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Plant-available P for maize and cowpea in P-deficient soils from the Nigerian Northern Guinea savanna – Comparison of *E*- and *L*-values

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Abstract

There are several indications that legumes are capable of accessing sparingly soluble phosphorus (P) in the soil through root-induced processes. We hypothesize that this plant-induced mobilization of P can be demonstrated if the plant accessible P assessed by isotopic dilution ('*L*-value') exceeds the corresponding values assessed in soil extracts ('*E*-values'). A greenhouse experiment was set up to assess if L/E ratios are affected by P supply and by crop type. The *L*- and *E*-values were determined in three P-deficient soils of the Nigerian Northern Guinea savanna (NGS), applied with various rates of TSP, for two cowpea varieties (*Vigna unguiculata* L., cv Dan-Ila and cv IT-82D-716) and maize (*Zea mays* L., cv oba super I) as a reference. Plants grown in control soils were severely P-deficient. Plant growth and shoot P uptake significantly increased with increasing P application in all three soils and for all crops, but relative yield and shoot-P responses to P application were similar between maize and cowpea. Both *L*- and *E*-values increased with increasing P application. Average L/E ratios for maize were 1.4 ± 0.3 and were unaffected by the P application. For cowpea in contrast, L/E ratios were 3.1 ± 0.2 (significantly larger than one) in one of the three control soils and significantly decreased to 1.3 ± 0.1 at largest P supply. Elevated L/E ratios in cowpea were not associated with an increase in P uptake compared to the other two control soils in which no increase in L/E ratio was observed. It is concluded that cowpea is able to access non-labile P under P-deficient conditions. However, this process cannot overcome P deficiency in these soils, probably because P uptake is limited by the small P concentration in the soil solution (1–2 μg P L^{-1}) and this limitation is not overcome by an increase in the accessible soil P quantity (*L*-value).

Introduction

Various legumes are able to access sparingly soluble phosphorus (P) in the soil (Kamh et al., 1999). This mobilization of P may be due to the exudation of organic acids or phosphatases and to pH changes in the rhizosphere. White lupin (*Lupinus albus*), for example, is characterized by cluster roots (proteoid roots), which excrete large amounts of citrate (Keerthisinghe et al., 1998). These rhizosphere processes enable legumes to take up P, which is inaccessible to other species. The use of such legumes may be particularly beneficial in legume-cereal rotation systems (Horst et al., 2001; Kamh et al., 1999). In addition to

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bringing extra nitrogen into the cropping system, the incorporation of such legumes in the cropping systems may lead to an improved utilization efficiency of soil and fertilizer P. Vanlauwe et al. (2000) found evidence for an enhanced P fertility in a maize-based rotation system. When rock phosphate was applied to *Mucuna pruriens* or *Lablab purpureus*, more P was available to the following maize crop and P uptake and grain yield were increased compared to a maize system where rock phosphate is added directly to the maize. However, these two legumes have little direct economical benefits. Therefore it is relevant to investigate P mobilization by legumes, which provide edible beans and a direct profit to farmers.

Isotopic dilution methods with P have been used to determine that fraction of soil P, which can exchange with phosphate anions in the soil solution. Since plant roots retrieve P directly from the soil solution, the determination of this so-called labile soil P yields information about the quantity of P that is accessible in the soil (Russell et al., 1954). Plant species characterized by rhizosphere processes, which enable them to draw P from sparingly soluble P sources, can be identified through isotopic dilution techniques. For such species, the isotopically exchangeable P assessed in a plant growth experiment ('*L*-value') exceeds the corresponding value in a soil extract ('*E*-value'). The *E*-value is determined by adding ^{32}P-orthophosphate to a soil suspension and measuring the isotopic dilution in the soil solution after a certain equilibration period (Dalal and Hallsworth, 1977; Fardeau, 1993; Russell et al., 1954). To determine the *L*-value, the soil is labeled with ^{32}P-orthophosphate and plants are grown to sample the specific activity (ratio of ^{32}P over ^{31}P) of the labile P in the soil (Larsen, 1952). The *L*-value equals the *E*-value, unless rhizosphere processes enable the plant to draw P from non-labile P sources in the soil. Such P-mobilizing rhizosphere processes decrease the specific activity of the P taken up by the roots. Various studies have shown that *L*- and *E*-values commonly do not differ if P supply by the soil is adequate. Morel and Plenchette (1994) observed that *L*-values for soybean (*Glycine max*) and barley (*Hordeum vulgare*) grown in loamy soil amended with NaH_2PO_4 at various rates were identical to the corresponding *E*-values. Frossard et al. (1994) grew common bentgrass (*Agrostis capillaris*) in soils with differing P status and concluded that *L*-values generally do not differ significantly from *E*-values but measurement difficulties occur in P-deficient soils. Bühler et al. (2003) found a satisfying agreement between *L*- and *E*-values for common bentgrass, grown in Colombian oxisols with different cropping and fertilization histories. However, increased *L*-values have been observed for white lupin (*Lupinus albus*) and pigeon pea (*Cajanus cajan*), which are typically known for their root-induced P-mobilizing processes. Hocking et al. (1997) showed a 4- and 2-fold increase in *L*-value for white lupin and pigeon pea, respectively compared to wheat (*Triticum aestivum*) grown in a P-deficient oxisol. Much less is known about the extent to which other legumes are able to access non-labile P in low-P soils.

Two methodological difficulties arise when comparing *E*- and *L*-values in low-P soils. On the one hand, the determination of the *E*-value becomes unreliable if P concentrations in the soil solution are near detection limits of standard colorimetric methods and when possible interference of silica, solution color or turbidity, or presence of colloids occur (Hamon and McLaughlin, 2002; Maertens et al., 2004). This situation is typically observed in highly weathered, P-deficient soils (Amer et al., 1969; Bühler et al., 2003; Dalal and Hallsworth, 1977; Wolf et al., 1986) and for which a better understanding of P availability and P acquisition by crops is particularly needed. The approach of Maertens et al. (2004) allows assessment of the labile P pool in these soils by combining isotopic exchange with resin extraction. An anion exchange membrane (AEM) samples P in the soil solution and a fraction of the isotopically exchanged P from the solid phase. Specific activity (SA) is then measured in the resin extract and assumed equal to the one in solution. The *L*-value, on the other hand, is often difficult to assess in low-P soils, as P-deficient plants remain small and a relatively large fraction of the P in the plant shoot is derived from the P in the seed (Amer et al., 1969). This contribution of seed P is difficult to estimate. Braum and Helmke (1995) assumed all seed P to be translocated to the plant shoot which represents a worst case scenario that allows *L*-values to be compared between species.

