important in highly weathered soils and volcanic ash soils. One alternative to increase soil Pi availability is to apply high amounts of soluble Pi fertilizers. However, most of the soluble Pi ions added are adsorbed or precipitated and, consequently, soluble Pi fertilizers have low efficiency in these soils. The use of mycorrhizal fungi helps plant roots in nutrient uptake (particularly Pi) increasing thus the effectiveness of these fertilizers. Another alternative is the use of rock phosphates, but their low solubility discourages their use. The combined used of mycorrhizal fungi and microorganisms capable of dissolving Pi compounds can increase the agronomic effectiveness of these materials. The concomitant use of both types microorganisms represents a cost-effective and environment friendly alternative to enhance the effectiveness of soluble and insoluble Pi fertilizers.*

***Key words*:** Phosphate fixation, Rock phosphate, Sorption isotherms, Mycorrhizal fungi, Phosphate solubilizing microorganisms.

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1. INTRODUCTION

The low availability of phosphate (Pi) in the soil is one of the most serious constraints on tropical agriculture (Wakelin *et al*., 2004; Oberson *et al*., 2006). This is due to reactions of *adsorption* of soluble Pi ions (where it is available for plant uptake) onto the surface of soil minerals (clays and oxides) where it is held in unavailable forms and *precipitation* of Pi ions with iron (Fe) and aluminum (Al) ions (Smith, 2002; Khan *et al*., 2007). This problem is particularly important in highly weathered soils and in soils formed from volcanic ash. As a result of that in tropical soils most of the Pi applied in soluble fertilizers became unavailable for plant use in the short term (Osorio and Habte, 2009; Batti and Yamar, 2010). In these soils the efficiency of soluble Pi fertilizers is low 5–10% and, consequently, it is necessary to apply high doses, which discourages their use by poor farmers of under-developed countries (Reddy *et al*., 2002).

Another alternative consists of the use of rock phosphates (RP), valuable, non-renewable, and finite resources for agriculture and other applications (Vassilev *et al*., 2009; Vassileva *et al*., 2010). They are world-wide used with a current growing demand rate ~3%; however, their low solubility also restricts their use.

There are increasing concerns about the decline of global RP reserves (Dibb, 2004). Recent predictions suggested that the world´s reserves of easily mining RP will last 100–125 years from now (Gilbert, 2009). This threatens the food security at global scale; in fact, some authors have predicted a potential phosphate crisis. We must to develop viable strategies to increase Pi fertilizers use efficiency.

There are soil microorganisms capable of increasing plant root Pi uptake: (*i*) *arbuscular mycorrhizal fungi* (AMF) form symbiotic association with plant roots that increase water and nutrient uptake, particularly those of limited diffusion (*e.g*., Pi, Cu, Zn) increasing thus the effectiveness of soluble Pi fertilizers (Osorio and Habte, 2013) and (*ii*) *Pi solublizing microorganisms* (PSM) can dissolve insoluble RP applied increasing its agronomic effectiveness (Osorno, 2013). Both types of microorganisms can be used as biofertilizers to enhance plant Pi uptake especially when they are concomitantly inoculated.

Our objective in this chapter is to discuss that the co-inoculation with both types of soil microorganisms represent a cost-effective and environment friendly alternative to enhance the effectiveness of soluble and insoluble Pi fertilizers in tropical soils.

2. PHOSPHATE DEFICIENCY IN TROPICAL SOILS

Plant roots uptake phosphate ion ($H_2PO_4^-$) dissolved in the soil solution; however, its concentration is quite low (0.001–0.3 mg L^{-1}). Highly weathered

soils and volcanic ash soils of the tropics usually exhibit low concentration of soluble Pi (<0.1 mg L^{-1}) (Scervino *et al*., 2010), which limits plant productivity in agricultural crops, grassland, and forestry. The low availability of Pi in tropical soils is due to a series of reaction that remove soluble Pi into the soil solid phase, which has been called Pi fixation (Barber, 1995; Collavino *et al*., 2012).

2.1. Soil Phosphate Fixation

Sanchez and Logan (1992) estimated that in the tropics the soils that exhibit high Pi fixation capacity occupy 1018 million ha. In tropical America there are 659 million ha affected, 210 in Africa, and 199 in Asia. The term Pi-fixation is used to describe two types of reactions that remove bioavailable Pi from the soil solution (Collavino *et al*., 2012): (*i*) *Pi adsorption* on the surface of soil minerals (clays and oxides) (Fig. 1) and (*ii*) *Pi precipitation* by cations such as Al^{3+} and Fe^{3+} in the soil solution (Havlin *et al*., 2004). Pi fixation is particularly a serious problem in highly weathered soils and those formed from volcanic ash (Trolove *et al*., 2003; Do Carmo Harta and Torrent, 2007).

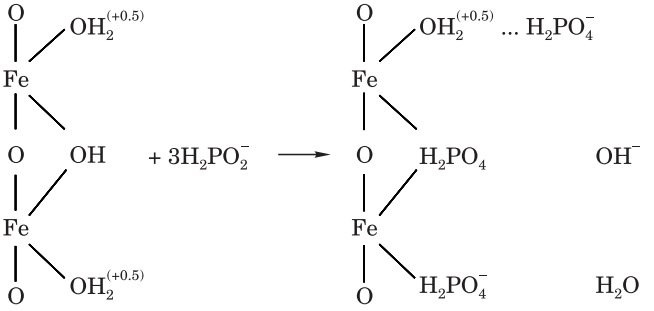


Fig. 1: Sites of Pi adsorption on the surface of an iron oxide-hydroxide. On the upper right Pi is weakly held by a positive charge [$-OH_2^{(0.5+)}$] (*non-specific adsorption*). In the lower right Pi is strongly held by a single bound and in the right center by two bounds (*specific adsorption*)

Phosphate adsorption is particularly strong on iron and aluminum hydrous-oxides (*e.g*., goethite, gibbsite) that predominate in the highly weathered soils of humid regions and acid savannas (Jones, 1981; Jackman *et al*., 1997; Hinsinger, 2001), most of them classified as Oxisol and Ultisols. In soils formed from volcanic ash (Andisols), minerals such as allophane, ferrihydrite, goethite, and humus-Al/Fe complexes are responsible for the strong Pi fixation (Parfitt, 1989; Schwertmann and Herbillon, 1992; Jackman *et al*., 1997; Shoji *et al*., 1993).

According to Bohn *et al*. (1985) the mechanisms of Pi adsorption are: (*i*) *non-specific adsorption* that consists of electrostatic attraction exerted by

positive charges on the surface of soil minerals by $-OH_2^+$ groups. In this sites the Pi is weakly held and can be exchangeable with other anion (*e.g.*, SO_4^{2-}, NO_3^-, Cl^-) from the soil solution becoming thus available for root uptake and (*ii*) *specific adsorption* occurs when Pi ions form single (monodentaded) or double bounds on the surface of soil minerals while replace OH^- or OH_2^+ (Fig. 1). In this type of adsorption Pi is strongly held that is not longer considered available for plant roots.

In general, the soil capacity to adsorb Pi ions is as follows: Andisols >Ultisols, Oxisols >...>Mollisols, Vertisols> Histosols.

