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Title: The performance of DGT versus conventional soil phosphorus tests in tropical soils – An isotope dilution study

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key words: phosphorus deficiency, soil fertility, isotopically exchangeable phosphate

Running title: Conventional soil tests, but not DGT, extract plant-inaccessible P.

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Abstract

- *Background and aims*

A soil test that samples nutrients only from fractions that are accessible to plants will predict availability and uptake more robustly than empirical tests. This can be tested by comparison of the isotope ratios (specific activity, SA) of the nutrient between plant and the soil extract. This study was set up to assess this requirement for the diffusive gradients in thin films technique (DGT), recently proposed as a soil P test, in comparison with conventional soil P tests viz. Olsen, Colwell, Bray-1, Mehlich-3, ammonium oxalate, anion exchange membranes (AEM) and 0.01 *M* $CaCl_2$ solution.

- *Methods*

Maize (*Zea mays* L.) was grown in two P-deficient soils from western Kenya with contrasting P sorption characteristics, amended with a low and a high P rate and labelled with ^{33}P.

- *Results*

The SA in the plant shoot corresponded with that of the extracts of the different soil tests, except $CaCl_2$ and ammonium oxalate extracts, at the low P rate in the soil with low P sorption capacity. For the high P rate on this soil, differences in SA between maize shoot and soil test were small for all established soil tests, but significant for the Colwell, Bray-1, Mehlich-3 and AEM test. The SA in the soil extracts was significantly smaller than that in the maize shoot for the strongly P-sorbing soil at both P rates for all conventional tests, including AEM. This indicates that these tests extracted P from a pool that is not accessible to the plant. For the DGT test, however, there was no difference in SA between the maize shoot and the soil test, for any of the treatments.

- *Conclusions*

Most conventional soil tests can extract a fraction of P which is not available to maize. The DGT technique, however, only samples P from the plant-accessible pool.

Acronyms: AEM – Anion Exchange Membrane, C_{DGT} – time-averaged concentration in solution at the surface of the DGT device, DGT - Diffusive Gradients in Thin films, DM – Dry Matter, ICP-OES – Inductively Coupled Plasma Optical Emission Spectrometer, MBL – Mixed Binding Layer, R – dose of ^{33}P applied to the soil, RY – Relative Yield, S:L – Solid:Liquid, SA – Specific Activity.

Keywords: phosphorus deficiency, soil fertility, isotopically exchangeable phosphate

Introduction

The measurement of P availability in highly weathered, tropical, low-P soils remains a scientific challenge to date. Many of these soils are P deficient, i.e. the availability of P to plants in these soils is too low to sustain optimal plant growth. These P-deficient soils require substantial P inputs to achieve economically acceptable crop yields. Unfortunately the use of fertilizer entails considerable (economic) risks for most smallholder farmers. In these regions, a need exists for soil P tests that are able to identify P-deficient fields, give an estimation of the P status of the soil and guide farmers in their fertilization strategies.

Several extraction methods with contrasting procedures have been proposed to provide a measure of P availability in soils (Sibbesen 1983). Soils are often extracted under conditions (e.g. ionic strength and composition, pH, solid:liquid (S:L) ratio) far from rhizosphere conditions. Despite their widespread use, failure of established soil testing methods to predict P availability to plants over a range of different soil types has been reported frequently (Bertrand et al. 2003; Holford et al. 1985; Mason et al. 2010; McBeath et al. 2005; Moody et al. 1990; Saggar et al. 1990). The extracted amount of P is operationally defined by the chemical extractant used (Abrams and Jarrell 1992) and may mobilize plant-available P together with significant amounts of unavailable forms (Demaria et al. 2005; Fardeau et al. 1988; Menon et al. 1989). Moreover, the determination of P becomes unreliable if P concentrations in the chemical extract are near detection limits. This is a problem typically observed in highly weathered, P-deficient soils extracted with water or 0.01 *M* $CaCl_2$ solution (Amer et al. 1969; Bühler et al. 2003; Maertens et al. 2004; Wolf et al. 1986). Traditionally Bray-1 and Mehlich-3 extractants are used for acidic soils with high concentrations of aluminium phosphates, while, the Olsen extractant is originally developed for calcareous soils, though it is also reported to work relatively well on acidic soils (Pierzynski 2000).

These drawbacks have generated interest in new soil testing procedures for measuring plant-available P. Diffusive gradients in thin films (DGT) is a passive sampling technique that has been successfully applied to aquatic systems for measurement of trace metal concentration (Zhang and Davison 1995; Zhang et al. 1998b), later for phosphorus measurements (Zhang et al. 1998a) and more recently for predicting crop response to applied P on a wide range of soils (Mason et al. 2010; McBeath et al. 2007; Menzies et al. 2005; Tandy et al. 2011). Similarly to the anion exchange resin methods (AEM), the DGT technique attempts to mimic the physico-chemical uptake of P by plant roots by providing a sink for free phosphate (Mason et al. 2010). As during plant uptake, the continuously removal of P from solution results in a continuous replenishment of P in the soil solution by the solid phase (Davison et al. 2007). Although both techniques rely on the same basic principle, there are important differences. In DGT, a layer of ferrihydrite binding gel with strong affinity for P is introduced behind a diffusive hydrogel layer and an overlying protective filter membrane. Unlike resins which are deployed in soil suspensions, the DGT device can be placed directly onto a saturated

soil paste. The amount of P accumulated on the binding layer then depends on the concentration of P in the soil pore water adjacent to the deployment unit and on the extent of re-supply from the solid phase to the solution within the deployment period. In contrast with the resin methods, the DGT techniques have been reported to be less vulnerable to anionic interferences, pH, contact time between soil suspension and resin, and the S:L ratios used (Mason et al. 2008; Sousa and Coutinho 2009).

