

Plant availability of isotopically exchangeable and isotopically nonexchangeable phosphate in soils

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Abstract

Isotopically exchangeable P (IEP) is usually considered to be completely plant-available and the major source of P for plant uptake. The aim of the present study is to test whether plants can, besides IEP, also use non-IEP and if part of the IEP has an equilibrium concentration in soil solution which is below the minimum concentration, C_{Lmin} , and can therefore not be taken up by plants. A pot experiment was carried out with maize for two years on two soils, an acid sandy and a neutral loamy soil, either without P fertilizer or fertilized with ten P sources of different solubility. Throughout both years of the study, pots were kept moist either without plants or planted twice with maize (*Zea mays* L., cv. Athletico). At the end of the experiment, plant P uptake, P concentration in the soil solution (C_L), and P accessible to isotopic exchange within 5 d (E_{5d}) were measured. Plant growth decreased the E_{5d} which was about equal to P uptake by maize for most treatments in the acid soil. But for some treatments, *i.e.*, five in the acid and eight in the neutral soil, P uptake was up to 50% larger than the decrease of E_{5d} , indicating that plants had, besides IEP, also used P from non-IEP sources. At adequate P supply, both soils had an E_{5d} of about 100 mg P (kg soil)⁻¹, but about 30 to 40 mg kg⁻¹ of this IEP had an equilibrium P concentration in the soil solution below C_{Lmin} of 0.1 $\mu\text{mol L}^{-1}$ at which P would actually not be plant-available. This study shows that plants take up P mainly from IEP, but not the whole IEP is plant-available. Furthermore, plants may also use P from non-IEP sources.

Key words: minimum P concentration (C_{Lmin}) / P in soil solution / recycled P products / P uptake

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1 Introduction

Isotopically exchangeable phosphorus (IEP) is the soil phosphorus (P) that exchanges more or less rapidly with the P in the soil solution. Together with P in the soil solution, IEP is the most likely available P source for plant uptake (Hamon et al., 2002). Because plants take up P directly from the soil solution (Barber, 1995), IEP seems to be the immediate source of P that replenishes the P in soil solution (Morel and Plenchette, 1994). However, part of the IEP may not be available to plants if its equilibrium concentration is equal or lower than the minimum concentration (C_{Lmin}) at which no net P uptake by plants will occur. This C_{Lmin} for P is about 0.2 $\mu\text{mol L}^{-1}$ for maize (Barber, 1995; Jungk et al., 1990; Bhadoria et al., 2004), 0.1 $\mu\text{mol L}^{-1}$ for soybean, oil seed rape, and sugar beet (Barber, 1995; Buhse, 1992; Jungk et al., 1990), and 0.01 $\mu\text{mol L}^{-1}$ for ryegrass (Breeze et al. 1984). This means that the portion of IEP or other compounds whose equilibrium concentration is below the values mentioned, *i.e.*, below 0.2–0.1 $\mu\text{mol L}^{-1}$, may actually not be plant-available.

When adding water-soluble phosphate fertilizer to the soil, *e.g.*, triple superphosphate (TSP), it dissolves in the soil solution followed by sorption processes which involve surface reactions and diffusion into the P-sorbing soil particles (Barrow, 2008) as well as precipitation (Sample et al., 1980). Thus, part of the P penetrates into pools that are not in a

rapid exchange with the P in soil solution (Hamon et al., 2002). The latter means that some part of P added to the soil will not readily be available to plants and not being isotopically exchangeable in a short time. The precipitation of P will depend on the P concentration in soil solution and the activity product of P and the relevant metals (*e.g.*, Ca, Fe, Al) in solution (Hamon et al., 2002).

In contrast to water-soluble P compounds a sparingly water-soluble P fertilizer, *e.g.*, phosphate rock (PR) or magnesium ammonium phosphate (called MAP in this paper), will become part of the IEP only if it dissolves in the soil solution because ions of nondissolved particles do not exchange with ions of the soil solution (Machold, 1963; Russell et al., 1954). Thus, the effect of a P fertilizer on IEP depends on the reactivity of the P products but also on soil conditions such pH and concentration of ions that may react with P (Chien and Menon, 1995).

The amount of soil P that exchanges with labeled P increases with time (Barrow and Shaw, 1975b). Therefore, the IEP of a soil is not a fixed amount but the time allowed for the exchange reaction to take place has always to be considered. However, a large proportion of the exchange reactions already occur in the first few days (Russell et al., 1954; Barber, 1995; Machold, 1962; Morel and Plenchette, 1994).