In acid soils (pH<5.5), *Pi precipitation* occurs with active forms of aluminum [Al^{3+}, $Al(OH)^{2+}$, $Al(OH)_2^+$] and iron [(Fe^{3+}), $Fe(OH)^{2+}$, $Fe(OH)_2^+$] (eq. 1 and 2) (Smith, 2002). In neutral and alkaline soils (pH>6.5) it occurs mostly with calcium (Ca^{2+}) (Bohn *et al*., 1985). Initially, Pi ions precipitate to form initially amorphous (non-crystalline) compounds, which became crystalline over time (Brady and Weil, 1999). Amorphous minerals are slightly more soluble than their crystalline forms because they have smaller particle size, and consequently greater surface area. For instance, the crystalline mineral variscite ($AlPO_4.2H_2O$) has a surface area of 1.54 $m^2\ g^{-1}$ (Taylor and Gurney, 1964) and its solubility product (K_{sp}) is $10^{-30.5}$ (Bache, 1963). On the other hand, its amorphous aluminum-phosphate counterpart has a surface area of 10.5 $m^2\ g^{-1}$ (Juo and Ellis, 1968) and a K_{sp} of $10^{-28.1}$ (Veith and Sposito, 1977). In alkaline soils, Pi compounds are similarly transformed to more insoluble forms. Initially Pi ions precipitate to form calcium-monohydrogen-phosphate, $Ca(H_2PO_4)_2$ (K_{sp}= $10^{-6.6}$) (Stumm and Morgan, 1995), which is then converted to calcium-orthophosphate ($CaHPO_4$) (K_{sp}= 10^{-24}), and finally to apatite ($Ca_5(PO_4)_3OH$; K_{sp} =$10^{-55.9}$) (Snoeyink and Jenkins, 1980).

$$H_2PO_4^- + Al(OH)_2^+ \leftrightarrow AlPO_4.2H_2O \quad ...(1)$$

$$H_2PO_4^- + Fe(OH)_2^+ \leftrightarrow FePO_4.2H_2O \quad ...(2)$$

In general, there are three major types of soil Pi minerals: aluminum phosphate (Al–Pi), iron phosphate (Fe–P) and calcium phosphate (Ca–P) (Osorio, 2012) (Table 1). The dominance of these compounds depends mainly on the degree of soil weathering. In lowly weathered soils (*e.g.*, Mollisoles, Vertisoles) there are high contents of calcium and neutral or alkaline pH; consequently, Ca–Pi compounds are dominant usually as primary minerals (apatite, francolite). In highly weathered soils (*e.g.*, Oxisols and Ultisols), as the weathering proceeds, the aluminosilicate minerals are dissolved and those structural elements released into the soil solution (Ca^{2+}, Mg^{2+}, K^+, Na^+, Al^{3+}, Fe^{3+}, among others) (eq. 3–6). The ions of Ca^{2+}, Mg^{2+}, K^+, and Na^+ are easily leached out in humid regions, leaving $Al^{3+}$and Fe^{3+} as the dominant cations that then react with Pi ions.

$$KAlSi_3O_8 \text{ (microcline)} + 8H_2O \leftrightarrow K^+ + Al(OH)_2^+ + 3H_4SiO_4 + 2OH^- \quad ...(3)$$

$$CaAl_2SiO_6 \text{ (pyroxene)} + 8H^+ \leftrightarrow Ca^{2+} + 2Al^{3+} + H_4SiO_4 + 2H_2O \quad ...(4)$$

$$Mg_5Al_2Si_3O_{10}(OH)_8 \text{ (chlorite)} + 16H^+ \leftrightarrow 5Mg^{2+} + 2Al^{3+} + 3H_4SiO_4 + 6H_2O \quad .(5)$$

$$Mg_{0.2}(Si_{3.81}Al_{1.71}Fe(III)_{0.22}Mg_{0.29})O_{10}(OH)_2 \text{ (montmorillonite)} + 6.76H^+ \leftrightarrow 0.49Mg^{2+} + 1.71Al^{3+} + 0.22Fe^{3+} + 3.81H_4SiO_4 \quad ...(6)$$

The soil Pi compounds, as well as the applied Pi fertilizers, are dissolved in a different way according to soil pH. Thus, Ca–Pi compounds are more easily dissolved as the pH decreases, while Al–Pi and Fe–Pi are dissolved when the pH increases (Fig. 2). The acid dilution for these compounds is showed in reactions 7–11.

Table 1: Soil mineral Pi fractionation of tropical soils of Colombia (COL) and Hawaii (HI)

Soil	***Available Pi**** **(%)**	***Al–Pi* (%)**	***Fe–Pi* (%)**	***Ca–Pi* (%)**
Lowly weathered				
Vertisol (Lualualei,HI)	0.5	16.7	27.7	55.0
Mollisol (Neira, COL)	0.6	35.4	11.1	52.9
Highly weathered				
Oxisol (Molokai, HI)	0.03	32.6	58.2	9.1
Oxisol (Wahiawa, HI)	0.5	24.0	67.8	7.7
Oxisol (Paaloa, HI)	0.9	22.4	49.4	27.3
Oxisol (Halii, HI)	0.5	20.7	61.4	17.4
Oxisol (Makapili, HI)	0.7	6.6	78.3	14.3
Oxisol (Kapaa, HI)	0.6	43.9	44.5	11.0
Oxisol (Carimagua, COL)	0.3	10.3	87.6	1.8
Ultisol (Caucasia, COL)	0.2	8.8	90.9	0.0

*Soluble and weakly adsorbed

Source: Osorio (2008, 2012)

$$Ca_5(PO_4)_3OH \text{ (hydroxilapatite)} + 7H^+ \leftrightarrow 5Ca^{2+} + 3H_2PO_4^- + H_2O \quad (K=10^{14.46}) \quad ...(7)$$

$$Ca_5(PO_4)_3F \text{ (fluorapatite)} + 6H^+ \leftrightarrow 5\ Ca^{2+} + 3H_2PO_4^- + F^- \ (K=10^{-0.21}) ...(8)$$

$$CaHPO_4.2H_2O \text{ (brushite)} + H^+ \leftrightarrow Ca^{2+} + H_2PO_4^- + 2H_2O \ (K=10^{0.63}) \quad ...(9)$$

$$FePO_4.2H_2O \text{ (strengite)} + 2H^+ \leftrightarrow Fe^{3+} + H_2PO_4^- + 2H_2O \ (K=10^{-6.85}) ...(10)$$

$$AlPO_4.2H_2O \text{ (variscite)} + 2H^+ \leftrightarrow Al^{3+} + H_2PO_4^- + 2H_2O \ (K=10^{-2.50}) \quad ...(11)$$

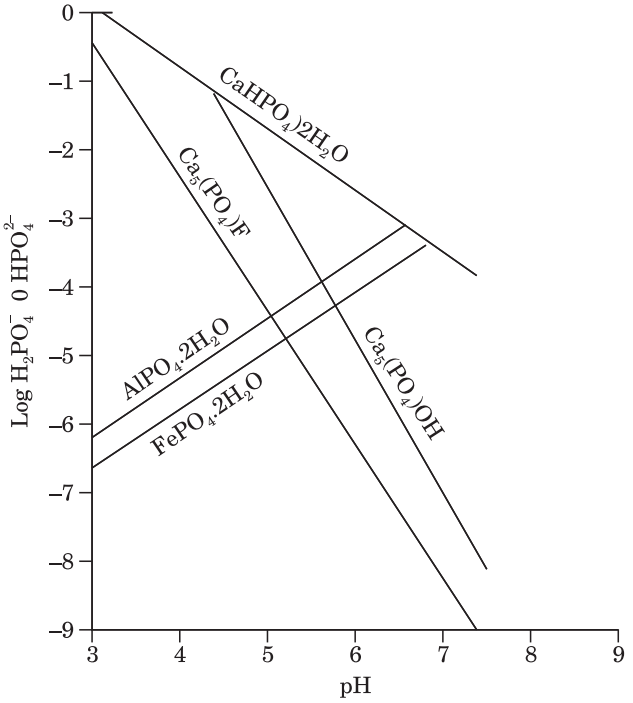


Fig. 2: Solubility of calcium phosphates, variscite ($AlPO_4.2H_2O$) and strengita ($FePO_4.2H_2O$) as a function of pH. *Source*: Lindsay (2001)