Isotopic dilution techniques allow quantifying the plant-available P in soils. Larsen (1952) proposed a procedure for measuring this available or labile pool based on the specific activity (SA) of P in a plant grown on a soil labelled with radioactive P. This plant-labile pool or L value represents the P participating in the soil-solution dynamics under plant growth conditions (Schneider and Morel 2000). Alternatively, the isotopically exchangeable pool can also be determined using the specific activity of P in the solution of soil labelled with radioactive P, i.e. the E value (Fardeau 1993; Russell et al. 1957). A conceptual requirement for soil tests predicting nutrient availability to plants is that these tests sample soil nutrients from the plant-accessible pool. Comparison of specific activity of the nutrient between soil extract and plant grown in radioisotope labelled soil can be used to verify this. For example, Tiller et al. (1972) demonstrated that the isotope ratio of zinc (Zn) was equal in soil EDTA extracts as in plants, suggesting that the EDTA extract samples Zn from the plant-accessible Zn pool in soils. As far as we know, no such comparison of specific activity between soil extracts and plants has been made for phosphorus. However, some studies have shown that the SA of extracts ($NaHCO_3$, EDTA, Bray-2, Truog extraction) is often lower than the SA measured in water/dilute salt extract (Demaria et al. 2005; Fardeau et al. 1988; Kato et al. 1995), with results strongly varying depending on soil properties and type of extractant used. The specific activities of soil solution and plants usually correspond, as indicated by an agreement between E and L values (Frossard et al. 1994; Morel and Plenchette 1994). This correspondence is expected, provided that plants do not access sources of P which are not isotopically exchangeable under the conditions of the E value measurement, by for example changing the rhizosphere chemistry (Hocking et al. 1997). Since specific activities in plants and soil solutions usually correspond, the lower SA in soil extracts than in a water/dilute salt extract indicates that the extracts sample P which is not accessible to plants.

The objective of this study was to compare the SA of plants grown on ^{33}P-labeled soil with that of P sampled by DGT or by conventional soil P tests, in highly weathered, P-deficient soils. For this purpose, maize (*Zea mays* L.) was grown on two P-deficient soils from western Kenya with contrasting P sorption capacity to which P was added at two rates.

Materials and methods

Soil P testing methods

For the P sorption isotherms, aliquots of 3 g dry soil were mixed with 30 mL 0.01 *M* $CaCl_2$ solution with increasing P concentrations. The soil suspensions were shaken 16 h on an end-over-end shaker, centrifuged and subsequently filtered over a 0.45 µm filter paper. The P concentration in the filtrate was determined colorimetrically using the malachite green method (Van Veldhoven and Mannaerts 1987) and absorbance was measured at 630 nm using a spectrophotometer (Perkin Elmer, Lambda 20, 1 cm path length). The quantification limit for this colorimetric method is 8 µg P L^{-1}. The P sorption isotherms of the soils used, Teso and Sega, are described by a Freundlich equation (Figure 1).

The soil P tests used in this experiment are described in Table 1; more details on the DGT and AEM method are given below. Since fresh soil samples were used, the equivalent of the required dry soil was weighed in order to maintain the S:L ratio as prescribed in the protocol. The P concentration in the different extracts of all conventional soil P tests was determined by Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES, Perkin Elmer, Optima 3300 Dual View) at 213.617 nm after filtration of the extract over a 0.45 µm filter paper. To 5 mL of extract, 15 mL of scintillation cocktail (ULITMA Gold XR) was added and samples were counted in a Packard Tri-Carb 1600CA liquid scintillation counter. Values were corrected for counting efficiency (97.7 %), background and radioactive decay. The ICP-OES was preferred over the colorimetric method to detect the same form of P for both radioactive as stable P, i.e. the total dissolved fraction.

The AEM-extractable P fraction was measured by weighing an aliquot of fresh soil equivalent to 0.5 g dry soil into a 40-mL centrifuge tube and adding 30 mL of ultrapure milli-Q water (18.2 MΩ) and two AEM strips 6 × 1 cm^2 (product 55164 2S; BDH Laboratory supplies, Poole, England). The resin strips were pre-treated to obtain the HCO_3^- form by soaking in 0.5 *M* $NaHCO_3$ for at least 24 hours. The suspensions were mounted on an end-over-end shaker for 16 hours at 20°C. Thereafter, the resin strips were removed and rinsed with milli-Q water. The membranes were transferred to a 40-mL centrifuge tube with 20 mL of 0.5 *M* HCl and shaken for at least 16 hours to extract the adsorbed phosphorus. The molybdate reactive P was determined colorimetrically using the malachite green method and ^{33}P was measured on the liquid scintillation counter as described earlier.

For the DGT method, the gel solution used for making the diffusive gels and mixed binding layers (MBL), containing both ferrihydrite and cation exchange resin Chelex-100, was composed of 15 vol. % acrylamide and 0.3 vol. % agarose derived cross-linker (DGT Research Ltd.). Both the diffusive and MBL gels were prepared as described by Zhang and Davison (1995) and Mason et al. (2005). We used plastic DGT devices designed for soil deployment with a 2.52 cm^2 exposure window. A 0.13

mm thick cellulose nitrate filter (0.45 µm) was placed on top of the diffusive gel (with a final thickness of 0.6 mm).