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Root activity, either through P uptake or exudation of H⁺ or organic compounds, may cause the dissolution of P compounds which had not been dissolved in the soil before (Gahoonia et al., 1992; Hinsinger, 2001; Neumann and Römheld, 1999). In a similar way, Ca accumulation or depletion due to Ca uptake affects the solubility of P in the rhizosphere (Devau et al., 2008). Furthermore, a reduction of the P concentration in the soil solution caused by plant P uptake will enhance the dissolution of formerly undissolved P fertilizers. Because of these different root actions P taken up by plants may originate not only from IEP but also from other sources.

The objectives of this research were (1) to assess whether plants, besides IEP, also use P from originally non-IEP sources, and (2) to estimate the amount of IEP that is below C_{Lmin} and therefore not plant-available.

2 Material and methods

2.1 Soils

The study was conducted with an acid sandy soil (pH (CaCl₂) 4.7) and a neutral loamy soil (pH (CaCl₂) 6.6). The sandy soil was collected from a long-time fallow site, and the loamy soil was under crop rotation. Soil surface samples (0–30 cm) were taken, air-dried, and sieved to pass 2 mm before the start of the experiment. The main soil characteristics are shown in Tab. 1. Plant-available P was extracted with calcium-acetate-lactate (CAL-P) (Schüller, 1969) and the measured values of 20–25 mg P kg⁻¹ indicate a low supply level. Organic matter (OM) was analyzed with the Walkley and Black oxidation procedure (Nelson and Sommers, 1982). Soil pH was measured in 0.01M CaCl₂ (1 : 2.5), and texture by means of the hydrometer method (Day, 1965).

2.2 Phosphorus compounds

In order to have P sources with different solubility and plant availability, the soils were fertilized with ten different P compounds, eight P products from recycling processes, and two commercial P fertilizers as references (triple superphosphate [TSP] and phosphate rock [PR], the latter provided by SeNa-Pro GmbH and imported from Syria). The recycled P products included materials obtained by chemical precipitation or by thermal processes. The chemically precipitated materials were: three magnesium ammonium phosphates (MAP) obtained from different sewage-sludge-treatment plants (MAP-Sb, MAP-Gf, and MAP-St), and a calcium phosphate precipitated from municipal-waste water (Ca-P). The ther-

mally obtained products were: an alkali sinter phosphate made from meat-and-bone meal (Sinter-P), a cupola furnace slag (Cupola slag) made from sewage sludge, a heavy metal-depleted sewage-sludge ash (Sl-ash), and a meat-and-bone-meal ash (MB-meal ash).

The main characteristics of the recycled P products and commercial P fertilizers are listed in Tab. 2. Total P was determined after digestion with H₂SO₄ and HNO₃ and measured colorimetrically. Water-soluble P was determined by shaking 5 g of material in 250 mL of water for 2 h, and measured colorimetrically. Citric acid-soluble P was extracted by shaking 2.5 g of material with 250 mL of 2% citric acid for 2 h and measured colorimetrically (Ostmann, 1995). For more details of the production process to obtain the different compounds see Cabeza et al. (2011).

2.3 Experimental setup

The greenhouse experiment was conducted with 5.5 kg of soil in 6-L pots with two maize plants for 2 years. In the first year, each P product was applied at a rate of 60 mg P (kg soil)⁻¹. There was also an unfertilized control. All treatments were incubated for 3 weeks before maize sowing. All P products were finely ground to pass through a 500- μ m mesh and mixed thoroughly with the whole soil in order to prevent concentrated spots of fertilizer.

Furthermore, for each treatment one pot was kept moist without plants for the duration of the experiment. The basal fertilization applied to each pot, including those without plants, in the first year consisted of 1 g K (K₂SO₄), 0.2 g Mg (MgSO₄ · 7 H₂O) and 0.5 g N (NH₄NO₃). In the second year, the soil of each pot was taken out and mixed thoroughly, but no P fertilizer was added. Two further applications of N were carried out as Ca(NO₃)₂ in doses of 0.3 g per pot per application in the first and second year. Pots were irrigated daily with demineralized water during the experiment to maintain a water content of 75% water-holding capacity; any leached water was returned to the pots. Each treatment with plants was replicated four times and for soils without plants, only one pot was used. Thus, the plant analyses and the determinations of P uptake consisted of four replicates. However, P concentration in soil solution as well as isotopically exchangeable P in pots with plants was determined from three randomly selected pots per treatment. On the other hand, in the pots without plants, soil samples for isotopically exchangeable P were taken from three different places from the same pot to obtain three replicates.

Table 1: Characteristics of the two soils used in the pot experiment.

Soil	Clay	Silt	Sand	Organic matter	pH(CaCl ₂) (1 : 2.5)	CAL-extractable P [§]
						/ %
Acid sandy	2.1	5.3	92.6	5.3	4.7	24.2
Neutral loamy	25.0	65.5	9.5	2.8	6.6	21.0

§ CAL, calcium acetate lactate solution.