Bühler et al. (2003) used common bentgrass and determined the *L*-values in the third cut to eliminate interference by seed P. For maize and legumes, however, it is necessary to estimate P contribution correctly to determine *L*-values since they generally have large P reserves in their seed. A method was proposed by Brookes (1982) in which ryegrass was grown in (P-free) sand culture to which increasing amounts of labeled P was added and seed P contribution could be determined based on the dilution of the labile P by the seed P in the plant shoot. By relating the amount of seed P in the shoot to the shoot dry biomass, *L*-values determined in plants grown in soil could then be corrected for seed P contribution. This technique allows determination of *L*-values in plants with limited P uptake. This is particularly relevant to study effects of P application and plant effects on P acquisition by maize and legumes grown in P-deficient tropical soils.

The main objective of this study was to determine P mobilization of a legume relative to a cereal crop in soils with varying P supply by comparing *L*- and *E*-values. Cowpea and maize were grown in three P-deficient soils to which P was added at various rates and *L*-values were assessed. These *L*-values were corrected for seed contribution to the shoot P using relations determined in a separate plant growth experiment. Changes in the corresponding *E*-values during the time of plant growth were followed independently in an incubation experiment.

Materials and methods

Plant growth experiment 1 (L-value determination)

L-values were determined for maize (*Zea mays* L., cv oba super I) and two cowpea varieties (*Vigna unguiculata* L., cv Dan-Ila and cv IT-82D-716), grown in pots in a greenhouse at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. Average minimum and maximum temperatures during plant growth were 22 and 43 °C and average relative air humidity was 75%. Plants were grown in three representative soils from the Northern Guinea savanna zone (length of growing period: 151–180 days) of Nigeria, sampled near the villages of Danayamaka (11°21′ N, 7°51′ E) and Kasuwan Magani (10°24′ N, 7°42′ E), and at the Shika farm (11°11′ N, 7°35′ E). The soils at Danayamaka, Kasuwan Magani and Shika were classified as a Ferric Lixisol (FAO–UNESCO 1990), a Ferric Acrisol with a petroferric phase (FAO, 1991) and a Haplic Lixisol (FAO–UNESCO 1990), respectively. Topsoil (0–20 cm) was sampled from fallow land in June 2003, sun-dried and passed through a 4 mm sieve. Selected soil properties are presented in Table 1. All three soils are characterized by a low P status.

The experiment was carried out in four replications and included P additions as triple super phosphate (TSP, 20.2% P) at rates of 12.5 mg P kg^{-1} and 37.5 mg P kg^{-1}. These rates are equivalent to either 15 or 45 kg P ha^{-1} for a 10 cm plough layer and a bulk density of 1.2 g cm^{-3}. Before application, the TSP was ground manually with a pestle. Per pot, 2 kg of dry soil was added; the TSP was thoroughly mixed with the dry soil. Basal macronutrients (66.6 mg Ca, 66.6 mg K, 41.6 mg Mg and 55.0 mg S per pot) and micronutrients (1.66 mg Fe, 1.66 mg Mn, 1.66 mg Zn, 1.66 mg Cu, 0.166 mg Co, 0.166 mg B and 0.166 mg Mo

Table 1. Selected soil properties of the soil sampled at Danayamaka, Kasuwan Magani and Shika (topsoil: 0–20 cm)

	Danayamaka	Kasuwan Magani	Shika
Sand (%)	49	49	47
Silt (%)	34	30	32
Clay (%)	18	22	22
PH (H_2O)	5.3	4.8	5.9
Organic C (g C kg^{-1})	4.6	11.9	7.6
Kjeldahl N (g N kg^{-1})	0.36	0.95	0.57
Total P (mg P kg^{-1})	69	113	75
CEC ($cmol_c$ kg^{-1})	2.9	4.0	4.8
Ca^{2+} ($cmol_c$ kg^{-1})	1.2	2.2	2.1
Mg^{2+} ($cmol_c$ kg^{-1})	0.5	0.8	1.0
K^+ ($cmol_c$ kg^{-1})	0.2	0.1	0.4
Bray-I P (mg P kg^{-1})	3.69	2.89	3.22
Olsen-P (mg P kg^{-1})	2.35	2.09	2.49
Fe_{ox} (mg Fe kg^{-1})	402	711	528
Al_{ox} (mg Al kg^{-1})	314	673	372

Particle size analysis, pH (H_2O) (1:1), total N, Bray-I P and Olsen P (IITA, 1982); organic C (Amato, 1983); CEC and exchangeable bases by AgTU method (Chhabra et al., 1975); ammonium oxalate-extractable Fe and Al (Schwertmann, 1964); total P was determined by ashing 1 g of soil at 550 °C during 12 h and extraction with 0.5 *M* H_2SO_4 during 16 h.

per pot) were applied to the dry soil as a nutrient solution containing $CaCl_2$, KCl, $MgSO_4$, $FeCl_3$, $MnCl_2$, $ZnCl_2$, $CuCl_2$, $CoCl_2$, $Na_2B_4O_7$ and Na_2MoO_4 salts. Consequently, a solution of carrier-free ^{32}P-orthophosphate was added and the soil was labeled at 500 kBq kg^{-1}. After each addition of TSP, nutrient solution or ^{32}P solution, the soil was thoroughly homogenized by hand. Soils were brought to a final moisture content of 16.5% w/w. These moisture contents corresponded to 83, 78 and 70% of the field capacity of the soils from Danayamaka, Kasuwan Magani and Shika, respectively. Seeds were selected for homogeneity with individual weight within 2% of median seed weight. Median seed weight, P concentration and total P content of the seeds are presented in Table 2. One day after soil labeling, four seeds were planted in every pot. Five days after emergence, seedlings were thinned to one plant per pot. Plants were watered daily in the morning; the pots were placed on a balance and water was added to the original moisture content. To the pots in which maize was grown, N was added as a solution of NH_4NO_3 at a rate of 75 mg N kg^{-1}. The N was split applied: 15% of the total rate at planting, 15% at 1 week after planting (WAP), 20% at 2 WAP, 25% at 3 WAP and 25% at 4 WAP. For maize, a control without N addition was included. Legumes were grown both with and without N addition.

