

Residual phosphorus effects and nitrogen \times phosphorus interactions in soybean–maize rotations on a P-deficient Ferralsol

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Abstract Legume-cereal rotations are an essential component of integrated soil fertility management in low-input cropping systems, but strategies are needed to increase phosphorus (P) fertilizer use efficiency in such systems. These may include preferential targeting of P to one of the crops in the rotation cycle, the use of P-efficient genotypes, and the optimization of the rates of P fertilizer used. A field trial was conducted to evaluate the effects of increasing P fertilizer rates (0, 11, 22 and 44 kg P ha⁻¹, added as triple super phosphate) applied to three soybean genotypes grown on a P-deficient Ferralsol, on the nitrogen (N) and P nutrition of a

subsequent maize crop. In addition, a greenhouse trial was set up to assess N, P and other rotation effects of three soybean genotypes on a subsequent maize crop relative to a maize–maize rotation at high and low P supply. In the field trial, soybean did not respond to increasing P rates, but residual P effects improved maize grain yields by up to 90 %. Ear leaf (field trial) and shoot (pot trial) P concentrations increased by applying N to maize, demonstrating important N \times P interactions. The pot trial did not reveal a positive rotation effect of soybean on maize beyond the mere N-benefit, showing that soybean was not able to improve P availability to maize after correcting for the N-effect. No variation in rotation effects on maize among soybean genotypes was observed. Because of the absence of effects of the soybean crop on P availability to maize, opportunities to increase P fertilizer use efficiency in soybean–maize rotations mainly reside in maximizing P uptake by each crop separately and in matching P fertilizer rates with crop demand.

Keywords Phosphorus fertilizer use efficiency · Soybean genotypes · Soybean–maize rotation · Residual effects · Nutrient interactions · Rotation effects

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Introduction

In many acidic, strongly weathered soils in Western Kenya, crop production is constrained by nitrogen

(N) and phosphorus (P) deficiency. Because of population pressure fields are often continuously cropped. In combination with low or absent use of fertilizers, this triggers a spiral of ever-decreasing soil fertility and crop production levels. The cultivation of legumes with a high N fixation potential is seen as a low-cost option to increase N availability in the soil and enhance production levels of other crops in the rotation cycle such as a subsequent maize crop. Growing legumes does, however, not necessarily lead to improved soil fertility (Vanlauwe and Giller 2006) and the net N contribution may vary widely across species and genotypes (Sanginga et al. 1997; Singh et al. 2003). Positive N balances after a season of legume cropping are obtained only when the amount of N fixed by the legume is larger than the amount of N removed with the grains at harvest (Giller and Cadisch 1995). In this context, the International Institute for Tropical Agriculture (IITA) developed ‘dual-purpose’ soybean (*Glycine max* (L.) Merr) genotypes which produce, beside good grain yields, a large amount of leafy biomass resulting in a low harvest index. Nevertheless, even when genotypes with a high N fixation potential and a low harvest index are used, N balances may remain low due to stresses such as drought, nutrient deficiencies or toxicities, soil acidity or high soil temperature which result in poor growth of the legume and/or poor growth of the rhizobia (Zahran 1999; Vanlauwe and Giller 2006). Among those stresses, P deficiency has been identified as a major factor limiting N fixation (Sanginga 2003). It is known that both nodulation and N fixation are highly dependent on P supply (Zahran 1999), and that soybean plants relying on N fixation require more P than those supplied with mineral N (Cassman et al. 1981). The application of considerable amounts of P fertilizers to the legume crop may be needed to overcome this problem. Moreover, on soils with low P availability, P application to the subsequent maize crop may equally be needed to ensure good grain yields. Ways to optimize the use efficiency of P fertilizers in soybean–maize rotations include fine-tuning of the quantities of fertilizer used and the use of phosphorus-efficient legume and maize genotypes. Further, there may be allocation opportunities to optimize P fertilizer use efficiency at the system level. Several authors have suggested that it may be beneficial to target P to the legume phase of a legume–cereal rotation compared to the more common practice

of targeting P to the cereal, which is often the staple crop and hence considered more important by farmers (Carsky et al. 2001; Zingore et al. 2008). Targeting P to the soybean phase of a soybean–maize rotation may increase levels of N fixation and lead to increased benefits to the maize crop in terms of N nutrition. In terms of P nutrition, it has been shown that a maize crop can largely benefit from residual P applied to a legume in the previous season (Zingore et al. 2008; Kihara et al. 2010).