2.2. Isotherm of Soil Phosphate Fixation

The use of isotherm of Pi sorption is a simple way to measure the soil capacity to fix Pi (Do Carmo Harta and Torrent, 2007). In our laboratory we used the method developed by professors Fox and Kamprath (1970) at North Carolina State University and University of Hawaii. Briefly, this consists of applying separately grading amounts of soluble Pi (*e.g.*, KH_2PO_4; 0–2000 mg P kg^{-1}) dissolved in 30 mL of 0.01 M $CaCl_2.2H_2O$ to aliquots of soils (3 g, dry basis) in plastic centrifuge tubes. Then, the tubes are shaken 30 min each 12 h for 6-days. After this incubation period, the tubes are centrifuged (15 min, 4000 rpm) and the supernatant filtered with filter paper (and membrane filters). The concentration of soluble at equilibrium is measured using the phosphomolybdate blue method (Murphy and Riley, 1962); the remaining non-soluble Pi is considered fixed into the soil particles. A graph is constructed to show the relationship between adsorbed Pi and soluble P. Juo and Fox (1977) proposed classify soil Pi fixation capacity according the amount of Pi required (mg kg^{-1}) to achieve a soil solution Pi concentration of 0.2 mg L^{-1} (Table 2). This concentration is considered a critical level to obtain 95% of the maximum yield of several agronomic crops.

Table 2: Categories of soil P fixation capacity and predominant soil minerals. The $P_{0.2}$ value is the amount of added P required to achieve a soil solution P concentration of 0.2 mg L^{-1}

Category	*$P_{0.2}$ value (mg P kg^{-1})*	*Predominant soil mineralogy*
Very low	<10	Quartz, organic materials
Low	10–100	2:1 clays, quartz + 1:1 clays
Medium	100–500	1:1 clays + oxides
High	500–1000	Oxides + volcanic ash moderately weathered
Very high	>1000	Amorphous material desilicated

Source: Juo and Fox (1977)

The isotherm of soil P fixation is also used to determine the amount of Pi fertilizers required (Hue and Fox, 2010). In this case, it is necessary to know the critical soil solution Pi level associated to a desired yield for a given crop (Table 3). Examples for this use are illustrated in the Fig. 3 with three Colombian soils (Mollisol, Oxisol, and Andisol). Whereas the Palmira soil (Valle del Cauca, Colombia) exhibited a low very high capacity to fix Pi ($P_{0.2}$= 64 mg kg^{-1}), the Carimagua soil (Vichada, Colombia) and the Chinchina soil (Caldas, Colombia) exhibited medium and very high capacity to fix P ($P_{0.2}$= 352 and 1658 mg kg^{-1}, respectively). These amounts of Pi coincide with the Pi requirements of soybean and tomato crops. In the case of corn, the Pi requirements for 95% of the maximum yield would be 3, 174, and 977 mg of P kg^{-1} for the Mollisol, Oxisol, and Andisol, respectively. If the corn yield desired is lower (75%) the soil solution Pi level should be 0.008 mg L^{-1}, consequently, the Pi required will be lower (0, 76, 604 mg of P kg^{-1}, respectively). In any case, the soluble Pi fertilizers must be applied in the root zone.

Table 3: Soil solution Pi concentrations required to achieve high relative yields for some tropical crops

Crop	*Soil solution P concentration (mg of P L^{-1}) to achieve the relative yield indicated*	
	75%	*95%*
Cassava	0.003	0.005
Peanut	0.003	0.010
Corn	0.008	0.025
Wheat	0.009	0.028
Cabbage	0.012	0.040
Potato	0.02	0.180
Soybean	0.025	0.200
Tomato	0.05	0.200
Lecttuce	0.10	0.300

Source: Fox *et al.* (1974)

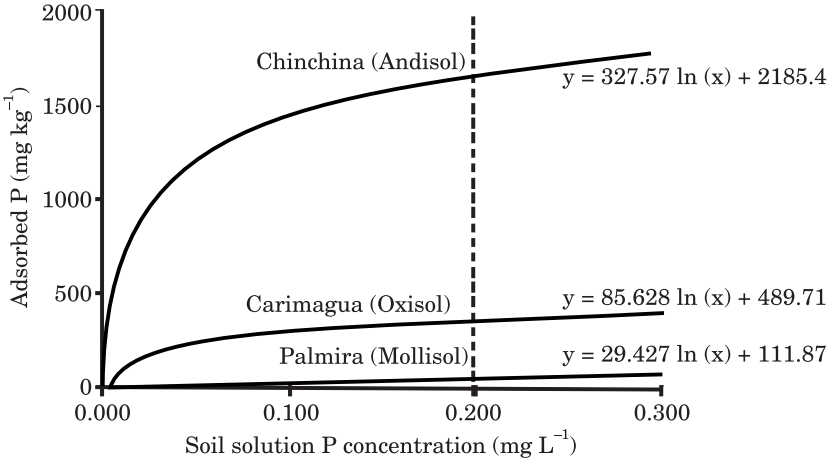


Fig. 3: Isotherms of Pi fixation for three soils of Colombia. The projection of the dashed line on the Y- axis shows the $P_{0.2}$ value, which measures the soil Pi fixation capacity. *Source*: Osorio (2012).

3. MANAGEMENT OF PHOSPHATE FERTILIZATION IN TROPICAL SOILS

3.1. Soluble Phosphate Fertilizers

Sanchez and Uehara (1980) discussed the strategy of *building-up and maintenance* to increase soil Pi availability of acidic tropical soils with high Pi fixation capacity. One strategy consists of applying a high dose of soluble Pi fertilizers (based on isotherm of soil P fixation) followed by small amounts of annual application (Engelstad and Terman, 1980). Although a great part of the added Pi is fixed, it may be released over several years, thus generating a residual effect. This strategy has been successfully used in tropical soils for sugarcane and pineapple (Hawaii), soybean (Brazil), and chrysanthemums, roses, carnations, and other ornamental crops (Colombia). However, the high soluble Pi fertilization rates that result from this method are not added by most farmers in developing countries due to the high cost of Pi fertilizers (Arcand and Schneider, 2006; Randhawa *et al*., 2006; Shigaki *et al*., 2006). The proportion of the added Pi taken up by the first crop is quite low, ranging from 5 to 10%. It means that 90–95% of the added soluble Pi fertilizer is fixed in the soils in chemical forms that slowly release Pi for plants (Engelstad and Terman, 1980).