Table 2: Phosphorus concentration and P solubility in the fertilizers and recycled P products.

Material	Total P	Proportion of total P soluble in	
		*water	†2% citric acid
Reference fertilizers		/ %	
Triple superphosphate (TSP)	20.1	90	–
Phosphate rock (PR)	11.8	< 0.01	17.0
Obtained by chemical processes			
Ca-P	11.1	4.1	48.6
MAP-Sb	11.0	1.1	51.0
MAP-Gf	9.6	0.8	47.0
MAP-St	11.8	1.9	43.6
Obtained by thermal processes			
Sinter-P	11.3	0.3	34.5
Cupola slag	2.9	n.d.	n.d.
Sl-ash	7.8	6.4	31.4
MB meal ash	16.4	0.1	23.8

* 5 g of material was extracted with 250 mL of water.

† 2.5 g of material was extracted with 250 mL of 2% citric acid.

n.d. not determined.

2.4 Soil and plant-material sampling

Before starting with the experiment, soils were analyzed for pH, organic matter, extractable calcium-acetate-lactate P (CAL-P), and texture (Tab. 1). Soil samples for P concentration in soil solution and isotopically exchangeable P were taken at the end of the second year from pots with and without plants and were air-dried and sieved to 500 μm . The maize plants were harvested at the beginning of flowering, at day 80 and day 70 after sowing for the first and second year, respectively. Plants were dried at 65°C until a constant weight was established.

2.5 Analytical procedure

2.5.1 Soil

The soil solution was obtained from the moist soil of the pots with and without plants. For this, a modified displacement procedure according to Adams (1974) was used. About 300 g of moist soil from the pots were put directly into cylinders of 30 cm height with a hole at the bottom. The soil column was covered by a 2 cm layer of sand, and water was added at the top drop-wise at about 5–10 mL h^{-1} . The first 20 mL of solution coming out at the bottom after several hours is the pure displaced soil solution as was controlled by a nonadsorbed reagent (KSCN) added to the water. Phosphorus concentration in the solution was measured colorimetrically according to Murphy and Riley (1962).

The isotopically exchangeable P after 5 d (E_{5d}) was determined in soil samples from the pots with and without plants.

For this, soil samples were air-dried and sieved to 500 μm and then 2 g of each treatment were weighed into 50-mL plastic tubes. Thereafter, 8 mL of distilled water and 7 mL of a carrier-free solution of $\text{H}_3^{33}\text{PO}_4$ with a radioactivity of about 0.04 to 0.06 MBq were added. The samples were shaken for 5 d. After shaking, samples were centrifuged at 4000 rpm. In the supernatant solution the ratio of ^{33}P and ^{31}P was determined. The P-ion concentration is often very low, and its determination would be very insecure. Thus, the solution was concentrated by a factor of five, similar to Pypers et al. (2006), using an anion-exchange resin. Ten-milliliter aliquots of supernatant were placed in 13-mL tubes with an anion-exchange resin strip (9 mm \times 15.5 mm, anion resin PC Acid 35, PCA Company, Germany) and shaken for 24 h. Then, the P on the resin strips was removed with 2 mL 0.5 M HCl by shaking for 2 h. From this solution ^{31}P and ^{33}P were determined. The P-ion concentration was colorimetrically measured according to Murphy and Riley (1962). Radioactivity of P in the HCl extract was measured with a liquid scintillation analyzer (Perkin Elmer TRICARB 2800 TR). The E_{5d} was calculated based on the relationship (Owusu-Bennoah et al., 2002):

$$E_{5d} = \frac{C_L}{r_{5d}} R,$$

where E_{5d} is the isotopically exchangeable P in mg per kg of soil after 5 days of shaking, C_L/r_{5d} is the ratio of the P concentration in the solution (C_L , mg L^{-1}) over the radioactivity in the solution remaining after 5 d of shaking (r_{5d} , Bq L^{-1}) and R is the total radioactivity introduced in Bq (kg soil) $^{-1}$. The method is based on the assumption that ^{31}P and ^{33}P have the same chemical behavior and that the relationship between ^{31}P and ^{33}P is maintained on anion-exchange-resin strips as it was found in the supernatant solution. The reaction of a labeled P with soil P is a dynamic time-dependent process. However, based on several findings (Russell et al., 1954; Machold, 1962; Barber, 1995; Pypers et al., 2006) it can be assumed that the IEP after 5 d of shaking includes the isotopically exchangeable P fraction relevant for P uptake of plants.

2.5.2 Plant material

Plant material was dried at 65°C until constant weight. Then, subsamples of plant dry matter were digested in concentrated HNO_3 at 180°C and the P concentration herein was measured colorimetrically with the molybdenum-vanadate method (Scheffer and Pajenkamp, 1952). Total P uptake over the 2 years was calculated from shoot dry matter and shoot P concentration.