Plants were harvested at 5 WAP; shoots were cut at 1 cm above the soil surface, and dried in an oven at 70 °C during 3 days. After determining the shoot dry weight, plant shoots were cut in pieces < 5 mm and an aliquot of 0.5 g of every shoot was digested in hot H_2SO_4 using a method adapted from Novozamsky et al. (1983). The P and N concentration in the digests were determined colorimetrically using automated analysis (TECHNICON™ AUTOANALYZER™ II System) by the method of Murphy and Riley (1962) for P and a method adapted from Searle (1984) for N. ^{32}P activity in the digest was determined by liquid scintillation counting. To 5 mL of digest, 15 mL of scintillation cocktail (ULTIMA Gold XR) was added and samples were counted in a Beckman LS5000CE liquid scintillation system. *L*-values, corrected for seed P, were calculated using

$$L = D/\mathrm{SA} = D/({}^{32}\mathrm{P}_{\mathrm{shoot}}/{}^{31}\mathrm{P}_{\mathrm{dfsoil}}) = D/({}^{32}\mathrm{P}_{\mathrm{shoot}}/({}^{31}\mathrm{P}_{\mathrm{shoot}} - {}^{31}\mathrm{P}_{\mathrm{dfseed}})) \quad (1)$$

with L the L-value (mg P kg^{-1}); D the applied dose (kBq kg^{-1}); SA the specific activity of the P in the plant shoot taken up from the soil [kBq (mg P)$^{-1}$]; $^{32}P_{shoot}$ the total ^{32}P activity in the shoot (kBq); $^{31}P_{dfsoil}$ the amount of P in the shoot taken up from the soil (mg P); $^{31}P_{shoot}$ the total amount of P in the shoot (mg P) and $^{31}P_{dfseed}$ the amount of P in the shoot translocated from the seed (mg P). All P quantities in the shoot are expressed per individual plant. The calculation of $^{31}P_{dfseed}$ is described below.

Table 2. Median seed weight, P concentration and total P content of the seeds of maize (cv oba super I) and cowpea (cv Dan-Ila and cv IT−82D−716)

	Median seed weight (g)	P concentration (%)	Total P content (mg P)
Maize			
Oba super I	0.254	0.363	0.923
Cowpea			
Dan-Ila	0.167	0.360	0.600
IT-82D-716	0.122	0.355	0.435

Plant growth experiment 2 (determination of seed P contribution in shoot)

In an additional plant growth experiment, the amount of seed P, which was translocated to the shoot was determined by a method adapted from Brookes (1982). The maize and cowpea varieties were grown in acid washed, P-free sand, to which increasing amounts of P with a known specific activity were applied. Sea sand was sampled on Lekki beach, Lagos, Nigeria. The pH (H_2O) (soil:solution = 1:1) of the sand after washing with H_2SO_4 and thorough rinsing equaled 6.13. The experiment was carried out in three replications. Per pot, 2.5 kg of sand was added. A basal nutrient solution containing Ca, K, Mg, S, Fe, Mn, Zn, Cu, Co, B and Mo was added; nutrients were applied at the same rates as in the first plant growth experiment. To both maize and legumes, N was split applied as a solution of NH_4NO_3 at a rate of 75 mg N kg^{-1}, similarly as in the first plant growth experiment. P was added as a solution of KH_2PO_4 at rates of 1.25, 4.5, 12.5 and 37.5 mg P kg^{-1}; a blank was included

as control. Consequently, a solution of carrier-free ^{32}P-orthophosphate was added and the sand was labeled at 500 kBq kg^{-1}. No ^{32}P was added to the control. The nutrients, ^{31}P and ^{32}P were manually mixed into the sand and after homogenizing thoroughly, the sand was brought to a final moisture content of 12% w/w. Planting, harvesting and analysis of the shoot biomass was done similarly as in the first plant growth experiment.

The amount of P in the shoot translocated from the seed was calculated as

$$^{31}P_{dfseed} = {}^{31}P_{shoot}(1 - (SA_{shoot}/SA_{initial})) \quad (2)$$

with $^{31}P_{dfseed}$ the amount of P in the shoot translocated from the seed (mg P); $^{31}P_{shoot}$ the total amount of P in the shoot (mg P); SA_{shoot} the ratio of $^{32}P_{shoot}$ over $^{31}P_{shoot}$ [kBq (mg P)$^{-1}$] and $SA_{initial}$ the initial ratio of ^{32}P over ^{31}P of the P applied to the sand [kBq (mg P)$^{-1}$]. As the sand did not contain any P, isotopic dilution of the added ^{32}P in the shoot was caused by P translocation from the seed. In the control, all P in the shoot originated from the seed.

Soil incubation experiment (E-value determination)

Changes in the *E*-value were assessed during 35 days in an incubation experiment. A 150 g aliquot of the three soils with the various treatments (0, 12.5, and 37.5 mg TSP-P kg^{-1}) was incubated at a constant moisture content of 16.5% w/w. The TSP was first added to the dry soil and thoroughly homogenized. Consequently, a basal nutrient solution containing N, Ca, K, Mg, S, Fe, Mn, Zn, Cu, Co, B and Mo was added; nutrients were added at the same rates as in the plant growth experiments. The soil was labeled with a carrier-free ^{32}P-orthophosphate solution at 20 kBq g^{-1} and thoroughly homogenized. Duplicate soil samples were incubated at 28 °C in closed containers, which were opened daily for aeration. After 8 h, and after 6, 13, 20, 27 and 34 days, a subsample was taken and the *E*-value was determined using a method adapted from Maertens et al. (2004). An aliquot of fresh soil equivalent to 2.5 g dry soil was weighed into 30 mL oak ridge polyethylene centrifuge tubes, 25 mL of distilled water was added (soil:solution ratio = 1:10) and samples were shaken during 16 h on a vertical shaker at 250 mot min^{-1}. Consequently, two AEM strips in HCO_3^--form (6×1 cm, product 55164 2S; BDH Laboratory supplies, Poole, England) were added to the suspension and the samples were remounted on the shaker for 8 h. The AEM strips were removed and rinsed with distilled water, transferred to 20 mL of HCl 0.5 *M* and shaken for another 16 h. The P concentration in the resin extract was determined using the method of Van Veldhoven and Mannaerts (1987). ^{32}P activity in the resin extract was determined by liquid scintillation counting. To 1 mL of the resin extract, 4 mL of scintillation cocktail (ULTIMA Gold XR) was added and samples were counted in a Beckman LS5000CE liquid scintillation system. The *E*-value was calculated as