Alternatively, the strategy of *sufficiency* is more common employed; this consists of applying moderate and frequent amounts of soluble Pi fertilizers at the crop establishment. In this case, no residual effect is expected and soluble Pi must be applied every time that a crop is planned. In comparison, the amounts

of Pi required in the *sufficiency* strategy are lower than in the *building-up and maintenance* strategy. Unfortunately, the crops yields are also lower. Some examples of the sufficiency strategy are illustrated in Table 4 for agronomical crops in Colombia. In this case, the method of Bray II is used to determine the soluble Pi required.

Table 4: Amounts of P required for agronomical crops in Colombia based on the concentration of soil Pi level extracted with the Bray-II method

Crop	***Soil P-Bray II* (*mg kg^{-1}*)**	***Required P* (*kg P ha^{-1}*)**
Rice	< 10	17–35
	10–20	9–17
	> 20	0–9
Potato	< 40	163–196
	40–60	131–163
	> 60	109–131
Cassava	< 10	44–54
	10–20	33–44
	> 20	0–33
Pineapple	< 10	33–44
	10–20	22–33
	> 20	0–22
Banana	< 12	60–80*
	12–20	40–60
	> 20	20–40
Brachiaria grass	< 5	22-33**
	5–10	11–22
	> 10	0–11
Kikuyo grass	< 10	22–33**
	10–20	11–22
	> 20	0–11
Cocoa	< 15	44–54***
	15–30	22–44
	> 30	0–22
Coffee	<10	26***
	10–20	17
	20–30	9
	>30	0

*Annual application, **application at establishment of the grassland, ***g plant^{-1} yr^{-1}.
Source: adapted from ICA (1992), Sadeghian (2008)

For instance, if a pineapple crop is going to be established in a soil with a P-Bray II value of 3 mg kg^{-1}, the amount of P required would be ~40 kg ha^{-1}

(Table 4). This represents an application of 200 kg ha^{-1} of diammonium phosphate (DAP, ~20% of P); it must be applied in bands near the roots to improve its effectiveness.

3.2. Rock Phosphate

Rock phosphate (RP) is a general term that describes different types of apatites [$Ca_{10}(PO_4)_6(F,OH,Cl)_2$], which are either employed directly as low-soluble Pi fertilizers or to produce more soluble Pi fertilizers (Zapata and Roy, 2007). The apatites have different type of elemental substitution Ca^{2+} by Na^{+1} and Mg^{2+}, and PO_4^{3-} by CO_3^{2-} (*e.g.*, $Ca_{10-x-y}Na_xMg_y(PO_4)_{6-z}(CO_3)_zF_2$), which produces different types of RP (Hammond and Day, 1992). Unfortunately, the low solubility of RP and its low agronomical effectiveness discourage its direct use (Rajan *et al*., 1996; Vassileva *et al*., 2000; Reddy *et al*., 2002, Pramanik *et al*., 2009). In spite of that, they are frequently used in soils with high Pi fixation capacity, because other more soluble Pi fertilizers are quickly fixed and are more expensive (Msolla *et al*., 2005; Randhawa *et al*., 2006; Yusdar *et al*., 2007). Satisfactory results have been obtained in acid soils particularly overtime (*i.e*., in the second and third season after its application).

There is an increasing interest in enhancing RP reactivity to obtain better, immediate, and consistent results through different treatments (Shrivastava *et al*., 2007; Ojo *et al*., 2007). Some of these treatments include fine grinding, partial acidulation with strong acids (eq. 12), thermal alteration, fusion with silica, sodium or magnesium carbonate; mixing it with barnyard manures, compost, and green manures (Sanchez and Uehara, 1980; Redding *et al*., 2006; Msolla *et al*., 2007; Yusdar *et al*., 2007; Shrivastava *et al*., 2007; Vassileva *et al*., 2010). Inoculation with arbuscular mycorrhizal fungi into soil amended with RP has been successfully used to enhance RP agronomic effectiveness (Manjunath *et al*., 1989). Another biotechnological approach consists of soil inoculation with Pi solubilizing microorganisms (PSM), whose production of organic acids accelerate the dissolution of RP (Whitelaw, 2000; Vassileva, 2003; Havlin *et al*., 2004; Ramírez and Osorio 2005; Jayasinghearachchi and Seneviratne, 2006; Osorio, 2008; Singh and Reddy, 2011). In addition, this has been proposed as a biotechnological alternative to produce more soluble Pi fertilizers (Bar-Yosef *et al*., 1999; Osorno, 2013). The use of these microorganisms will be discussed above.

$$Ca_5(PO_4)_3OH \text{ (hydroxylapatite)} + 7H^+ \leftrightarrow 3H_2PO_4^{2-} + 5Ca^{2+} + H_2O \qquad K=10^{14.5} \quad ...(12)$$

4. BIOFERTILIZERS THAT ENHANCE PLANT PHOSPHATE UPTAKE

The use of microbial inocula as biofertilizer is currently considered as a viable alternative to either improve the effectiveness of fertilizers or reduce fertilizer

dose (Khan *et al*., 2007). This approach is based on a more sustainable agriculture that involves environmental friendly practices to maintain an ecological balance in soils (Vessey, 2003: Borges *et al*., 2011). Several authors have used this biotechnological approach to enhance the effectiveness of Pi fertilizers (Oliveira *et al*., 2009). The most relevant types of microorganisms used have been arbuscular mycorrhizal fungi (AMF) (Manjunath *et al*., 1989) and Pi solibilizing microorganisms (PSM) (Kucey and Leggett, 1989; Whitelaw, 2000). Although the results reported when each microorganisms is inoculated separately, they can have synergistic effects when inoculated concomitantly (Osorio and Habte, 2013). Next, we will describe a series of studies that show the mechanisms of both types of microorganisms alone and together in increasing Pi fertilizer effectiveness in tropical soils.

Also, PSM may be used to produce industrially soluble Pi fertilizers (*e.g*., superphosphates) via acidulation of RP with organic acids as illustrated with some experimental results.

4.1. Use of AMF to Enhance Effectiveness of Phosphate Fertilizers

Plant roots can form a symbiotic association with soil fungi of the phylum *Glomeromycota* (Oehl, 2011). This association is termed "arbuscular mycorrhiza", which means "fungus-root" and is widely spread geographically as well as botanically. The fungal hyphae invade the cortical cells inter- and intra-cellularly where these form clusters of finely divided hyphae known as arbuscules (Habte, 2006); the arbuscules are believed to be sites of exchange of materials between the host and the plant.

Arbuscular mycorrhizal fungi (AMF) absorb, via extrarradical hyphae, nutrients such as N, P, K, Ca, S, Fe, Mn, Cu, and Zn from the soil solution to inside the plants roots (Vosatka and Albrechtova, 2009). The most consistent and important nutritional effect is to improve the uptake of immobile nutrients such as $H_2PO_4^-$. AMF are very effective in enhancing plant P uptake, particularly with plant species that lack phisiological or morphological mechanisms for efficient P uptake, such as fine-branched root systems and abundant root hairs, among others (Manjunath and Habte, 1991; Habte and Osorio, 2001).

4.1.1. *Response of mycorrhizal and non-mycorrhizal plants to RP*

Mycorrhizal hyphae have a higher affinity for absorbing Pi than roots. Schachtman *et al*. (1998) reported that the hyphae of *Gigaspora margarita* had an affinity constant for Pi (Km) of 2.5 mM (P: 0.077 mg L^{-1}), whereas most plants usually exhibited a Km of 6–44 mM (P: 0.19–1.36 mg L^{-1}), particularly those highly dependent on the mycorrhizal association (Nye and Tinker, 1977; Barber, 1995).