Soil samples (30 g) were moistened to saturation (glistening of water on the soil surface) at least 24 hours before deployment (Docekal et al. 2003; Menzies et al. 2005). The following day, DGT devices were deployed in a room at 20 °C (± 1 °C). Deployment times were selected according to expected accumulated mass on the MBL to avoid saturation of the MBL. According to Mason et al. (2005) the maximal capacity of the MBL is limited to about 12 µg P per gel. We included a safety margin and reduced deployment periods in order to accumulate maximally 6 µg P. The DGT was deployed 48 hours for the low P treatments of both soils. For the high P treatment 30 minutes and 8 hours deployment were chosen for Teso and Sega respectively. After deployment, the DGT units were removed and rinsed with milli-Q water to remove adhering soil particles. The DGT units were opened and the MBL retrieved. To know the total mass of P sorbed on the MBL, the MBL was eluted as described by Mason et al. (2005), in 1 mL of 1 *M* HCl. The P concentration in the eluent was measured colorimetrically using the malachite green method, while ^{33}P was measured on the liquid scintillation counter as described earlier. Two DGT blanks were included to correct for 'background' P. Accuracy and reproducibility of the DGT technique was checked using a solution with known P concentration (0.2 mg P L^{-1}) and a background concentration of 0.2 m*M* NaCl. The P was recovered on the MBL with 100–106 % recovery. Time-averaged concentration in solution at the surface of the DGT device, C_{DGT} [µg P L^{-1}], was calculated with following equation (Zhang and Davison 1995):

$$C_{DGT} = (M \Delta g) / (D A t) \qquad (1)$$

where M [µg P] is the measured accumulated mass of diffused phosphate in the MBL over the deployment period, Δg the thickness of the diffusive layer which includes both the diffusive gel and membrane filter [0.073 cm], D [cm^2 s^{-1}] the diffusive coefficient in the diffusive layer, A [cm^3] the exposed area of the gel and t [s] the deployment period. Values for D are temperature corrected and are provided by DGT research Ltd (5.42×10^{-6} cm^2 s^{-1} at 21 °C; www.DGTresearch.com). Notice that C_{DGT} is an intensity measure and is expressed as µg P L^{-1}. This in contrast with the established extraction procedures where extracted quantities are given in mg P kg^{-1} soil.

Plant growth experiment

Plants were grown in two representative soils from western Kenya, sampled near the villages of Teso (0°43'41''N – 34°23'34''E) and Sega (0°15'07''N – 34°12'16''E). The topsoil (0–20 cm) was air-dried and sieved through a 4-mm sieve. Selected soil properties are presented in Table 2. Soils were amended with a KH_2PO_4 solution at low (50 mg P kg^{-1}) and high (500 mg P kg^{-1}) P rates with three replicates. Per pot, 3 kg of dry soil was thoroughly mixed with the KH_2PO_4 solution. To each pot NH_4NO_3 (400 mg N kg^{-1} in 2 splits: 50 % at planting and 50 % 2 weeks after planting), macronutrients (62.5 mg Ca kg^{-1}, 27.5 mg Mg kg^{-1}, 10 mg S kg^{-1}) and micronutrients (1 mg Mn kg^{-1}, 0.5 mg Zn kg^{-1},

0.5 mg Cu kg^{-1}, 0.17 mg B kg^{-1}, 0.17 mg Mo kg^{-1}) were applied. In the low P treatments, KCl was added at 283 mg K kg^{-1}. No extra K was added to the soils treated with the high rate of KH_2PO_4, which equates to a dose of 631 mg K kg^{-1}. Measurements of electrical conductivity showed that the risk for salt stress was small (maximal 0.237 dS m^{-1} at the high P addition in a 1:5 soil:water extract). The day after nutrient mixing, a solution of carrier-free ^{33}P orthophosphate (^{33}P radionuclide 1 mCi, orthophosphoric acid in 1 mL HCl-free water, Perkin Elmer) was added to label the soil at 10.31 kBq kg^{-1}. An aliquot of the tracer solution was preserved to serve as the standard solution during later radio assays. The water contents of all soils/treatments were adjusted to a final moisture content of 70% field capacity (0.25 L kg^{-1} for Sega and 0.15 L kg^{-1} for Teso). One day after labelling with ^{33}P, three maize seeds (*Zea mays* L. cv. H513, average seed weight of 0.42 g and standard deviation of 0.05 g) were planted in every pot and thinned to 1 plant per pot 5 days after emergence. Plants were grown in a plant growth cabinet with controlled conditions (22°C during a 12 hours day and 18°C during a 12 hours night; constant relative air humidity of 60 %; light intensity of 40,000 lux). Pots were weighed daily and watered to restore the original moisture content. Plants were harvested 4 weeks after planting, shoots were cut about 1 cm above the soil surface and oven dried at 70 °C for 7 days. The P concentration in the shoots was determined after hot nitric acid digestion. An aliquot of 200 mg dried plant material was digested in 2 mL HNO_3 and diluted to 6 mL with Milli-Q water. The ^{33}P activity was determined by liquid scintillation counting. To 5 mL digest, 15 mL of scintillation cocktail (ULTIMA Gold XR) was added and samples were counted in a Packard Tri-Carb 1600CA liquid scintillation counter. Values were corrected for counting efficiency (97.7 %), background, radioactive decay and quenching in concentrated HNO_3. The total P content was determined by ICP-OES at 213.617 nm. The specific activities in the plant shoot were calculated and corrected for seed contribution to shoot P using equation 2:

$$SA_{shoot} = (^{33}P_{shoot} / P_{dfsoil}) = {}^{33}P_{shoot} / (P_{shoot} - P_{dfseed}) \qquad (2)$$

with SA_{shoot} the specific activity of P in the plant shoot taken up from the soil [kBq $(mg\ P)^{-1}$], $^{33}P_{shoot}$ the ^{33}P activity in the plant shoot [kBq $plant^{-1}$], and P_{dfsoil} the P in the plant shoot taken up from the soil [mg P $plant^{-1}$]. The latter was calculated from the difference between the total amount of P in the plant shoot, P_{shoot} [mg P $plant^{-1}$] and the P in the plant shoot translocated from the seed, P_{dfseed} [mg P $plant^{-1}$; see below].