2.6 Data analysis and experimental design

Statistical analysis was performed using Statistica 9.0 (StatSoft, Inc. Tulsa, USA). The experiment had a completely randomized design, with 11 treatments for soil with plants and 11 without plants. The fertilizers were considered as main factors, according to one-way ANOVA (Quinn and Keough, 2002). The ANOVA test was followed by a Tukey test at the 5% level of significance to separate the means. To compare

the P uptake by maize plants and E_{5d} at the same treatment the Student's *t*-test was used. Freundlich equation was used to fit the relationship between E_{5d} and P concentration in the soil solution (C_L) as suggested by Barrow (2008).

3 Results

Table 2 shows some properties of the fertilizers used. The total P concentration varied from 20.1% (TSP) to 2.9% (Cupola slag). The solubility in water varied from 90% (TSP) to less than 0.01% (PR). The solubility of the MAPs (magnesium ammonium phosphates) in water varied by a factor of 2 which clearly shows that even though all three compounds (MAP-Sb, MAP-Gf, and MAP-St) are named MAP, their prop-

erties differ widely. This is because they actually are no pure MAP but a mixture of MAP with other P compounds that precipitate during the treatment of sewage sludge. They differ because the composition of sewage sludge differs as well as the procedure to precipitate the MAP.

Table 3 shows the E_{5d} for soils without plants (E_{5d} Soil) and with plants (E_{5d} Plants) as well as the P uptake. The addition of P products to the soils without plants significantly increased the E_{5d} in most treatments in relation to the unfertilized control (P-0) in both soils. The increase of E_{5d} after the addition of TSP in the acid sandy soil was 53 mg P kg⁻¹ and in the neutral loamy soil 42 mg P kg⁻¹ showing that 2 years after its application 90% and 70% of the water-soluble TSP,

Table 3: Isotopically exchangeable P without plants (E_{5d} Soil) and with plants (E_{5d} Plants), change of isotopically exchangeable P (ΔE_{5d}) due to plant growth, P uptake (PU) by maize, and statistical significance for the difference between ΔE_{5d} and PU.

Treatments	E_{5d} Soil	E_{5d} Plants	ΔE_{5d}	PU	Difference (PU – ΔE_{5d})	P value
	mg P / (kg soil) ⁻¹					
Acid sandy soil						
P-0	50.6a§	26.1a	24.5abc	26.7a	2.2	0.171
TSP	103.8e	57.0cd	46.7de	40.4de	-6.3	<0.05
PR	74.0c	40.0b	34.0bcd	32.6bc	-1.4	0.320
Products obtained by chemical methods						
Ca-P	90.8d	55.0cd	35.8cd	38.6de	2.8	<0.05
MAP-Sb	87.3d	61.6d	25.7abc	42.5e	16.8	<0.05
MAP-Gf	87.6d	59.2cd	28.4bc	36.6cd	8.2	<0.01
MAP-St	102.3e	52.4c	50.0e	37.9de	-12.1	<0.001
Products obtained by thermal methods						
Sinter-P	89.5d	55.3cd	34.2bcd	38.5de	4.2	0.163
Cupola slag	70.4c	56.5cd	13.8a	30.0ab	16.1	<0.05
Sl-slash	60.1ab	38.3b	21.7ab	29.0ab	7.2	<0.01
MB meal ash	64.1bc	37.4b	26.7bc	27.8ab	1.1	0.419
Neutral loamy soil						
P-0	57.4a	42.2a	15.2ab	19.3a	4.1	<0.001
TSP	99.0f	62.7e	36.3de	41.6e	5.3	<0.01
PR	61.0ab	45.1ab	15.9ab	20.3ab	4.3	<0.05
Products obtained by chemical methods						
Ca-P	62.7ab	50.4bc	12.3a	25.2abc	12.9	<0.001
MAP-Sb	101.8f	61.3e	40.5e	39.6de	-0.9	0.662
MAP-Gf	83.6c	60.5de	23.1bc	34.6cde	11.5	0.199
MAP-St	92.6e	63.2e	29.4cd	40.7e	11.3	<0.05
Products obtained by thermal methods						
Sinter-P	86.0cd	55.2cd	30.8cd	33.9cde	3.0	0.093
Cupola slag	90.5de	59.9de	30.6cd	45.8e	15.2	<0.001
Sl-ash	66.8b	49.6b	17.2ab	29.7bcd	12.6	<0.001
MB meal ash	65.3b	49.7bc	15.6ab	25.9abc	10.3	<0.01

§ Different letters denote significant differences between treatments (Tukey, $P < 5\%$) and P values indicate differences between ΔE_{5d} and PU at the same treatment (Student's *t* test, $P < 5\%$).