$$E = D/SA_{resin} = D/(^{32}P_{resin}/^{31}P_{resin}) \quad (3)$$

with *E* the *E*-value (mg P kg^{-1}); *D* the applied dose (kBq kg^{-1}); SA_{resin} the specific activity on the resin [kBq (mg P)$^{-1}$]; $^{32}P_{resin}$ the ^{32}P activity in the resin extract (kBq L^{-1}) and $^{31}P_{resin}$ the P concentration in the resin extract (mg P L^{-1}).

Distribution coefficients (K_D, the ratio of P adsorbed over P in solution, L kg^{-1}) were determined on the control soils. To 3 g of dry soil, 29 mL of 0.01 *M* $CaCl_2$ was added and samples were shaken end-over-end during 16 h. Consequently, 1 mL of a carrier-free ^{32}P solution (0.9 MBq mL^{-1}) was added and the samples were remounted on the shaker. After 48 h of isotopic exchange, the samples were centrifuged (20 min at 3100 *g*) and filtered (MF-Millipore, 0.45 μm). ^{32}P activity in the filtrate was determined by liquid scintillation counting.

Statistical analyses

Regression analysis and analysis of variance (ANOVA) was done using the SAS program (SAS, 1999). Significance of difference between soils, treatments, species and varieties in P uptake and *L*-values and effects of N addition on P uptake and *L*-values was determined using the GLM procedure with the ESTIMATE statement. The uncertainty in the *L*-value due to the uncertainty in the estimated P_{dfseed} was taken into account when comparing *L*- and *E*-values and *L*-values among species. Underestimation of the seed contribution to the shoot P leads to an

overestimated *L*-value and *vice versa* (Equation 1). At small P uptake, a large proportion of P in the plant shoot is derived from the seed and the variability due to the estimation of P_{dfseed} outweighs the variability of the other parameters in Equation (1). To consider this, a confidence interval of *L*-values was derived by applying the 95% lower and upper bounds of P_{dfseed} in Equation (1), separately for every replicate. Two *L*-values were only considered significantly different, if the lower bound of the largest *L*-value (calculated using the upper bound of P_{dfseed}) was significantly ($P < 0.05$) larger than the upper bound of the smallest *L*-value (calculated using the lower bound of P_{dfseed}). This was tested by conducting a one-sided *t*-test using the TTEST procedure. Differences between *L*- and *E*-values were tested similarly for every treatment individually. The *L*-value was only considered significantly larger than the corresponding *E*-value if the difference between the lower bound of the *L*-value (calculated using the upper bound of P_{dfseed}) and the *E*-value was significantly larger than zero. Changes in time in resin-extractable P and *E*-value in the incubation experiment were examined using the GLM procedure with the REPEATED and LSMEANS statement.

Results

Soil incubation experiment (E-value determination)

Resin-extractable *P* was small in the three control samples (less than 1 mg P kg^{-1}) and was significantly ($P < 0.001$) increased by the application of TSP at both rates on the first day after application (11–16 mg P kg^{-1} at 37.5 mg P kg^{-1} and 3–5 mg P kg^{-1} at 12.5 mg P kg^{-1}). During the incubation period, resin-extractable P decreased readily and after 5 weeks, resin-extractable P was reduced by on average 60% in the treatments with TSP application (no data shown).

Changes in *E*-value as affected by the application of TSP during the incubation are presented in Figure 1. *E*-values in the three control soil samples were similar and small (4–6 mg P kg^{-1} at 8 h after labeling). *E*-values increased significantly ($P < 0.01$) with time during the first 2 weeks of incubation with 3.9–5.7 mg P kg^{-1} and reached an equilibrium of 9–10 mg P kg^{-1}. As such, the labile P quantity (*E*-value) after 35 days of incubation comprises 9–14% of the total P content of the control soils (Table 1). The *E*-value was significantly ($P < 0.001$) increased by the application of TSP at both rates. About 60–70% of the applied TSP-P was recovered in the *E*-value after 35 days of incubation. Similarly as in the controls, the *E*-value increased significantly during the first 2–4 weeks of incubation with 3.1–5.3 mg P kg^{-1}, except in the Shika soil with TSP applied at 37.5 mg P kg^{-1}, where the variation was comparatively larger and the *E*-value did not change significantly ($P < 0.05$) with time during the incubation period.

The K_D of the control soils equaled 3300, 4600 and 3600 L kg^{-1} for the soils from Danayamaka, Kasuwan Magani and Shika, respectively.

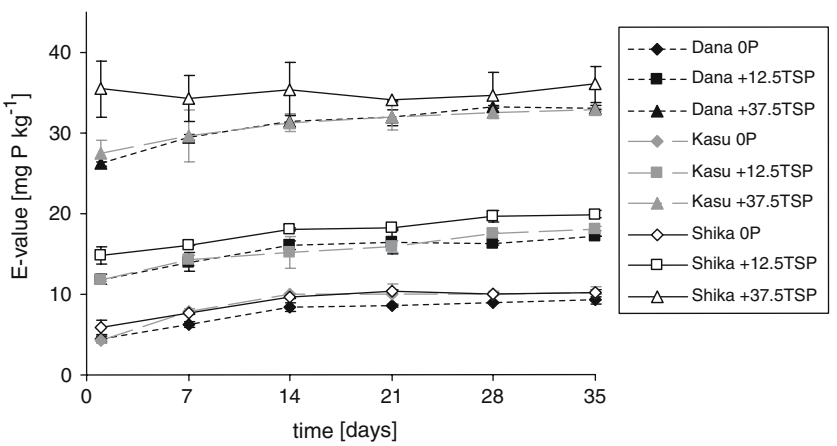

Figure 1. Isotopically exchangeable P during soil incubation in three soils from the NGS as affected by TSP application at rates of 12.5 and 37.5 mg P kg^{-1}. Error bars represent two standard deviations of the mean value ($n = 2$).