Because the ^{33}P dose applied for the plant test (10.31 kBq kg^{-1}) was 891-fold smaller than that in soil tests (9190 kBq kg^{-1}, see below), the specific activities of the plants were multiplied by 891 for comparison with the specific activity of the soil extractants. The soils were labelled at higher dose for the soil P tests than for the pot trial to allow sufficient detection limits of ^{33}P in the soil P tests such as $CaCl_2$ extract, while limiting radiation hazard in the pot trial.

The L value [mg P kg^{-1}] (plant-labile pool) was determined from the amount of radioisotope added and the specific activity in shoot and corrected for seed P contribution (equation 2, SA_{shoot} in kBq $(mg\ P)^{-1}$):

$$L = R / SA_{shoot} \qquad (3)$$

with R the ^{33}P dose applied in the plant growth experiment [kBq kg^{-1} soil].

Seed P ($^{31}P_{seed}$) of this cultivar (cv. H513) was determined in seed digests and is 2.4 g P kg^{-1} (± 0.1 g P kg^{-1}). The maize seeds selected in this experiment had an average seed weight of 0.42 g (standard deviation 0.05 g). As such, maximally 1 mg P can be translocated from the seed to the plant shoot. The fraction of seed P translocated to the shoot was determined by using the equation given by Pypers et al. (2006) based on and the growth of maize seedlings at varying P supply in a P-free sand culture:

$$P_{dfseed} = 0.77\ P_{seed}\ (1 - \exp\ (-1.973\ P_{shoot})) \qquad (4)$$

with P_{seed} the average P content in the seeds used [mg P $seed^{-1}$]. When the P supply is optimal, i.e. at both P rates for Teso and at the high P rate for Sega, it was predicted that the maximal amount of P (0.79 mg P $plant^{-1}$) was translocated from the seed to the shoot. Under P-deficient conditions, i.e. low P rate in Sega, less P (0.68 mg P $plant^{-1}$) was predicted to be translocated to the shoot. Even when the P concentration in shoot is small, the uncertainty of the prediction remains small. The 95 % confidence limits on P_{dfseed} for Sega at low P rate are 0.63–0.70 mg P $plant^{-1}$ resulting in small variations in the L values (56–69 mg P kg^{-1}).

Soil incubation experiment

Soil tests were applied to unplanted ^{33}P spiked soil. Unplanted soils were preferred to planted soils because soil tests are commonly applied pre-planting for predictive purposes. The specific activities of P for the different soil tests were measured after 1 and 4 week(s) incubation. The soil incubation experiment was carried out in two replicates. The nutrient solutions containing N, Ca, K, Mg, S, Mn, Zn, Cu, B and Mo were first mixed with 200 g of dry soil which was passed over a 4-mm sieve. Subsequently, the P solution was added at both low and high P rate, and the soils were thoroughly homogenized. The following day, both soils were labelled with ^{33}P (9190 kBq kg^{-1}) by adding a carrier-free ^{33}P orthophosphate solution. An aliquot of the tracer solution used was preserved to serve as the standard solution during later radio assays. The P treatments and the nutrients were applied identically as in the plant growth experiment. Soils were moistened to the same moisture contents and incubated in air tight pots in an incubation chamber at 20 °C (± 1 °C). Pots were opened weekly to allow gas-exchange. Subsamples for P analysis were taken after 1 and 4 weeks of incubation. Specific activities were calculated for each soil P test as follows:

$$SA_{P\text{-}test} = {}^{33}P_{P\text{-}test} / P_{P\text{-}test} \qquad (5)$$

with $SA_{P\text{-}test}$ the specific activity in the extract [kBq $(mg\ P)^{-1}$], $^{33}P_{P\text{-}test}$ the ^{33}P activity in the extract [kBq L^{-1}] and $P_{P\text{-}test}$ the P concentration in the extract [mg P L^{-1}].

The E value was calculated based on specific activity measured in the $CaCl_2$ extract:

$$E = R / SA_{CaCl2} \qquad (6)$$

with R the ^{33}P dose in the incubation experiment [kBq kg^{-1}].

Data and statistical analysis

Comparison of the SA of P in the plant with the SA of P extracted in the soil test allows assessing if the soil test extracts P that is not accessible to the plant. To do so, we simplify the partitioning of P by considering two pools: a (plant-)labile pool, i.e. the P pool that has the same SA as P taken up by the plants, and a non-labile pool with which there was no isotopic exchange during the plant experiment. This is an oversimplification, since in reality, there is no strict distinction between labile and non-labile pool. For instance, it is generally found that the E value increases (and SA decreases) in time, as isotopic dilution of the added ^{33}P with soil P progresses. However, after four weeks of equilibration, the changes in SA and E values are negligibly small (see also results), and the discretization in two pools, i.e. labile (L or E value) and non-labile pool, is therefore a useful and applicable concept. If the soil test only extracts P from the plant-labile pool, the SA in plant shoot and the soil extract will be the same, provided that the ^{33}P spiking rate was the same (or was corrected for different rates as we did here). However, if P is extracted from the non-labile pool (not exchanged with the isotope), this will result in a proportional decrease in the SA for the soil tests. This can be quantified based on following equations:

$$P_{P\text{-}test} = P_{df\ lab} + P_{df\ nonlab} \qquad (7)$$

where $P_{df\ lab}$ and $P_{df\ nonlab}$ are the concentrations [mg P kg^{-1}] of extracted P derived from the plant-labile and non-labile pool, respectively. The SA of P extracted from the labile pool equals SA_{shoot}. The SA of P extracted from the non-labile pool equals 0 (no isotopic exchange), hence, the mass balance for ^{33}P reads:

$$P_{P\text{-}test}\ SA_{P\text{-}test} = P_{df\ lab}\ SA_{shoot} \qquad (8)$$

Equations (7) and (8) can be rearranged to obtain following equations that allow calculating the amount of P extracted from labile and non-labile pool based on the measured specific activities:

$$P_{df\ lab} = P_{P\text{-}test}\ SA_{P\text{-}test} / SA_{shoot} \qquad (9)$$

$$P_{df\ nonlab} = P_{P\text{-}test} - P_{df\ lab} \qquad (10)$$

Analysis of variance (ANOVA) was performed using the general linear model procedure (GLM) of the SAS program (SAS Institute Inc. 2009) to determine differences in specific activity between soils and P treatments and between plants and soil extracts. SA_{shoot} was compared to $SA_{P\text{-}test}$ measured after 4 weeks incubation. This was done by a two-sided t-test (TTEST) ($\alpha = 0.05$).