respectively, were accessible to isotopic exchange within 5 d. Phosphate rock (PR) and Ca-P increased the E_{5d} in the acid soil only, although less than TSP, and were ineffective in the neutral soil. The MAP products significantly increased the IEP in both soils, with MAP-St in the acid and MAP-Sb in the neutral soil reaching levels equal to those reached by TSP. The thermal products Sinter-P and Cupola slag increased the E_{5d} in both soils. Sinter-P was very effective in both soils while Cupola slag was effective mostly in the neutral soil. The ashes (SI-ash and MB-meal ash) only slightly increased the E_{5d} in both the acid and the neutral soil. Plant growth decreased the E_{5d} in all treatments, *i.e.*, in the unfertilized (P-0) as well as in the fertilized soils.

In order to study to which extent P uptake may originate from IEP or from other sources, the decrease of IEP (ΔE_{5d}) due to plant growth was compared with P uptake. If plants acquired P only from the IEP, ΔE_{5d} and P uptake should be similar and the difference between P uptake and ΔE_{5d} would be close to

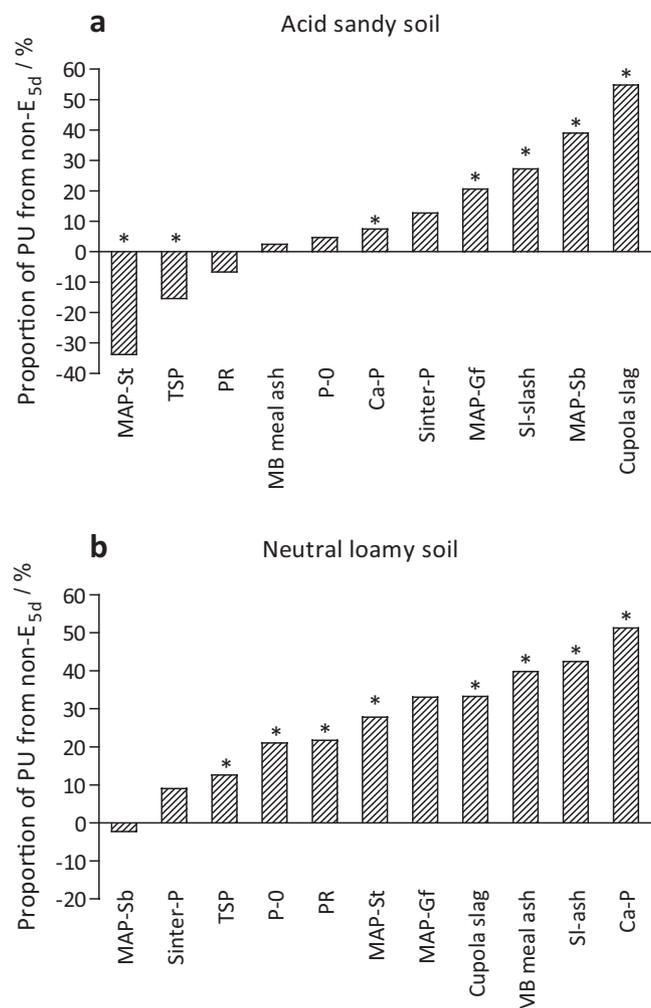


Figure 1: Proportion of P uptake (PU) of maize coming from P sources not accessible to isotopic exchange within 5 d (E_{5d}) in the acid sandy and neutral loamy soil. Phosphorus fertilizers were arranged according to the proportion of PU coming from P sources not accessible to isotopic exchange within 5 d (E_{5d}). * indicates that the proportion is significantly ($P = 5\%$) different from zero.

zero. It can be seen (Tab. 3) that in some cases the difference was not significantly different from zero, like for P-0, PR, Sinter-P and MB-meal-ash treatments in the acid soil or MAP-Sb, MAP-Gf, and Sinter-P in the neutral soil.

Figure 1 shows the proportion of P uptake coming from P fractions not accessible to isotopic exchange within 5 d. In the acid soil (Fig. 1a), in the unfertilized (P-0) as well as in four additional treatments P uptake from non-IEP was not different from zero, *i.e.*, PU was equal to ΔE_{5d} , meaning that P taken up by plants came from the IEP only. But in four cases, P uptake from P sources not exchangeable within 5 d was significant, reaching up to 50% of PU in Cupola-slag treatment. In the MAP-St and TSP treatments the proportion of PU coming from non-IEP was negative, meaning that PU was smaller than the decrease of E_{5d} caused by plant growth. In the neutral loamy soil (Fig. 1b) in most cases, except three, there was a significant contribution of the non- E_{5d} fraction to PU reaching up to 40%–50% in the ashes and the Ca-P.