Some authors have proposed the use of AMF to increase efficiency in plant Pi uptake (Mosse, 1981). For instance, Manjunath *et al*. (1989) studied the

effectiveness of *Glomus aggregatum* to enhance plant Pi uptake of *Leucaena leucocephala* grown in a Hawaiian Oxisol fertilized with RP (0.17–2.72 g kg^{-1}). Plant dry weight and shoot P concentration did not increase significantly in uninoculated soils. In contrast, in inoculated soils with *Glomus aggregatum* there was a significant increase in plant dry weight (Fig. 4a). In similar studies, Herrera (unpublished data) and Ramírez *et al*. (2013) found that the effectiveness of RP addition in increasing plant growth of pimenton (*Capsicum annuum*) seedlings (Fig. 5a) and cowpea (*Vigna unguiculata*) (Fig. 5b) grown in a Colombian Oxisol, was significantly increased by the AMF inoculation with *G. fasciculatum*. The results show clearly that the effectiveness of RP in increase plant performance was increased if the mycorrhizal fungus was present, even in short periods of time (~60 days). In fact, in mycorrhiza-free plant there was no response to RP addition.

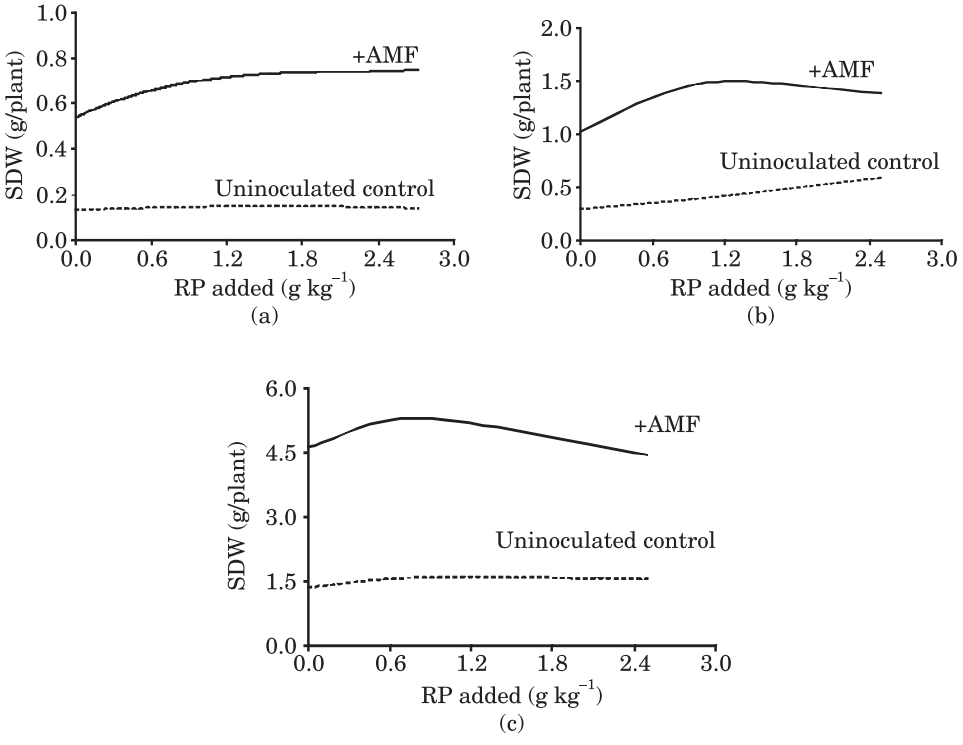


Fig. 4: Shoot dry weight (SDW) of *L. leucocephala* (*a*), *Capsicum annuum* (*b*), and *Vigna unguiculata* (*c*) as a function of RP added and AMF inoculation. *Source*: Manjunath *et al*. (1989), Herrera (unpublished) and Ramírez *et al*. (2013), respectively

Our results contrast with early results obtained by several researchers of RP effectiveness (Espinosa *et al*., 1987; Martínez *et al*., 1987; León, 1990; León *et al*., 1995), in which RP effectiveness was low; also, in these studies crop

response to RP addition was detected after several months (at least 6 months). In our studies, RP effectiveness is evident after in short periods of time.

In addition, it has been claimed that RP should be applied only in acidic soils (soil pH <5.5), because at low soil pH this will dissolve faster (Havlin *et al*., 2004). However, in our experiments the RP has been effective in increasing mycorrhizal plant P uptake and growth even in soils with pH ≥6.0. Several conditions can explain the better results when the mycorrizal association is present: (*i*) it is clear that the elongated hyphae can capture soluble P at longer distance than the root alone, (*ii*) the hyphae is more efficient than roots in taking up P from the soil solution, and (*iii*) the decline of soluble P around RP particles promotes their dissolution (Manjunath *et al*., 1989). Presumably, the mycorrhizal hyphae exhibit a more active proton exudation than roots alone, which will favor a faster RP dissolution (Vassilev *et al.,* 2001).

4.1.2. *Response of mycorrhizal and non-mycorrhizal plants to soluble P fertilizers*

The inoculation with AMF can increase the effectiveness of soluble P fertilizers to promote plant growth as illustrated in Fig. 5. In this series of experiments the soil was amended with grading amounts of a soluble P fertilizer (*e.g*., $Ca(H_2PO_4)_2$, KH_2PO_4) in order to obtain increasing soil solution P concentrations. In addition, the soil was either inoculated with an AMF (*G. fasciculatum*) or uninoculated (control). The results indicated that the plant growth increased with the increase in solution P level as a result of the addition of a soluble P fertilizer (except in *P. ligularis*); however, the effect was significantly higher when the soil was concomitantly inoculated with AMF. For instance, at a soil solution P of 0.02 mg L^{-1} the shoot dry weight of mycorrhizal *Leucaena leucocephala* was about 7-fold higher than in non-mycorrhizal *Leucaena*. In the case of coffee (*Coffea arabiga*), sweet granadilla (*Passiflora. ligularis*), and lulo (*Solanum quitoense*) the respective increases were 3.0, 3.7, and 1.7 times. Notice that in the case of non-mycorrhizal leucaena the plant did not respond until the soil solution P reached a value of 0.03 mg L^{-1} (Fig. 5*a*). In the case of non-mycorrhizal sweet granadilla there was not response to the addition of soluble P fertilization (Fig. 5*c*).

In this way, to obtain the maximal plant growth of non-mycorrhizal coffee the soil required an addition of 2880 mg of KH_2PO_4 per kg; the same level of plant growth could be obtained in mycorrizhal coffee with only 611 mg of KH_2PO_4 per kg (Fig. 5*b*). This represents a reduction of 79% in the P fertilizer dose. In the case of lulo same calculations suggested a reduction in 66% of the P fertilization dose (Fig. 5*d*).

Optimal response to mycorrhizal inoculation can be achieved at a soil solution P concentration of 0.02 mg L^{-1}. The amount of soluble P required to achieve such concentration can be easily determined through an isotherm of soil phosphate fixation.