These values indicate that the sorption capacities of the three soils are similar and relatively large. P concentrations in the extract were smaller than the detection limit of the malachite green method (8 μg P L^{-1}) but could be calculated using the *E*-value and the K_D-value (Maertens et al. 2004) and equaled 1 μg P L^{-1} for the Danayamaka and Kasuwan Magani soil and 2 μg P L^{-1} for the Shika soil. As such, all three soils are P deficient as shown by the small labile P quantity (*E*-value) and the small P intensity (P concentration in soil solution).

Plant growth experiment 2 (determination of seed P contribution in shoot)

Shoot dry weight was significantly increased by the addition of the P solution. For maize, shoot dry weight varied between 0.3 and 3.5 g dry matter (DM). For the cowpea varieties Dan-Ila and IT-82D-716, shoot dry weight varied between 0.4 and 5.3 g DM and between 0.3 and 3.6 g DM, respectively. Total shoot P contents were small (< 0.3 mg P) when no P was applied and increased up to 5.1, 5.9 and 3.2 mg P for maize, cowpea cv Dan-Ila and cowpea cv IT-82D-716, respectively, when P was added at a rate of 37.5 mg P kg^{-1}. The amount of P derived from the seed was plotted as a function of the total amount of P in the shoot (Figure 2). By non-linear regression, an exponential function (Equation 4) was fitted to the data.

$$^{31}P_{dfseed} = A \times (1 - EXP(B \times {}^{31}P_{shoot})) \qquad (4)$$

Parameter estimates for the exponential relation are presented in Table 3. The model fitted predicts that the percentage of seed P translocated to the shoot increases from about 30% of the total seed P for maize and about 40% of the total seed P for cowpea under most P-deficient conditions to a maximum of 77% for maize and 90% for cowpea at optimal P supply. These exponential relationships were used to correct *L*-values for seed P contribution in the first plant growth experiment.

Plant growth experiment 1 (L-value determination)

Shoot dry weights varied between on average 1.1 and 6.8 g DM for maize and between 0.8 and 3.5 g DM for cowpea. Both maize and cowpea were P-deficient in the control soils. Maize leaves showed a purplish coloration; cowpea plants dropped their older leaves. P concentrations in the biomass were generally smaller than 0.1%. The application of TSP at a rate of 37.5 mg P kg^{-1} significantly ($P < 0.001$) increased shoot dry weights and no signs of P deficiency were observed. Shoot dry weights in treatments with TSP applied at 12.5 mg P kg^{-1} were, in general, larger than in the control (no data shown) but smaller than in the treatment with TSP addition at a rate of 37.5 mg P kg^{-1}. Regression analysis showed a significant ($P < 0.001$) increase in shoot dry matter with the rate of applied TSP, which was different among species. The slope for maize was significantly ($P < 0.001$) larger than for both cowpea varieties. However, the relation between relative shoot dry matter (shoot dry matter divided by the average shoot dry matter in the treatment with 37.5 mg P kg^{-1} TSP) and the rate of TSP applied did not differ among species.

Shoot P uptake by maize and the two cowpea varieties were comparable and responded similarly to P application (Table 4). Largest shoot P contents were observed in treatments with TSP applied at a rate of 37.5 mg P kg^{-1}. Total shoot P contents in the treatments with TSP at a rate of 12.5 mg P kg^{-1} were smaller than in the treatment with TSP at 37.5 mg P kg^{-1}, but generally larger than the control value. Significant ($P < 0.05$) effects of N application on total shoot P content of cowpea were only observed in TSP treatments at 37.5 mg P kg^{-1} in the Danayamaka soil for both cowpea varieties and in the Shika soil for the IT-82D-716 variety; shoot P uptake was smaller when no N was added. Regression analysis showed a significant increase ($P < 0.001$) in shoot P content with the rate of TSP applied, which slightly but significantly ($P < 0.05$) differed among species. The slope was significantly larger for maize than for the cowpea varieties. The relation between relative shoot P content (shoot P content divided by the average shoot P content in the treatment with 37.5 mg P kg^{-1} TSP) and the applied TSP rate did not differ among species.

Average seed P contributions, calculated using Equation (4) and parameters in Table 3, are also presented in Table 4. For both maize and cowpea, the seed contribution to shoot P is considerable in the control soils: the proportion

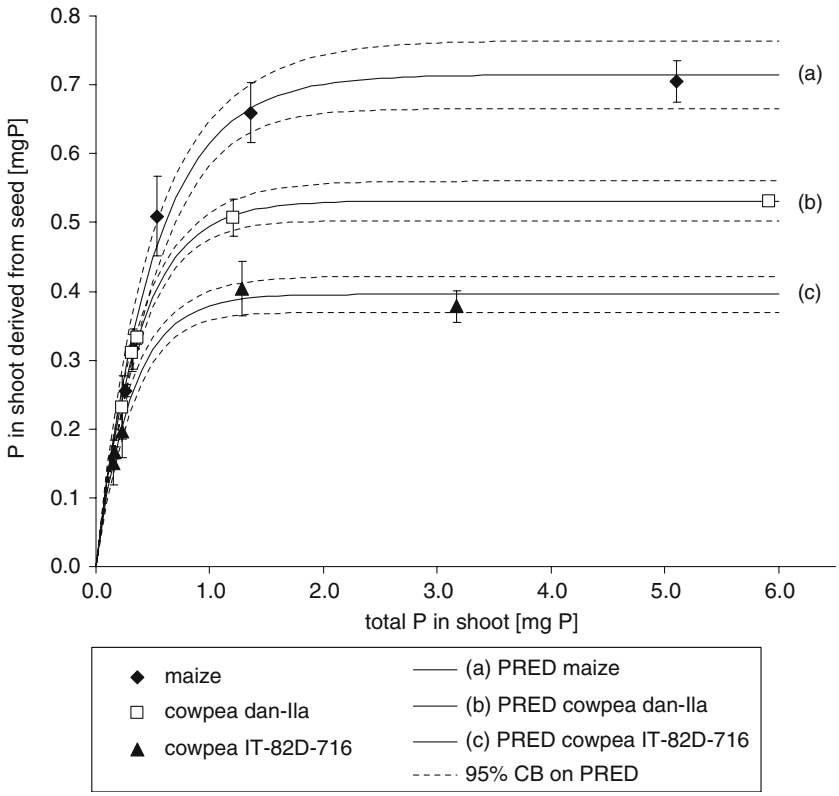

Figure 2. P in shoot derived from seed as a function of total P in shoot for maize and two cowpea varieties grown in P-free sand with different additions of labeled P solutions. Solid lines represent predicted values using non-linear regression; dotted lines represent upper and lower 95% confidence bounds for the mean predicted value. Error bars represent two standard errors of the mean value ($n = 3$).

of P in the plant shoot derived from the seed is larger than 50%. At the largest P application rate however, the seed contribution to shoot P is relatively much smaller compared to the controls.