Figures 2 and 3 show the relationships between E_{5d} and the P concentration in the soil solution (C_L) of the moist soil directly after the last harvest of maize for the acid and neutral soil, respectively. In both soils, the data of the planted and unplanted soils fit in the same curve. The relationship was described by a Freundlich isotherm:

$$E_{5d} = aC_L^b,$$

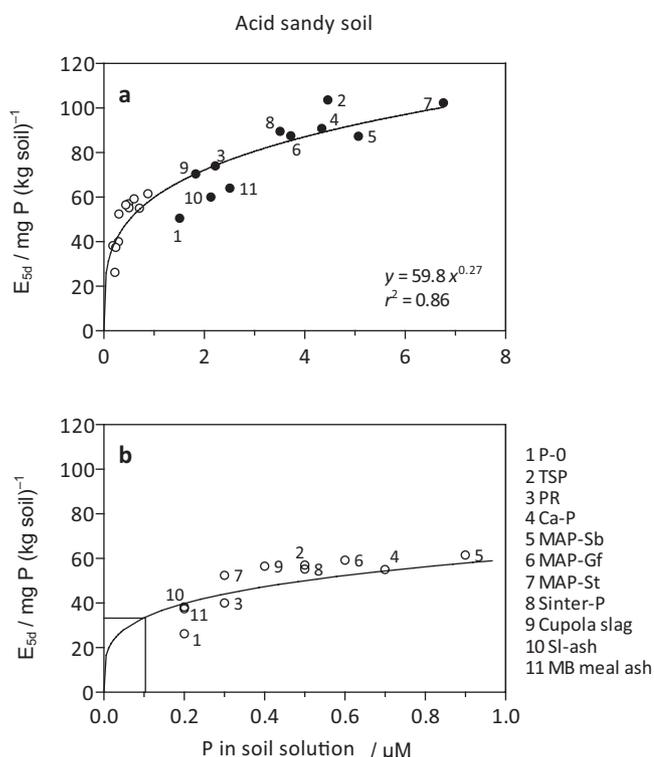


Figure 2: Isotopically exchangeable P (E_{5d}) as a function of the P concentration in the soil solution (C_L) in the acid sandy soil. Closed symbols stand for soils without and open symbols for soils with plants. Numbers indicate the treatment as listed on the right side. The data were fitted with the Freundlich equation shown in the figure. a) shows the whole and b) the low-concentration range.

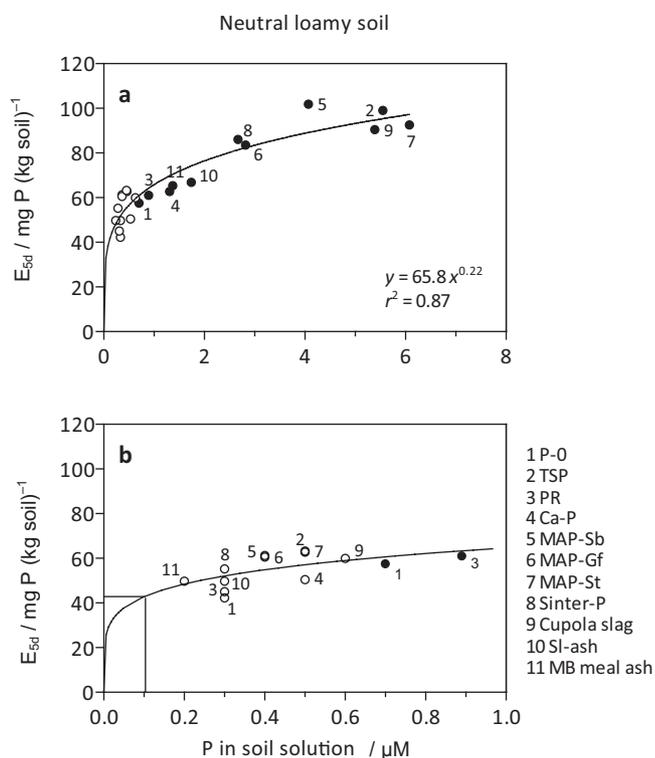


Figure 3: Isotopically exchangeable P (E_{5d}) as a function of the P concentration in the soil solution (C_L) in the neutral loamy soil. Closed symbols stand for soils without and open symbols for soils with plants. Numbers indicate the treatment as listed on the right side. The data were fitted with the Freundlich equation shown in the figure. a) shows the whole and b) the low-concentration range.

in which C_L is the concentration of P in the soil solution, and a and b are coefficients.