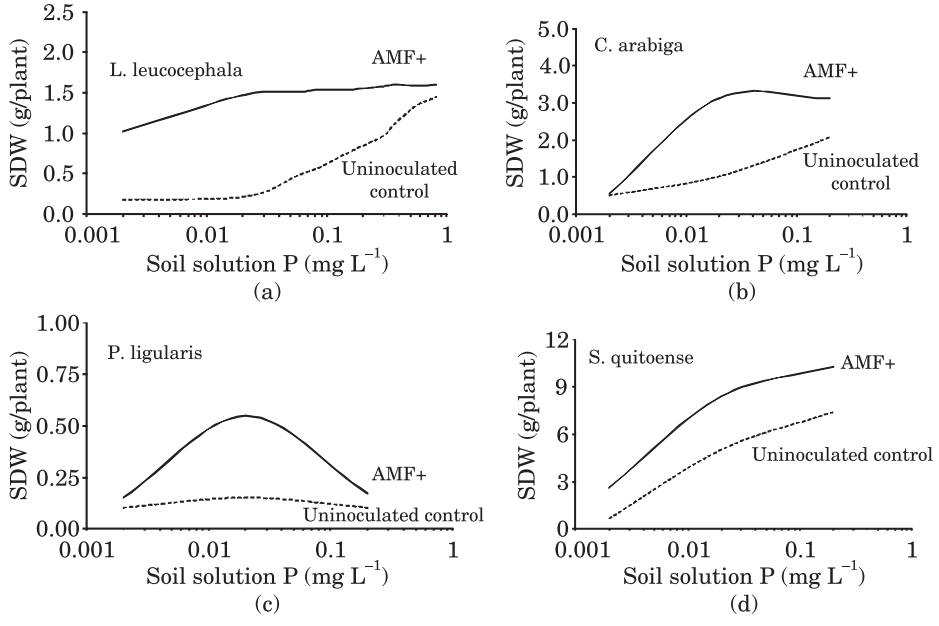


Fig. 5: Shoot dry weight (SDW) of tropical plants (*L. leucocephala, C. arabiga, S. quitoense,* and *P. ligularis*) as a function of soil solution P concentration and AMF inoculation with *G. fasciculatum*. *Source*: Habte and Manjunath *et al*. (1987), Rodriguez and Osorio (unpublished), Corredor and Osorio (unpublished), and Gonzalez and Osorio (2008)

4.2. Use of PSM to Enhance RP Effectiveness

Many soil microorganisms are involved in soil Pi transformation, contributing thus in the biogeochemical cycle of Pi (Chen *et al*., 2006). These microorganisms release Pi from organic compounds (Ramirez, 2005; Alikhani *et al*., 2006; Tao *et al*., 2008; Tallapragada *et al*., 2012) and inorganic compounds (Rao, 1992; Gyaneshwar *et al*., 2002, Oliveira *et al*., 2009). In the first mechanism, the microorganism releases extracellular phosphatase enzyme that hydrolyzes the ester bound (C–O–P) (Oberson *et al*., 2001) (Fig. 6.). In the second mechanism, the soil microorganism releases low molecular weight organic acid (*e.g*., citric acid and oxalic acid) that dissolve Pi compounds, mostly Ca–Pi (Selvakujmar *et al*., 2013) (Eq. 13). In some cases, a microorganism is capable of carrying out both mechanisms (*e.g*., *Aspergillus, Penicillium, Mortierella*) (Tao *et al*., 2008). However, once Pi ions have been released they can be either absorbed by plant roots or soil microorganisms (*e.g*., mycorrhizal fungi) or fixed into the soil solid phase (adsorbed by clays/oxides or precipitated with Al/Fe ions) (Osorio, 2012). Both types of microorganisms can be easily isolated from soils or plant rhizosphere with proper culturable media (Bashan *et al*., 2012; Ramirez and Kloepper, 2010) (Fig. 7).

$$Ca_5(PO_4)_3OH + 7H^+ + 5\ citrate \leftrightarrow 3H_2PO_4^{2-} + 5\ citrate\text{-}Ca^{2+} + H_2O \quad K=10^{37.9} \quad ...(13)$$

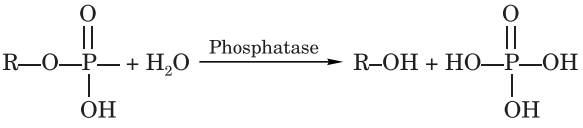
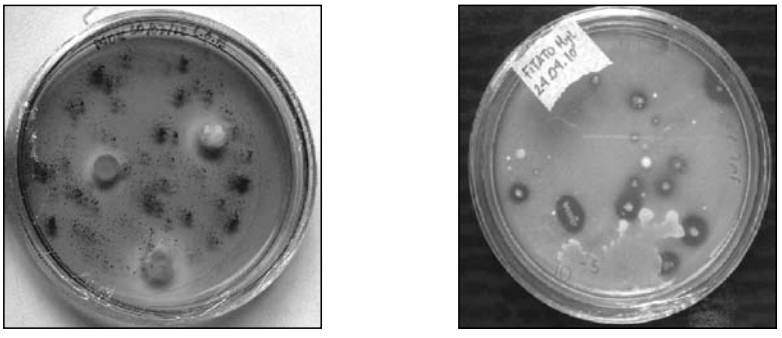


Fig. 6: Phosphatase enzyme breaks the ester bound and thus releases phosphate into the soil solution



(a) (b)

Fig. 7: Petri dishes contained culture media for isolation of RP solubilizing microorganisms (*a*) and microorganisms with phytate activity (*b*). Notice the halos around the most active microbial colonies in both media

Among the most effective bacterial PSM are species of the genera: *Pseudomonas* (Kim *et al*., 1998; Bar-Yoseph *et al*., 1999; Rosas *et al*., 2006), *Enterobacter* (Kim *et al*., 1998; Vasquez *et al*., 2000), *Bacillus* (Kim *et al*., 1998; Vasquez *et al*., 2000; Chen *et al*., 2006), *Burkholderia* (Song *et al*., 2008; Tao *et al*., 2008), *Serratia* (Chen *et al*., 2006; Hameeda, 2006), *Citrobacter* (Patel *et al*., 2008), *Xanthomonas* (Sharan *et al*., 2008), *Rhizobium* (Alikhani *et al*., 2006), *Azospirillum* (Rodriguez *et al*., 2004), *Lebsiella* (Chung *et al*., 2005). Effective fungal PSM belong to *Penicillium* (Reyes *et al*., 2001; Wakelin *et al*., 2004; Morales *et al*., 2007), *Aspergillus* (Vassilev *et al*., 1997; Vassileva *et al*., 1998; Whitelaw, 2000; Bojinova, 2008) and *Mortierella* (Osorio, 2003, Zhang *et al*., 2011; Osorio and Habte, 2013). Also, some yeasts and actinomycetes species have been reported as effective PSM (Caroline, 1994, Beauchamp and Hume, 1997, Atlas and Bartha, 1998; Hamdali *et al*., 2008).

Although bacteria have received great attention, several authors (Arora and Gaur, 1979; Kucey, 1983; Osorio and Habte, 2009) have indicated that fungi may be consistently more effective than bacteria in solubilizing Pi. It seems that after several subcultures bacteria PSM lose their ability to solubilize Pi compounds, while fungal subcultures retain this capacity (Whitelaw, 2000;

Rashid *et al.*, 2004). Moreover, the elongated growth of hyphae allow fungi to have a rapid and abundant contact onto the surface of RP particles (Bermanec *et al.*, 2012) and even inside RP particles (Fig. 8). However, Alam *et al*. (2002) indicate that the fungi can immobilize more Pi than bacteria.