In addition to rotation effects related to improved N uptake and to residual P effects, other rotation effects may have an impact on productivity in a soybean–maize rotation. Maize yield increases ranging from 20 to 130 % have been reported for maize grown after soybean relative to maize grown after maize (Sanginga et al. 2002). These rotation effects were mostly ascribed to a N carry-over effect, but some authors have suggested that rotational effects of legumes on a subsequent crop go beyond improved N nutrition (Vanlauwe et al. 2000a, b; Sanginga et al. 2002; Kihara et al. 2007). Kamh et al. (2002) found that some legumes enhanced maize dry matter yield and P uptake of subsequent maize plants, and attributed the positive rotation effect to a capacity of these legumes to mobilize P that is otherwise unavailable for plant uptake. Most likely, the enhanced P supply to maize resulted from P released by decomposition of the residues of the legume which acquired P from pools that are unavailable to maize. It has been shown that some species, including several legumes, feature root-induced mechanisms such as rhizosphere acidification and the exudation of organic anions or phosphatases which may enhance the availability of P in the rhizosphere (Hinsinger 2001; Richardson et al. 2011). Further, it has been shown that rotations with legumes may affect mycorrhizal infection rates of a subsequent cereal crop, which may in turn enhance P uptake (Alvey et al. 2001). In addition, Pypers et al. (2007) showed that the rotation effect of mucuna on a subsequent maize crop even goes beyond improved N and P nutrition, and suggested that other beneficial effects play a role. Indeed, it has been reported that incorporating legumes into the cropping cycle may lead to other rotation effects such as the improvement of soil physical and biological properties, which may affect growth factors other than N and P nutrition (Fyson and Oaks 1990; Latif et al. 1992; Alvey et al. 2001). A distinction can be made between the different

rotation effects by referring to the ‘N effect’, the ‘P effect’ and ‘other rotation effects’. For soybean, the N effect on a subsequent cereal crop such as maize is well documented, but it is not known whether soybean is able to improve P availability to a next maize crop.

We conducted a field study on a highly weathered, P-deficient Ferralsol in Western Kenya, where we tested the effect of increasing levels of P applied to three different soybean genotypes on a subsequent maize crop in terms of N and P nutrition. With this experiment, we aimed at assessing whether N uptake by maize is enhanced by applying P to a previous soybean crop, whether residual P effects on maize occur in such a system, and how these effects depend on the soybean genotype and P rate used. In addition, a greenhouse trial was set up with the same soil to evaluate rotation effects of soybean on maize. In the latter experiment, soybean–maize rotations were compared with maize–maize rotations, and different nutrient treatments were imposed in the second season in order to distinguish between N, P and other rotation effects. The specific objectives of this study were (1) to assess whether increased P rates applied to soybean lead to improved N and or P nutrition of a subsequent maize crop (2) to evaluate P fertilizer use efficiency of different P rates applied to three different soybean genotypes in a soybean–maize rotation, and (3) to determine N, P and other rotation effects of three soybean genotypes on maize.

Materials and methods

Field trial

First season: trial installation and management

A field trial was established at the onset of the short rainy season in September 2010 in the village of Nyabeda, Siaya district, Nyanza province in West Kenya (N 00°07′32.5″, E 034°24′15.8″, 1,320 m.a.s.l.). The soil at this site is deep and red with diffuse horizon boundaries and was classified as a Ferralsol (FAO-ISRIC 2006). A composite top soil (0–15 cm) sample was taken, air-dried and passed through a 2 mm sieve. Soil characteristics are presented in Table 1. The soil has a low pH, is low in resin-extractable P, and has a high P buffering capacity. Three soybean genotypes were used: TGx 1740-2F, TGx 1895-33F and Namsoy 4M. TGx