Table 3. Parameters estimates for the exponential function (Equation 4) fitted by non-linear regression, relating P in the shoot derived from the seed to the total P in the shoots of maize and two cowpea varieties

	n	R^2	A (mg P)	B (mg P^{-1})
Maize				
Oba super I	15	0.94	0.715	−1.973
Cowpea				
Dan-Ila	13	0.97	0.531	−2.674
IT-82D-716	14	0.96	0.396	−3.177

n = number of observations; R^2 = pseudo $R^2 = 1 - SS$ (residual)/SS (corrected). Three data points were not considered in the regression as for those points, P in shoot derived from seed exceeded the total P content of the seed.

The proportion of P in the plant shoot derived from the seed is less than 10% at the application rate of 37.5 mg P kg^{-1}. This has important implications for the calculation of the *L*-values. In the control soils, the error on the *L*-value is dominated by the error on the estimated seed P contribution due to the uncertainty on parameters 'A' and 'B' in Equation (4). At the largest P application rate contrarily, the error due to estimation of seed contribution to shoot P becomes negligible compared to the variation between replicate *L*-values, because of the larger P uptake from the soil and the smaller proportion of P in the plant shoot derived from the seed.

The *L*-values for maize and cowpea, corrected for seed contribution, are presented in Table 5. The *L*-values for control soils in maize were small: 10–20 mg P kg^{-1} in all soils. *L*-values were significantly ($P < 0.01$) larger in treatments with TSP application. The *L*-values for cowpea were

Table 4. Total P (mg P) in shoots of maize and two cowpea varieties grown in three soils from the NGS as affected by TSP application at rates of 12.5 and 37.5 mg P kg^{-1}, with and without an N application at a rate of 75 mg N kg^{1} ($n = 4$) Numbers between brackets are amounts of P derived from the seed (mg P), calculated using Equation (4) and parameters in Table 3

		Maize (oba super I)	Cowpea (Dan-Ila)		Cowpea (IT-82D-716)	
Danayamaka						
0P	+N	1.15 (0.64) c	0.72 (0.41) b		0.65 (0.33) b	
	−N	1.33 (0.66)	1.10 (0.49)	C	0.95 (0.36)	C
12.5TSP	+N	3.88 (0.71) b	nd		nd	
	−N	nd	2.94 (0.53)	B*	2.40 (0.39)	B*
37.5TSP	+N	8.46 (0.71) a	7.52 (0.53) a		7.32 (0.40) a	
	−N	nd	4.56 (0.53)	A*	5.71 (0.40)	A*
Kasuwan Magani						
0P	+N	0.65 (0.51) b	0.74 (0.45) b		0.49 (0.30) b	
	−N	0.76 (0.56)	0.65 (0.44)	C	0.52 (0.30)	B
12.5TSP	+N	1.79 (0.68) b	nd		nd	
	−N	nd	2.82 (0.53)	B	1.92 (0.39)	B
37.5TSP	+N	7.77 (0.71) a	8.17 (0.53) a		7.93 (0.40) a	
	−N	nd	8.11 (0.53)	A	6.45 (0.40)	A
Shika						
0P	+N	1.11 (0.63) c	0.84 (0.47) b		0.85 (0.37) b	
	−N	0.93 (0.60)	0.86 (0.47)	C	0.84 (0.35)	B
12.5TSP	+N	2.57 (0.70) b	nd		nd	
	−N	nd	3.84 (0.53)	B	2.24 (0.40)	B*
37.5TSP	+N	7.61 (0.71) a	6.01 (0.53) a		8.08 (0.40) a	
	−N	nd	6.55 (0.53)	A	5.98 (0.40)	A*

nd: not determined; small letters represent significant ($P < 0.05$) differences in total shoot P between P treatments with N application (+N) within soil and within plant variety; capital letters represent significant ($P < 0.05$) differences in total shoot P between P treatments without N application (−N) within soil and within plant variety; * represents a significant ($P < 0.05$) effect of N application on shoot P content within P treatment, soil and plant variety.

larger than for maize, but only in some treatments and soils. In the control soils from Danayamaka and Kasuwan Magani, some significantly larger *L*-values were observed for cowpea. Also in the treatment with TSP at 12.5 mg P kg^{-1} in the Kasuwan Magani soil, the *L*-value observed for the cowpea variety Dan-Ila was significantly ($P < 0.05$) larger than the *L*-value for maize. In the treatments with TSP at a rate of 37.5 mg P kg^{-1}, *L*-values for maize and cowpea were not significantly different in any of the soils.

Discussion

We observed significant effects of N application on the P uptake of both cowpea varieties grown in the Danayamaka and Shika soils with TSP applied at a rate of 37.5 mg P kg^{-1}. P uptake was larger when 75 mg N kg^{-1} was applied as NH_4NO_3. The N concentrations in the cowpea biomass were larger ($P < 0.05$) when N was applied (on average 3.4% N) compared to when no N was applied (on average 1.8% N). As N application did not affect *L*-values, P availability was not influenced by N application. At a rate of 37.5 mg P kg^{-1}, P availability was not limiting growth and the P uptake increases with N application due to a direct N effect. Probably, fixation of atmospheric N could not provide N at a rate allowing maximal growth, contrary to an application of NH_4NO_3. In the Kasuwan Magani soil however, biomass N concentrations were larger (3.4% without N vs. 4.3% with N application) and no significant N effects on P uptake were observed.