Fig. 8: SEM photographs showing RP particles either: untreated (*a*) and treated with PSM. Notice the high degree of corrosion due to the organic acid attack on the RP surface (*b*), and the colonization of a RP particle by hyphae of a PSM (A. Zapata and N.W. Osorio, unpublished)

Different mechanisms have been proposed to explain the microbial RP solubilization:

- Production of organic acids (Bar-Yosef *et al.*, 1999; Hameeda *et al.*, 2006; Marschner, 2008)
- Proton excretion due to NH_4^+ assimilation by microorganisms (Whitelaw, 2000)
- Formation of calcium-Chelates at the surfaces of RP (Welch *et al*., 2002)

In addition, it has been reported that organic acids can compete with or desorb Pi ions on the surface of soil minerals (He and Zhu, 1998; Osorio and Habte, 2013).

Several authors have reported beneficial effects with the PSM inoculation on plant P uptake and grwoth of diverse plant species grown in soils of tropical, subtropical, and temperate zones (Table 5). The effects are higher on plant P uptake than in plant growth, there are several reasons that explain this: (*i*) most of these studies have been conducted with seedlings or plantlets that acumulate P in the first stages of growth, (*ii*) plant growth depends on other factors (water and other nutrient availability, light, etc.). In general, in temperate soils the increases with PSM on plant P uptake are higher than in tropical soils, likely due to the higher P fixation in tropical soils. However, this contrast of soil types and their influence can be also observed in the tropical zone. For instance, Osorio and Habte (2001) reported that the plant P uptake of seedlings of non-mycorrhizal leucaena increased by 14% with a PSM inoculation (*Mortierella* sp.) in a Hawaian Oxisol (medium P fixation); in a similar experiment established in a Mollisol (low P fixation) Osorio (2008)

reported an increase of 59% with the same PSM. The results reported by Dupponois *et al.* (2006) are higher (56–74) perhaps due to the lower P fixation expected in sandy soils. In the temperate soils the contrast in also clear, Wakelin *et al.* (2004) reported an increase in wheat P uptake of 34–76% in a sandy soil of Australia (low P fixation), while Whitelaw *et al.* (1997) registered an increase of only 8% in an Ultisol (persumably with high P fixation capacity).

On the other hand, the presence of AMF seems to have an important role in the magnitude of the plant response. For instance, the increase in plant P uptake by PSM inoculation raised from 14% in non-mycorrhizal leucaena to 40–73% with mycorrhizal leucaena (Osorio and Habte 2001; Osorio 2008; Londoño 2010) with the same PSM (*Mortierella* sp.) (Table 5). This synergism between AMF and PSM will be discussed below in more detail.

4.3. PSM for RP Bioacidulation

Phosphorus containing fertilizers have an important role in agriculture. Conventionally soluble Pi fertilizers are obtained from RP (Goenadi *et al.*, 2000). The PSM can be used in a biotechnological process aiming to improve RP agronomic effectiveness and reduces both production cost and environmental pollution in making soluble P fertilizers (Stewart and Howell, 2003; Smith and Moore, 2005; Khan *et al.*, 2007). The bioacidification of RP is a green, clean, and innovative alternative that might make attractive this material for agricultural use (Borges *et al.*, 2011). Bar-Yosef *et al.* (1999) proposed the use of a bacterial PSM to dissolve RP by acidification (gluconic acid) and thus produces a more soluble P fertilizer (superphosphate type).

It seems that under *in vitro* conditions PSM can dissolve as much as 40% of the RP in only 5–7 days (Osorio, 2008; Osorno, 2013). Among several factors that control the efficiency of RP bioacidification are RP type and particle size, RP amount in suspension, microbial composition of culture media, type of microorganisms (PSM), stirring conditions, temperature, pH, energy sources for PSM and incubation time (Cunningham and Kuiack, 1992; Narsian and Patel, 2000; Ates *et al.*, 2002; Adham, 2002; Haq and Iqbal, 2003; Nahas, 2007; Xiao *et al.*, 2008; Osorno, 2013).

As mentioned above, the major mechanism in PSM activity is the production of organic acids (*e.g.*, citric acid, oxalic acid) (Bar-Yosef *et al.*, 1999; Hameeda *et al.*, 2006; Marschner, 2008). It has been found that the production of these acids depends mainly on C and N sources (Madigan, 2004; Reyes *et al.*, 2006; Nahas, 2007).

We have found that under *in vitro* conditions *A. niger* and *Mortierella* sp. are more efficient in dissolving RP if C is supplied as glucose and N as NH_4^+ (Fig. 9*a*, *b*). Glucose seems to be the most easily C source for both fungi (Hameeda *et al.*, 2006; Sharan *et al.*, 2008; Nisha and Venkateswaran, 2011;

Table 5: Increase in plant P uptake and shoot dry weight (SDW) of diverse plant species by PSM inoculation in soil of tropical, subtropical, and temperate zones

PSM	*Soil/site*	*Plant*	*Increase* (%)		*Reference*(*s*)
			P uptake	*SDW*	
***Tropical zone*:**					
Arthrobotrys ollgospora	Sandy soil, Senegal	*Acacia holoserica*	56–74	—	Dupponois *et al.*, 2006
Mortierella sp.	Oxisol, Hawaii, RP added	Non-mycorrhizal *L. leucocephala*	14	19	Osorio and Habte, 2001
Mortierella sp.	Mollisol, Colombia	Non-mycorrhizal *L. leucocephala*	59	31	Osorio, 2008
Mortierella sp.	Oxisol, Hawaii, RP added	Mycorrizal *L. leucocephala*	73	28	Osorio and Habte, 2001
Mortierella sp.	Oxisol, Colombia, RP added	Mycorrizal *L. leucocephala*	33	24	Londoño, 2010
Mortierella sp.	Oxisol, Colombia, RP added	Mycorrizal *L. leucocephala*	40	15	Osorio and Habte, 2013
Mortierella sp.	Oxisol, Colombia	*Vigna unguiculata*	54	22	Ramirez *et al.*, 2012
Unknown	Acidic soil, Taiwan	*L. leucocephala*	20–24	—	Young *et al.*, 1990
***Temperate zone*:**					
P. radicum	Sand soil, Australia	*Triticum aestivum*	34 to 76	—	Wakelin *et al.*, 2004
P. radicum	Ultisol, Australia	*Triticum aestivum*	8	—	Whitelaw *et al.*, 1997
P. albidum	Volcanic soil	*Trifolium pratense*	—	38	Morales *et al.*, 2007
Aspergillus sp.	Turkey	*Fragaria ananassa*	—	114	Gunes *et al.*, 2009
A. awamori	Field soil	*Vigna radiata*	263	502	Jain *et al.*, 2012
E. aerogenes	Argentina	*Phaseolus vulgaris*	—	80	Collavino *et al.*, 2010
Enterobacter sp.	Spain	*Lactuca sativa*	—	34	Vassilev *et al.*, 2001
Enterobacter sp.	Calcareus soil Spain	*Medicago sativa*	125	—	Toro *et al.*, 1989
Mesorhizobium mediterraneum	Calcareus soil, Spain	*Cicer arietinum*	100	—	Peix *et al.*, 2001
P. thomii	Vermiculite-perlite subtrate	*Mentha piperita*	200	—	Cabello *et al.*, 2005
P. jessenii	Spain	*Cicer arietinum*	—	14	Valverde, 2006
Unknown	Sand-vermiculite	*Medicago sativa*	—	159	Piccini and Azcon, 1987

Osorno and Osorio, 2012). On the other hand, the excess of NH_4^+ causes an excess of positive charge in the cytoplasm, which is offset by increasing the H^+ pump into the external solution (Roos and Luckner, 1984; Illmer and Schinner, 1995; Slayman *et al*., 1990; Cooke and Whipps, 1993). Another mechanism to increase negative charge in the cytoplasm consists in diverting some organic anions (*e.g*., citrate) from the Krebs cycle to it, which although decreases microbial growth can improves RP bioacidification (Habte and Osorio, 2013; Osorno, 2013). Conversely, excessive NO_3^- uptake by the fungi is compensated by the release of HCO_3^- or OH^- to the external medium, which prevents RP dissolution.Similar results have been widely published by several authors (Nahas *et al*., 1996; Kara and Bozdemir, 1998; Reyes *et al*., 1999).