Results

Plant growth response to applied P fertilizer

Maize plants grown on soils from Sega with P applied at the low rate showed P deficiency symptoms: purplish coloration (anthocyanin pigment) of the leaves and stunted growth. Application of 500 mg P kg^{-1} soil significantly increased the DM yields and shoot P concentrations. P deficiency symptoms were no longer observed at the high P rate (Table 3).

Plants grown on Teso soils at low P showed no signs of P deficiency, but DM yield was significantly increased by high P application. The shoot P concentrations at the low P rate (Table 3) were below a general critical value of 2.5 mg P $(g\ DM)^{-1}$ (Reuter and Robinson 1997). At the high P rates, shoot P concentrations were significantly increased. The combined effects of the high P dose on yield and shoot P concentration resulted in distinct increases in the total shoot P uptake for which the seed contribution was negligible.

Phosphorus extracted with the conventionally used soil P tests and DGT

Although a small decrease in the amount of P extracted was observed between one week and four weeks of incubation, this was not significant in any of the treatments. Therefore, only the results of the second sampling, 4 weeks incubation, are shown (Table 6). The addition of P significantly increased extractable P compared to unamended soils. Throughout all P tests, the amount and recovery of added P in soil extracts was larger in Teso than in Sega. This difference in extractability of added P between these soils can be ascribed to the difference in P sorption capacity (Figure 1).

Among all soil P tests, oxalate extractions yielded the largest extractable P concentration (Table 6). This is not surprising since the oxalate method is also used for the estimation of the soil's amorphous Al and Fe content and is able to dissolve Al and Fe oxides in which P may be fixed in non-exchangeable form. The amount of P extracted in Mehlich-3 test was similar to that in Bray-1 method. Both extractants are acidic and contain reactive fluoride which enhances the release of P from Al-phosphates and at the same time suppresses the re-adsorption of P by soil colloids (Pierzynski 2000). Though both the Colwell and Olsen test are based on extraction with a 0.5 *M* $NaHCO_3$ (pH 8.5) solution, more P was extracted in the alkaline Colwell than in the Olsen test. This is most likely due to larger extraction periods and smaller S:L ratios used with Colwell (Pierzynski 2000). The least aggressive extractant is $CaCl_2$, which is reflected in the low amounts of P extracted (Table 6). In the low P treatment of Sega the phosphate concentration in the $CaCl_2$ extract was below quantification limits of the ICP-OES (Table 7).

Measured DGT concentrations are given in Table 7. For Teso at high P rate, the MBL was nearly saturated despite the short deployment period used for this treatment (30 minutes). Consequently, the

measured C_{DGT} may be an underestimate of the actual value. Shorter deployment periods were not assessed since C_{DGT} can only be calculated precisely when a linear concentration gradient is established within the diffusive gel. The minimum deployment time needed to use Equation 1 is 1 h according to Zhang et al. (1995); shorter deployment periods can be used if non-steady state conditions are allowed. Notice that this lack of steady state and possible saturation of the gel do not impede estimating the SA of P in the DGT. The ratio of ^{33}P over ^{31}P bound on the MBL gel is independent of the loading on the gel, thus even when the gel is saturated SA can easily be estimated.

Isotopic composition in the different soil extracts

The SAs measured in the different soil extracts did not significantly change between one and four week(s) incubation (Table 4). Similarly, Pypers et al. (2006), Hedley et al. (Hedley et al. 1982) and Bühler et al. (2003) showed that E values increase significantly the first hours or days after addition of ^{33}P solution, and remained stable at longer incubation times. The reaction between soil and the introduced isotope is very rapid and continues for a long time but at a decreasing rate (Barrow 1991). When slow reactions are negligible E and SA values should be nearly constant (Hedley et al. 1982) We compared the specific activities measured after 4 weeks incubation with the SA in the shoot to have equal equilibration periods(Table 5). Many authors (e.g. Frossard et al. 1994) prefer to determine SA values 1, 10 and 100 minutes after isotope addition and extrapolated over several weeks. Although this technique has proved to be relatively successful, it entails a large uncertainty on the SA value (Bühler et al. 2003).

From the SA in the extracts, the amount of P extracted from the labile pool or non-labile pool could be calculated using equations 9 and 10 (Table 6). In both soils at high P rate and in the Teso soil at low P rate, the amount of P extracted from the non-labile pool was relatively small (Table 6) Up to 25 % of the extracted P originates from the non-labile phase. However, in the Sega soil, at low P rate, the amount of P extracted from the non-labile pool was for many extractants over half of the total P extracted, as indicated by the much smaller SA in these extracts than in the plant shoot (Table 6).

Discussion

L value determination

L values are measures for the quantity of labile P in a soil. Even though both soils have similar L values at same P addition rates (Table 3), the P deficiency was much more pronounced for the Sega soil. This is related to its lower P intensity and stronger sorption (Figure 1) because of the larger Al and Fe oxide content (Table 2). This observation indicates that the labile pool (L value) is not a good indicator for P-deficiency, as it is a quantity parameter, i.e. an indicator of the total buffering pool.