The relative shoot P content of maize was well correlated with P in solution (0.01 *M* $CaCl_2$ extraction), HCO_3^--resin extractable P and the *E*-value measured 8 h after labeling. Correlation coefficients (r) varied in between 0.87 and 0.90 (all significant at $P < 0.001$). This indicates that the *E*-value as assessed by the protocol of

Table 5. The *L*-values corrected for seed contribution for maize and two cowpea varieties grown in three soils from the NGS as affected by TSP application at rates of 12.5 and 37.5 mg P kg^{-1}, with and without an N application at a rate of 75 mg N kg^{-1} (n = 4) and corresponding *E*-values with standard errors ($n = 2$) determined in a separate incubation experiment, averaged over 14–35 DAP

		L-value			*E*-value
		Maize (oba super I)	Cowpea (Dan-Ila)	Cowpea (IT-82D-716)	
Danayamaka					
0P	+N	8.7 B	11.0 AB	12.2 AB*	8.8 (±0.16)
	−N	7.5 B	10.9 AB	16.1 A	nd
12.5TSP	+N	23.1 A*	nd	nd	16.5 (±0.32)
	−N	nd	22.5 A*	21.9 A	nd
37.5TSP	+N	43.8 A	43.2 A*	40.6 A*	32.4 (±0.12)
	−N	nd	45.9 A*	45.5 A*	nd
Kasuwan Magani					
0P	+N	20.8 AB	28.6 AB*	30.9 AB*	10.5 (±0.31)
	−N	17.9 B	33.3 A*	29.6 AB*	nd
12.5TSP	+N	27.4 B*	nd	nd	16.7 (±0.15)
	−N	nd	35.9 A*	29.5 AB*	nd
37.5TSP	+N	46.8 A*	44.6 A*	47.3 A*	32.1 (±0.58)
	−N	nd	43.3 A*	42.2 A*	nd
Shika					
0P	+N	18.1 A	21.6 A*	23.2 A*	10.1 (±0.14)
	−N	15.2 A	15.9 A	17.5 A	nd
12.5TSP	+N	24.7 A	nd	nd	18.9 (±0.24)
	−N	nd	27.2 A*	24.8 A	nd
37.5TSP	+N	41.9 A	40.0 A	42.4 A*	35.1 (±1.45)
	−N	nd	42.5 A	39.8 A	nd

nd: not determined; capital letters represent a significant ($P < 0.05$) difference in *L*-value between species or varieties within soil and P treatment; * represents an *L*-value significantly differing ($P < 0.05$) from the corresponding *E*-value. All comparisons take into account the error on the *L*-values due to the uncertainty in the estimated seed P contribution.

Maertens et al. (2004) correctly reflects treatment effects and that the isotopically exchangeable P pool correlates with the available P pool in these three soils from the NGS.

In Figure 3, the ratio of the *L*-value for maize and cowpea in the control and TSP treatments to the average *E*-value during the last 3 weeks of incubation (14–35 days after labeling) was plotted vs. the total shoot P content. Most of the P uptake occurred during these last 3 weeks and *E*-values did not change with time during that period (Figure 1). As such, this *E*-value should correspond with the *L*-value, if no P mobilization by the plant occurred. Average L/E ratios for maize were 1.4 ± 0.3 and did not differ significantly between P treatments. Only in the TSP treatments in the Danayamaka and Kasuwan Magani soil, *L*-values were slightly but significantly ($P < 0.05$) larger than the *E*-values. For both cowpea varieties, *L*-values corresponded to *E*-values in the Danayamaka and Shika soil, similarly as observed for maize. In the Kasuwan Magani soil however, L/E-ratios were 3.1 ± 0.2 in the control and 2.0 ± 0.1 in the TSP treatment at 12.5 mg P kg^{-1}, both significantly ($P < 0.05$) larger than 1. In the TSP treatment at 37.5 mg P kg^{-1}, the L/E ratio was only slightly larger than 1, similarly as in the other two soils. This increase in *L*-value indicates that when P deficiency occurs, cowpea can exert certain mobilization mechanisms and access non-labile P in the Kasuwan Magani soil. Maize is unable to access this non-labile P. In the two other soils, cowpea is unable to access non-labile P.

The observed P mobilization by cowpea agrees with numerous other studies in which legumes are found to access non-labile P (Braum and Helmke, 1995; Hocking et al., 1997, Horst

et al., 2001). This mobilization of P can be attributed to the secretion of organic acids or phosphatases (Kamh et al., 1999) or to pH changes in the rhizosphere (Gahoonia and Nielsen, 1992). Various authors also found these mobilization mechanisms to occur only under P deficiency stress (Hoffland et al., 1989; Keerthisinghe et al., 1998; Neumann and Römheld, 1999; Zhang et al., 1997). Jones (1998) suggested that it was unlikely that more P is mobilized than the plant's own requirements and P mobilization mechanisms are regulated by the internal P concentration. Our results in the Kasuwan Magani soil confirm this, as P mobilization by cowpea was only observed in treatments with suboptimal P supply.

Although the increase in *L*-value signifies an increase in the soil P pool that can be accessed, there were no indications that P uptake by the legume was increased relative to the P uptake by maize. In the control and TSP treatment at 12.5 mg P kg^{-1} in the Kasuwan Magani soil, cowpea plants remained P-deficient and the relation between relative P uptake and the TSP rate applied did not differ between cowpea and maize. Although the control *L*-value equaled almost 70% of the *L*-value in the treatment with TSP applied at a rate of 37.5 mg P kg^{-1}, P uptake remained more than 10-fold smaller. Most likely, the P concentration in the soil solution (P intensity) was limiting P uptake. The uptake of P by legumes is predominantly controlled by the P intensity (Moody et al., 1983, 1990), but the P quantity (the soil P in the solid fraction, which can readily exchange with P in the soil solution) also affects P uptake. The P concentration in solution required to obtain a certain P uptake decreases as the P quantity in equilibrium with the P in solution increases (Holford and Mattingly, 1976). Through P mobilization mechanisms exerted by the legumes, some non-labile P may become exchangeable, but because of the relatively large sorption capacity of the soil, the P concentration in the soil solution may increase only very little. In the control in the Kasuwan Magani soil, the *L*-value in cowpea cv Dan-Ila was 15 mg P kg^{-1} larger than the *L*-value in maize and equal to the *E*-value in the TSP