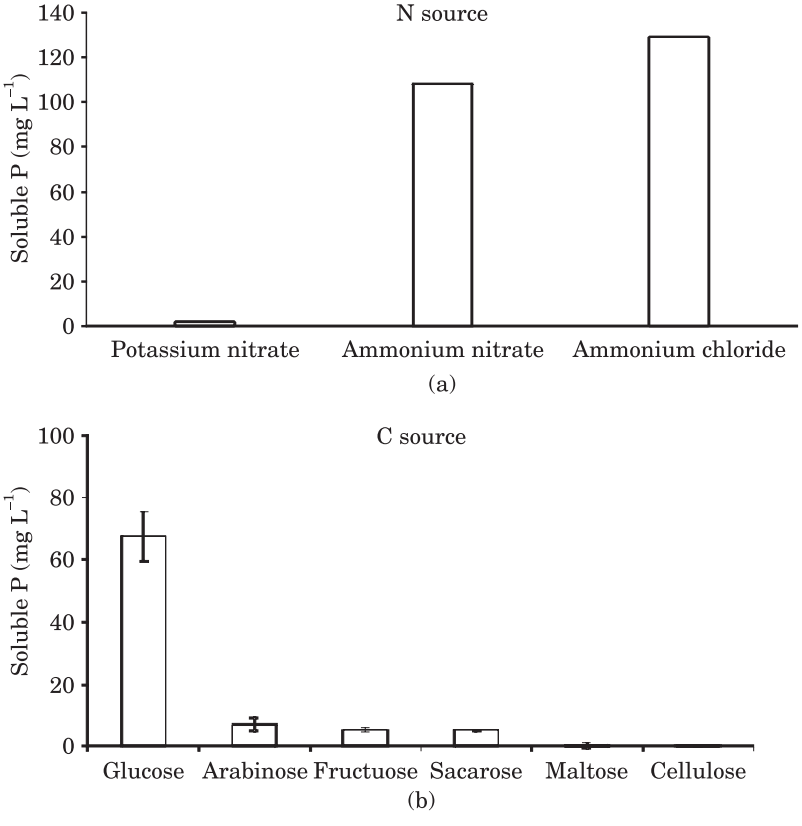


Fig. 9: Effect of C and N source on the ability of a fungus P solubilizer for increasing the concentration of soluble P by dissolving RP. *Sources*: Habte and Osorio (2013) and Osorno (2013)

Currently, we are investigating other factors that seem to be important (glucose and ammonium concentration, type and particle size, type of microorganisms, incubation time, among others).

4.4. Synergistic Effects of AMF and PSM to Enhance RP Effectiveness

It has been shown that the dual inoculation with AMF and PSM can increase the RP effectiveness beyond the effect of the AMF inoculation alone. Osorio and Habte (2013) evaluated the effects of single and dual inoculation with the AMF *G. fasciculatum* and the PSM *Mortierella* sp.on plant P uptake and growth of leucaena grown in a Colombia Oxisol at Carimagua. The addition of RP increased slighty the plant growth of leucaena seedlings; however, the effect of RP addition was significantly higher when *G. fasciculatum* (AMF+) was inoculated and even higher when both microorganisms (AMF+PSM+) were concomitantly coinoculated (Fig. 10a). The results were higher with at rate of 1.2 g of RP per kg of soil. At this level the AMF inoculation (AMF+) increased the shoot dry weitgt by 2.8-times and the dual inoculation (AMF+PSM+) by 3.2-times over the uninoculated control. The P use efficiency of non-mycorrhizal leucaena was only 0.33, but it was increased by 11-times with the mycorrhizal inoculation and by 13-times with the dual inoculation. Comparable results were obtained by Londoño (2010) in a similar experiment with leucaena grown in a Colombian Oxisol at Santander de Quilichao (Fig. 10*b*). In this case, at the RP addition rate of 1.2 g kg^{-1} the increase in plant P uptaje was 6.6 with AMF alone (AMF+) and 8.2 with both fungi (AMF+PSM+).

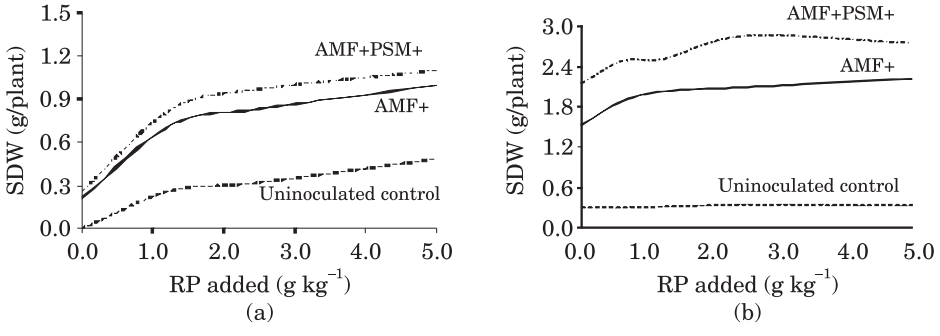


Fig. 10: Shoot dry weight (SDW) of *L. leucocephala* as a function of the RP level added and the inoculation with *G. fasciculatum* (AMF+) and the dual inoculation with *G. fasciculatum* and *Mortierella* sp. (AMF+PSM+) in two Colombiana Oxisols. *Sources*: (*a*) Osorio and Habte (2013) and (*b*) Londoño (2010)

These synergistc effects are associated to the complementary roles of each type of microorganism. This is, the PSM dissolve RP releasing thus $H_2PO_4^-$ ions into the soil solution, which are absorbed by the mycorrhizal hyphae that then transfer them into the plant roots, avoinding the P refixation by soil minerals.

5. CONCLUSIONS

Soil phosphate is a critical factor for plant nutrtion and growth in tropical soils; this can be overcome by use of P fertilizers. However, there are some limitations: soluble P fertilizers have low efficiency due to the strong P fixation that many of these soils and high rates of addition are required. Insoluble P fertilizers as rock phosphates have low effectiveness and acidulation is recomended, which increases cost production. The biotechnological alternative of using arbuscular mycorrhizal fungi and P solubilizing microorganisms can increase the effectiveness of P fertilizers in tropical agriculture. In addition, PSM can be used to bioacidify RP and thus produce more soluble fertilizers. Fortunatelly, there are comercial formulations of both types of microorganisms, which are available and are currently being used for farmers in many countries.

6. ACKNOWLEDGEMENTS

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