Isotopic composition in the different soil extracts

The $CaCl_2$-extractable concentration, in contrast with the other established soil P tests, is an intensity parameter, measuring the P concentration in a solution that has similar ionic composition as indigenous soil solution (Degryse et al. 2009; Van Raij 1998). This explains why the concentrations measured in the $CaCl_2$ extract are much lower than the P concentrations measured in the other extracts (Table 6). The stronger sorption in Sega soil results in lower solution concentration which can be seen from the lower $CaCl_2$-solution concentration and DGT-concentration (Table 7).

Many authors have reported difficulties in determining SA or E values in water or $CaCl_2$ extracts of soils that either have a high P sorption capacity, low P levels or a large amount of colloidal P (Aigner et al. 2002; Amer et al. 1969; Hamon and McLaughlin 2002; Maertens et al. 2004; Tran et al. 1988; Wolf et al. 1986). Also in this study, the P concentration in the $CaCl_2$ extract could not be measured for the Sega soil at the low P rate, since the P concentration in the extract was below quantification limits. With the DGT technique, this problem is circumvented by the pre-concentration step on the MBL layer during the deployment of the DGT. Moreover, deployment periods can be increased in order to accumulate more P on the MBL.

The principle of both AEM and DGT is in strong contrast with conventional soil P tests where a chemical reagent is used to dissolve a fraction of the solid phase by increasing S:L ratios which disturbs equilibrium, changing pH and introducing anions that complex, precipitate or desorb phosphate (Sibbesen 1983). The SAs were significantly different between DGT and AEM when P was applied at the low P rate (Table 4). The DGT includes the diffusional limitations of P towards the binding gel unit (Degryse et al. 2009), in contrast with AEM which is deployed in agitated suspensions where desorption of P is more induced (Mason et al. 2008). Our results indicate that the AEM may desorb non-isotopically exchangeable P in soils with small quantities of labile P (Table 6).

Comparing ^{33}P specific activities in shoot to that in each soil test

Since maize is unlikely to access non-labile P by rhizosphere processes, the specific activity measured in maize plants should equal the specific activity measured in the extract of the soil P test, if the soil test extracts P from the same pool as the maize roots.

In both soils at high P rate and in the Teso soil at low P rate, the amount of P extracted from the non-labile pool was relatively small (Table 6). Contrastingly, for Sega soils at the low P rate, the amount of P extracted from the non-labile pool was over half of the total P extracted for many extractants. This indicates that a significant portion of non-labile P was extracted by these chemical extractants, including the resin strips. This also confirms observations made by Fardeau et al. (1988), Kato et al. (1995) and Demaria et al. (2005) that different soil P tests extract up to 90 % more P than

the isotopically exchangeable P after a relatively short exchange period. As a result, P availabilities will be overestimated using the soil P tests based on chemical extraction.

This is confirmed when SA_{shoot} is compared with $SA_{P\text{-}test}$. The SA in the plant shoot corresponded with that of the extracts of the different soil tests, except $CaCl_2$ and ammonium oxalate extracts, at the low P rate in the soil with low P sorption capacity. For the high P rate on this soil, differences in SA between maize shoot and soil test were small for all established soil tests, but significant for the Colwell, Bray-1, Mehlich-3 and AEM test. The SA in the soil extracts was significantly smaller than that in the maize shoot for the strongly P-sorbing soil at both P rates for all conventional tests, including AEM, This indicates that these tests extracted P from a pool that is not accessible to the plant. In contrast, the DGT technique only samples P from the plant-labile pool, as can be seen from the agreement in SA between extracted P and P in the plant shoot (Table 4).

Numerous studies on soils with high P sorption capacity have shown that DGT and AEM give better estimation of plant-available P than conventionally used soil P tests, because they sample P from solution in a similar way as plant roots without chemical alteration of the soil (Mason et al. 2010; McBeath et al. 2007; Qian et al. 1992; Sibbesen 1978). Mason et al. (2010) showed that relative DM yields as well as were much better predicted by the DGT sampler than by the AEM method in field trials. This can be explained by (i) the presence of a diffusive layer in the DGT units which limits the maximum flux of P from the soil to the binding layer in a similar way plants control P fluxes (Mason et al. 2010); (ii) the diffusional limitations of P acquisition by plants which are considered in the DGT measurement in contrast with AEM deployed in agitated suspensions (Degryse et al. 2009); and (iii) the lower anionic interferences with DGT compared to the AEM method (Mason et al. 2008). Results in this study demonstrate that AEM, with given resin-soil ratio and equilibration period, extracts a significant portion of P inaccessible to plants from soils at low P status, in contrast to DGT.

In summary, strong chemical extractants extract a vast amount of the solid labile pool, but may also extract significant portions of the non-labile P pool that does not rapidly buffer the P concentration in solution when soils are poor in P and have a large P sorption capacity. The amount of non-labile P extracted varies with the soil type and the type of chemical extractant used. In contrast, we found that the DGT technique sampled P from the plant-available fraction only.