The extrapolation of the curves down to the origin allows deriving the E_{5d} at C_{Lmin} (P concentration below which no net P uptake by plants occurs). Assuming a C_{Lmin} for P of $0.1 \mu\text{mol L}^{-1}$ (see section 1) results in an IEP of about 30 mg kg^{-1} for the acid sandy soil and more than 40 mg kg^{-1} for the neutral loamy soil which would not be available to plants.

4 Discussion

Two years after applying water-soluble TSP about 90% of it stayed isotopically exchangeable within 5 d of shaking in the acid soil and 70% in the neutral soil (Tab. 3). This was more than the value of about 45% found by Barrow and Shaw (1975b) after only 220 d of incubation and 5 d of shaking. On the other hand, Javid and Rowell (2010) using 13 high-pH soils (pH 7.3–8.7) recovered as IEP less than 5%, and sometimes even less than 0.003% of the P applied to the soils 1 year before and stored at 25°C . This shows that the degree of recovery of P added to a soil as IEP depends largely on the mineralogy and chemical conditions of the soils.

The application of fertilizers other than TSP caused in most cases a smaller increase of the E_{5d} value than the water-soluble TSP. This indicates that not all the P of the respective fer-

tilizers dissolved in the soil but most likely stayed in its original form. It cannot be excluded that the fertilizer P did dissolve in soil solution but may have precipitated again due to other compounds in the fertilizer as, e.g., Fe or Ca salts. The MAPs, even though considered as of low solubility (Bhuiyan et al., 2007; Weast, 1970) should have dissolved completely because pure MAP has a solubility of $937 \mu\text{mol L}^{-1}$, which is much higher than the P equilibrium concentration in the soil solution of $6\text{--}7 \mu\text{mol L}^{-1}$ (Fig. 2 and Fig. 3). However, MAP products were not completely dissolved which shows that besides magnesium ammonium phosphate they also contained other P compounds of lower solubility than pure MAP, as suggested by the Ca and Fe concentrations (8.6% and 5.5%, respectively) found in MAP-Gf. The other MAPs had a Ca concentration of around 1% and Fe was below 1%.

The addition of PR and Ca-P increased the E_{5d} value only in the acid soil because the low soil pH and the lower Ca concentration favor the dissolution of Ca phosphates in acid soil conditions (Chien and Menon, 1995; Kanabo and Gilkes, 1987). Accordingly, in the neutral soil with a higher pH and Ca concentration, no dissolution or, as mentioned above, a dissolution but reprecipitation of these products occurred, i.e., no increase of the E_{5d} value.

Sinter-P increased the E_{5d} value in both soils, while Cupola slag only in the neutral soil. These differences may be attributed to the Al and Fe impurities that Cupola slag contains (Scheidig et al., 2009), which are of low solubility in acid conditions (Havlin et al., 2005). For ashes (SI-ash and MB-meal ash), the E_{5d} value was low in both soils, probably because of the high amount of Ca that they contain in addition to the Al and Fe impurities, which in the case of SI-ash is about 12% (Al + Fe; Data not shown).

In order to assess whether plants, besides IEP, also use P from originally non-IEP sources, we compared the change of E_{5d} (ΔE_{5d}) due to plant growth with the P uptake of the plants (Tab. 3). In the acid soil, at P-0 P uptake was almost equal to ΔE_{5d} which would indicate that plants used E_{5d} as sole source of P. This finding agrees with Morel and Plenchette (1994) and Frossard et al. (1994) who found that plants only absorb P from the IEP pool. But in other cases, e.g., MAP-Sb or Coupola slag, the P uptake was much larger than ΔE_{5d} indicating that besides E_{5d} plants used other P sources as well. These sources could be: (1) isotopically exchangeable P but of slow accessibility (not reached within 5 d of shaking as used in our experiment; Barrow and Shaw, 1975a; Barrow, 1991) or (2) precipitated P compounds or nonsolved P fertilizers which, according to Machold (1963), are not accessible to isotopic exchange. However, the activity of the root may have dissolved them making them accessible for plant uptake. A transfer from slowly to faster accessible positions due to plant uptake would be strongest in the P-0 treatment because at low P supply more P would be removed from the positions of slow access. This is supported by Pypers et al. (2006) who found that cowpea used non-IEP only at low P supply but not when P supply was adequate.

In the acid soil, a transfer of P from positions of slow to positions of faster access during the time of the experiment did

not happen since at P-0 ΔE_{5d} and P uptake were equal (Tab. 3) and therefore the portion of P uptake coming from non-IEP was equal to zero (Fig. 1a). In several other treatments of the acid soil the contribution of non-IEP was also not significant. However, there were treatments, mainly Cou-pola slag, MAP-Sb, and SI-ash, where plants also used significant amounts from P sources not accessible to isotopic exchange as indicated by P uptake being larger than ΔE_{5d} (Tab. 3). In these treatments, only a small portion of the applied fertilizer dissolved in the soil without plants, as indicated by the smaller increase of E_{5d} as compared to TSP (Tab. 3). The undissolved portion was not accessible to isotopic exchange during the two years of the experiment but part of this non-IEP was used by the plants.