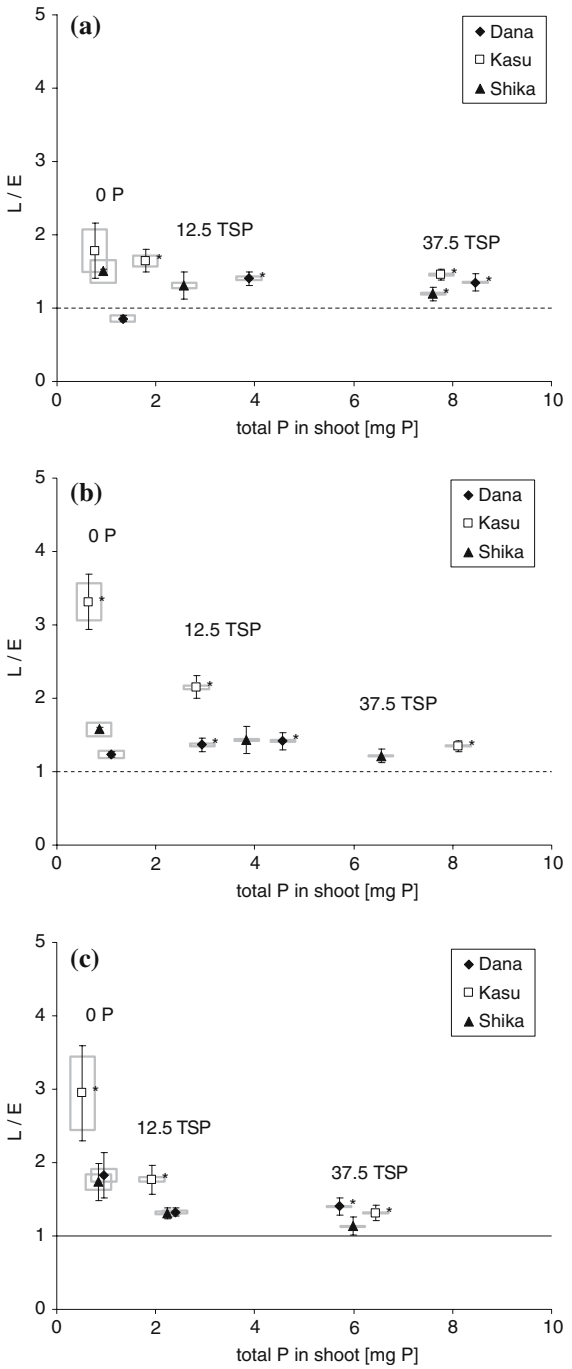

Figure 3. Ratio of *L*-value and *E*-value plotted vs. total P content of the shoot for maize cv oba super I (a) and cowpea cv Dan-Ila (b) and cv IT−82D-716 (c) grown in three soils from the NGS with TSP application at rates of 0, 12.5 and 37.5 mg P kg^{-1}. *L*-values were corrected for seed P contribution. Error bars represent two standard errors of the mean ($n = 4$). Rectangular bars represent the range in mean L/E ratio originating from the error on the *L*-value due to the estimation of seed contribution to shoot P. * represents an L/E ratio significantly differing ($P < 0.05$) from 1.

treatment at 37.5 mg P kg^{-1}. As such, the P quantities in the control and in the TSP treatment at 37.5 mg P kg^{-1} were similar for cowpea. The P intensities however, differed significantly. After 8 h of incubation, a subsample was taken and the P concentration was determined in a 10:1 0.01 *M* $CaCl_2$-extract after shaking during 48 h on a vertical shaker. These concentrations equaled 0.001 mg P L^{-1} in the control and 0.14 mg P L^{-1} in the TSP treatment at 37.5 mg P kg^{-1}. It is unlikely that through rhizosphere processes, cowpea was able to increase the P intensity in the rhizosphere of the control to the same level as in the TSP treatment at 37.5 mg P kg^{-1}. Consequently, rhizosphere processes caused a larger dilution of the ^{32}P isotope in the soil, and the P taken up by the plant roots had a smaller specific activity. The P uptake however remained unaffected and small. Because of the very small P intensity, the observed increase in the accessible P quantity for cowpea in the Kasuwan Magani control soil is not sufficient to significantly increase P uptake.

Our results show evidence that cowpea can access non-labile P but these varieties are most likely not as efficient in mobilizing P as other legumes such as white lupin or pigeon pea. P stressed white lupin excretes large amounts of citrate (Keerthisinghe, 1998), while pigeon pea is known to excrete piscidic acid (Ae et al., 1993) and to have a large phosphatase activity (Kamh et al., 1999). Hocking et al. (1997) showed that *L*-values in white lupin and pigeon pea were 4- and 2-fold larger than in a cereal crop like wheat; P uptake was 10- and 2-fold larger, respectively. In our study, we observed a 2-fold increase in *L*-value for cowpea compared to maize in the control of the Kasuwan Magani soil. However, the *L*-values in maize grown in our control soils (15 mg P kg^{-1}) were small compared to the *L*-value in wheat grown in the soil studied by Hocking et al. (1997) (250 mg P kg^{-1}). Likely, the quantity of P mobilized by the legumes in our study was not sufficient to increase P uptake.

We have shown that cowpea is able to access non-labile P, contrary to maize. However, different varieties need to be tested for their ability to mobilize non-labile P. Recent studies have investigated genotypic differences of cowpeas in P efficiency. Sanginga et al. (2000) showed that there exist large differences in P requirements and P use efficiency of the cowpea lines available at IITA. Using a modeling approach and measurements of root and root hair length, Krasilnikoff et al. (2003) found large differences between cowpea lines to dissolve and absorb non-Olsen P. They showed that the development of an extensive root system with long root hairs was the main strategy of acquiring soil P for varieties IT-82D-716 and Dan-Ila. Other varieties showed to rely more on root-induced processes to mobilize sparingly soluble P. In our study, both IT-82D-716 and Dan-Ila were able to access non-labile P, but this did not enable them to increase P uptake. It is therefore relevant to investigate more cowpea lines for their ability to access and utilize non-labile P. By incorporating P mobilizing legumes, which improve the use efficiency of fertilizer P in these soils with large sorption capacity, overall P fertility in a rotation system with maize could be improved. In conclusion, there is scope for identifying P mobilizing varieties through the use of isotopic dilution techniques. Detection limitations and interferences can be overcome and P mobilization by legumes can be assessed in low-P soils.

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