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Figures and tables

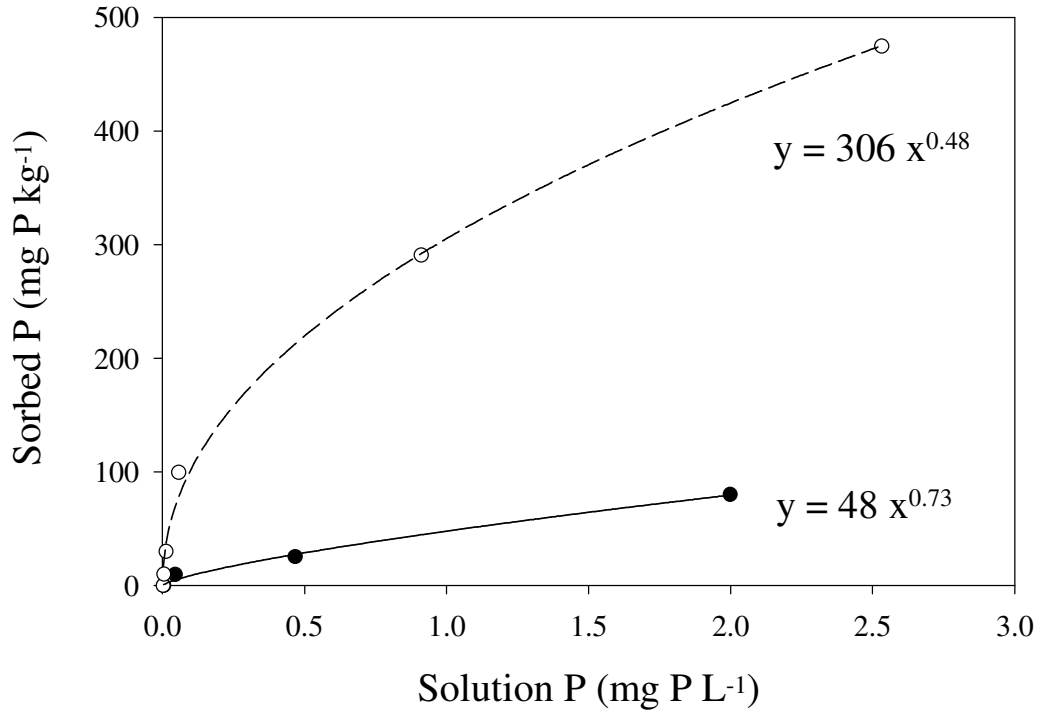

Figure 1 P sorption isotherms of Teso (●) and Sega (○) with corresponding Freundlich equations.

Table 1 Summary of soil P tests.

Soil P test	Chemical extractant	Extraction time	S:L	Reference
			g mL^{-1}	
Oxalate	0.2 *M* $(COONH_4)_2$ in 0.14 *M* $(COOH)_2$	2 h	1:50	Schwertmann (1964)
Olsen	0.5 *M* $NaHCO_3$ at pH 8.5	30 min	1:20	Olsen et al. (1954)
Colwell	0.5 *M* $NaHCO_3$ at pH 8.5	16 h	1:100	Colwell (1963)
Bray-1	0.025 *M* HCl in 0.03 *M* NH_4F, pH 2.6	5 min	1:10	Bray (1945)
Mehlich-3	0.2 *M* CH_3COOH, 0.25 *M* NH_4NO_3, 0.015 *M* NH_4F, 0.013 *M* HNO_3 in 0.001 *M* EDTA, pH 2.5	5 min	1:10	Mehlich (1984)
AEM	Anion exchange membrane	16 h	1:60	Amer et al. (1955), Sibbesen (1977)
$CaCl_2$	0.01 *M* $CaCl_2$	2 h	1:10	Houba et al. (1996)
DGT	Ferrihydrite based binding gel	30 min to 48 h	saturation	Zhang and Davison (1995), Mason et al. (2005)

Table 2 Selected properties of the topsoil (0–15 cm) sampled at Teso and Sega.

	Teso	Sega
Soil type[a]	Lixisol	Ferralsol
Sand (%)[b]	82	23
Silt (%)[b]	12	12
Clay (%)[b]	6	65
pH[c]	4.6	4.2
CEC ($cmol_c\ kg^{-1}$)[d]	3.2	5.7
Olsen-P (mg P kg^{-1})	< 1	3
AEM-P (mg P kg^{-1})	1.7	2.8
Al_{ox} (mg Al kg^{-1})[e]	270	1400
Fe_{ox} (mg Fe kg^{-1})[e]	380	1600
P_{ox} (mg P kg^{-1})[e]	30	60

[a] Major soil groupings (FAO 1990), . [b] particle size analysis by pipette method (Day 1965) ; [c] pH (1:10) determined in 0.01 *M* $CaCl_2$; [d] CEC determined at soil pH by AgTu method (Chhabra et al. 1975); [e] ammonium oxalate-extractable Al, Fe and P (Schwertmann 1964).

Table 3 Shoot dry matter (DM) yield, shoot P concentration and P uptake in shoot (P_{shoot}) for maize grown in two soils amended with 50 mg P kg^{-1} (low) or 500 mg P kg^{-1} (high). The L value is the plant-labile pool as determined from the specific activity in the plant grown on the ^{33}P spiked soil. Means values and standard error of mean (between brackets) of three replicates.

Soil	P rate	DM yield	Shoot P concentration	P_{shoot}	L value
		g $plant^{-1}$	mg P (g DM)$^{-1}$	mg P $plant^{-1}$	mg P kg^{-1}
Teso	Low	4.49 (0.88)	2.00 (0.06)	8.9 (1.5)	68.7 (3.8)
	High	7.05 (1.06)	8.95 (1.46)	60.1 (2.9)	439.7 (7.1)
Sega	Low	0.73 (0.04)	1.34 (0.06)	1.0 (0.1)	61.6 (1.05)
	High	6.70 (0.32)	3.45 (0.51)	23.3 (4.0)	433.7 (10.8)

Table 4 Specific activities of ^{33}P (SA) measured in the soil extracts of different soil P tests after 1 and 4 weeks incubation for both soils amended with low (50 mg P kg^{-1}) or high (500 mg P kg^{-1}) P dose. An asterisk (*) represents a significant difference (t-test at $P < 0.05$) between specific activity in the shoot and the soil extract after 4 weeks incubation. The specific activity in the plant shoot was corrected to the same ^{33}P rate as used for the soil tests (9190 kBq kg^{-1}). Standard error of mean between brackets.