In the neutral soil, even though most of PU came from the E_{5d} , a majority of the treatments also showed a significant P uptake from non-IEP (Fig. 1b). In this soil at P-0, in contrast to the acid soil, plants absorbed up to 20% from the P fraction that did not exchange with ^{33}P within 5 d (Fig. 1b). This P could have come from the P fraction that was only slowly accessible (see above) or from P compounds not accessible to isotopic exchange, such as precipitated Ca phosphates (Machold, 1962, 1963). In other treatments, the proportion of P uptake coming from non-IEP was larger than the 20% of the P-0 indicating that at least the portion of PU in excess of the 20% came from P fractions not accessible at all to isotopic exchange. This was most evident with Ca-P which apparently remained undissolved in the soil without plants, *i.e.*, no increase of the E_{5d} , but it increased P uptake (Tab. 3).

The P uptake from the non-IEP occurred mainly when the fertilizer only partly dissolved in the soil or did not dissolve at all (change of E_{5d} about nil). There was a general trend that the less a fertilizer dissolved in soil the higher the uptake from the non-IEP. Here, the roots may have dissolved part of this non-dissolved product and then absorbed it which thereby appears as P uptake from the non-IEP.

Other studies on the possible use of non-IEP by plants have been based on the comparison of E and L values. Frossard et al. (1994) found that the E value (based on the specific activity, SA, of P in soil solution, the same as the E_{5d} in our experiment) and the L value (based on the SA in the plant) of *Agrostis capillaris* L. growing in ten very different soils for 13 weeks were about equal, *i.e.*, $L/E \approx 1$, indicating that IEP is the available P for these species. In contrast, Vu et al. (2010) found that at low P supply wheat and chickpea had an L/E ratio of 2.0 to 2.5 in two soils but below 1 in another soil while canola always showed an L/E ratio of 1 or less. At adequate P supply in all cases L/E was around 1. Braum and Helmke (1995) found a three times higher L value for white lupin than for soybean. An L/E ratio larger than 1 indicates that plants also used P sources that were not accessible to isotopic exchange. This is usually attributed to the mobilization or solubilization of soil P by root exudates which are well known for legumes such as cowpea (Pypers et al., 2006) or white lupin (Dinkelaker et al., 1989). Whether this mobilization occurs depends, as seen above, on plant species, soil type or P supply of the soil. In our experimental approach a 50% uptake from non-IEP sources like in the Ca-P treatment in the

neutral soil (Fig. 1b) would be equivalent to an L/E ratio of 2. This uptake from non-IEP sources may be due to exudates of maize roots that were able to solubilize the nondissolved fertilizer residues (Gaume et al., 2001). But it could also be due to the strong decrease of the P concentration in soil solution (C_L , see Figs. 2 and 3) facilitating the dissolution of P compounds with a low solubility product.

Figures 2 and 3 show for each soil that the E_{5d} value as a function of C_L fits a single curve that can be described by a Freundlich equation which was extrapolated down to the origin assuming that at no IEP C_L would also be zero. All the data from P fertilizers of different solubility (Tab. 2), with and without plants, fit to the same curve which indicates that the fertilizers had reached equilibrium with the IEP, which would finally control C_L . In relation to plant growth and uptake kinetics, C_L covered a wide range. In some treatments, C_L was several times higher than K_m values found for crops of around 2–3 $\mu\text{mol L}^{-1}$ (Barber, 1995; Bhadoria et al., 2004) which are above the C_L needed for maximum plant growth (Bhadoria et al., 2004; Buhse, 1992). In other treatments, C_L reached down to values of 0.2 $\mu\text{mol L}^{-1}$, which are already close to $C_{L\text{min}}$. Even though $C_{L\text{min}}$ represents only a small portion of the C_L with TSP, the amount of E_{5d} remaining at this concentration is still high, representing 30% to 40% of the well P-supplied soil (TSP; Figs. 2 and 3). The latter is because the buffer curve is not linear but has a strong curvature in the low concentration range. We suggest that the amount of E_{5d} remaining in the soil at $C_{L\text{min}}$ would not be available to plants.

5 Conclusions

Maize plants mainly use isotopically exchangeable P but in some cases, and more so in neutral and probably in alkaline soils as well or when P compounds of low solubility are added to the soil, also use nonisotopically exchangeable P. However, not all the IEP is plant-available because part of it, even though in equilibrium with the soil solution, has an equilibrium P concentration which is below the minimum P concentration, $C_{L\text{min}}$, for P uptake by plants.

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