	Teso				Sega			
	Low P		High P		Low P		High P	
	1 week kBq kg^{-1}	4 weeks kBq kg^{-1}	1 week kBq kg^{-1}	4 weeks kBq kg^{-1}	1 week kBq kg^{-1}	4 weeks kBq kg^{-1}	1 week kBq kg^{-1}	4 weeks kBq kg^{-1}
Oxalate	108 (2)	100 (2)*	17.0 (0.1)	17.6 (0.7)	58.8 (0.7)	63.3 (1.8)*	16.0 (0.7)	15.7 (0.3)*
Olsen	137 (2)	128 (1)	18.5 (0.1)	17.2 (0.7)	56.2 (1.9)	49.6 (3.1)*	15.6 (0.2)	15.8 (0.3)*
Colwell	130 (1)	122 (5)	18.2 (0.4)	16.8 (0.6)*	62.5 (1.9)	58.2 (4.5)*	17.0 (0.5)	16.8 (0.6)*
Bray-1	147 (3)	149 (1)	17.6	18.0 (0.1)*	78.3	84.5 (1.9)*	15.4 (0.3)	16.6 (0.2)*
Mehlich-3	147 (2)	142 (1)	17.8 (0.3)	17.7 (0.3)*	90.1 (3.1)	87.2 (5.7)*	16.2 (0.1)	16.8 (0.2)*
AEM	185	168 (7)	19.8 (0.1)	19.1 (0.3)*	110.2 (5.3)	104.7 (2.3)*	18.1 (0.3)	18.0 (0.4)*
$CaCl_2$	170 (5)	166 (26)*	21.2 (0.1)	20.7 (0.1)	nd.	nd.	18.7 (0.5)	18.7 (0.5)*
DGT	133 (1)	135 (2)	19.6 (0.3)	20.1 (0.1)	149.4 (0.6)	147.4 (0.4)	20.1 (1.3)	19.9 (2.1)
shoot		135 (8)		20.9 (0.3)		149.2 (2.6)		21.2 (0.5)

nd.: not determined (P concentration in extract below quantification limit).

Table 5 Ratio of the specific activity in shoot to that in the extract for both soils amended with low (50 mg P kg^{-1}) or high (500 mg P kg^{-1}) P dose. Standard error of mean between brackets.

	Teso		Sega	
	Low P	High P	Low P	High P
Oxalate	1.35 (0.08)	1.19 (0.05)	2.36 (0.08)	1.35 (0.04)
Olsen	1.05 (0.06)	1.21(0.05)	3.01 (0.19)	1.35 (0.05)
Colwell	1.10 (0.07)	1.24 (0.05)	2.56 (0.20)	1.26 (0.05)
Bray-1	0.90 (0.05)	1.16 (0.02)	1.77 (0.05)	1.27 (0.04)
Mehlich-3	0.95 (0.05)	1.18 (0.03)	1.71 (0.03)	1.26 (0.04)
AEM	0.80 (0.06)	1.10 (0.02)	1.42 (0.04)	1.18 (0.04)
$CaCl_2$	0.79 (0.05)	1.01 (0.02)	nd.	1.13 (0.04)
DGT	1.00 (0.06)	1.04 (0.02)	1.01 (0.02)	1.06 (0.12)

Table 6 Total P extracted using different soil P tests on ^{33}P labelled soils amended with 50 mg P kg^{-1} (low) or 500 mg P kg^{-1} (high) after 4 weeks incubation. The amount of P extracted from the labile and non-labile pool was calculated based on the specific activity in the extract compared to that in the shoot of maize grown on these soils (Equation 9 and 10). The percentages denote the fraction of extracted P that is derived from the labile and non-labile pool.

		Low P treatment					High P treatment				
Soil	Soil test	P extracted mg P kg^{-1}	From labile mg P kg^{-1}	From non-labile mg P kg^{-1}	From labile %	From non-labile %	P extracted mg P kg^{-1}	From labile mg P kg^{-1}	From non-labile mg P kg^{-1}	From labile %	From non-labile %
Teso	Oxalate	75	56	19	74	26	482	406	76	84	16
	Olsen	30	28	1.5	95	5	290	239	51	82	18
	Colwell	53	48	5.0	90	9	455	366	90	80	20
	Bray-1	28	31	-3.0	111	-11	378	325	53	86	14
	Mehlich-3	35	37	-1.9	105	-5	352	298	54	85	15
	AEM	21	26	-5.2	125	-25	346	315	31	91	9
	$CaCl_2$	2.2	2.2	-0.6	126	-26	96.2	90.3	5.9	94	6
Sega	Oxalate	83	35	48	42	58	408	301	107	75	25
	Olsen	22	7.3	15	33	67	178	132	45.7	74	26
	Colwell	52	20	32	38	62	312	247	64.6	79	21
	Bray-1	16	9.0	7.1	56	44	160	127	33.1	75	25
	Mehlich-3	14	7.8	6.7	54	46	184	145	39.7	81	19
	AEM	14	9.7	4.3	69	31	199	169	30.0	86	14
	$CaCl_2$	nm.					7.1	6.26	0.83	86	14

nm.: not measureable; below quantification limit (< 0.003 mg P kg^{-1}).

Table 7 The P concentration in the 0.01 *M* $CaCl_2$ extract, the DGT-measured concentration, and the E value in the two soils amended with 50 mg P kg^{-1} (low) or 500 mg P kg^{-1} (high). Means values and standard deviation (between brackets) of two replicates.

Soil	P rate	C_{CaCl2}	C_{DGT}	E value
		mg P L^{-1}	mg P L^{-1}	mg P kg^{-1}
Teso	Low	0.22 (0.02)	0.090 (0.004)	54 (2)
	High	9.61 (0.07)	> 26 (1)*	444 (3)
Sega	Low	nm.	0.0045 (0.0001)	nm.
	High	0.71 (0.02)	0.44 (0.03)	491 (12)

* the MBL was possibly saturated (capacity set at 6 µg P), the C_{DGT} can thus be an underestimate.
nm.: not measurable, below quantification limit (< 0.03 mg P L^{-1}).