1740-2F is an early- to medium maturing genotype from the breeding program of IITA (International Institute of Tropical Agriculture) and is commonly used by soybean farmers in West Kenya. Namsoy 4M is a medium-maturing genotype from the breeding program of Makerere University in Uganda and also popular among farmers in West Kenya for its high grain yield and large leafy biomass production. TGx 1895-33F is a late-maturing genotype from the breeding program of IITA, but less commonly used as it has only recently been released by KARI (Kenya Agricultural Research Institute). The soybean genotypes were sown in combination with four levels of P: a control treatment without P (No P), a full P rate (FPR) of 44 kg P ha⁻¹, 1/2nd of the full P rate (50 % FPR), i.e. 22 kg P ha⁻¹ and 1/4th of the full P rate (25 % FPR), i.e. 11 kg P ha⁻¹. Based on the recommended P fertilizer rate in West Kenya, which is 18–22 kg P ha⁻¹, it was expected that the FPR (44 kg P ha⁻¹, i.e. twice the recommended rate) would fully alleviate P deficiency and result in maximal P supply. The experiment was carried out in three replicates and replicates were blocked. Within the replicate blocks, a split-plot design was adopted, with P rates blocked in the whole plots, and genotypes planted in the sub plots. At each site, whole plots were randomized within the replicate blocks, and sub plots randomized within the main blocks. Sub plots measured 7.5 × 6 m and consisted of 15 lines of 6 m long (interline spacing of 0.5 m). Paths of 0.75 m and 1 m were provided between sub plots/whole plots and replicate blocks respectively. In each replicate block, 1 fallow plot (7.5 × 6 m) was included for assessment of biological nitrogen fixation (BNF). Fields were ploughed by animal traction and subsequently tilled with hoes by a team of farmers. All plots received a basal application of 50 kg potassium (K) ha⁻¹ as Muriate of Potash (MOP). P was applied as triple super phosphate (TSP). MOP and TSP were applied in furrows of about 10 cm depth and covered with little soil. Soybean seeds were inoculated with a peat-based rhizobial inoculant (Legumefix Ltd., containing the commercial rhizobia strain USD 110), drilled into the furrows, and covered with soil. Three weeks after sowing, soybean plants were thinned to 1 plant per 7.5 cm. The fields were weeded by a team of farmers twice during the experiment. The amount of daily rainfall was measured with a rain gauge in Nyabeda installed close to the field. Cumulative rainfall in Nyabeda during the season was 723 mm, and there were no serious drought spells

Table 1 Selected soil characteristics of the trial site in Nyabeda (Nyanza province, Kenya)

pH ^a	5.1
clay ^b (%)	38
silt ^b (%)	13
sand ^b (%)	49
Total N ^c (%)	0.16
Total C ^c (%)	1.97
CEC ^d (cmol _c kg ⁻¹ soil)	8.6
exch Ca ²⁺	4.4
exch Mg ²⁺	1.5
exch K ⁺	0.4
P _{AEM} ^e (mg P kg ⁻¹ soil)	2.7
P _{ox} ^f (mg P kg ⁻¹ soil)	52
Fe _{ox} ^f (mg Fe kg ⁻¹ soil)	3,000
Al _{ox} ^f (mg Al kg ⁻¹ soil)	1,300
Mn _{ox} ^f (mg Mn kg ⁻¹ soil)	3,500
PBC ^g (mg P kg ⁻¹ soil)	26

^a pH determined in 0.01 M CaCl₂ (1:5)

^b Particle size analysis by the hydrometer method (Day 1965)

^c Total nitrogen and carbon determined by elemental analysis after dry combustion (ANCA-GSL Preparation Module 20e20 Stable Isotope Analyser; Europa Scientific, Crewe, Cheshire, UK)

^d Effective cation exchange capacity determined by the silver-thiourea method (Chhabra et al. 1975)

^e Anion exchange membrane extractable P (Sibbesen 1983)

^f Ammonium oxalate extractable P, Fe, Al and Mn (Schwertmann 1964)

^g P buffering capacity by the method of Ozanne and Shaw (1968)

affecting vegetative growth (planting to podding) (Fig. 1a). At about 1 month after sowing, wrinkling of the youngest leaves of the soybean plants was observed. TGx 1740-2E was most severely affected, followed by TGx 1895-33F, while Namsoy 4M was less affected. The symptoms were believed to be the result of a disease, and an insecticide was sprayed twice on all the plots to prevent further spread of the disease by insects. After spraying, symptoms became less severe. The disease could however not be identified and the possibility that the symptoms were wholly or partly the result of a nutrient disorder (e.g. K-induced Mg deficiency) could not be excluded.

First season: plant sampling and harvest

At mid-podding, i.e. when plants have formed pods but pods are not filled with grains yet (Nov–Dec 2010,

date depending on the genotype), a representative section of 0.75 m along a plant line (at least 0.5 m away from the plot border) was selected in each plot, and all plant shoots in the section were cut and stored in a paper bag. Shoots were dried at 65 °C to constant weight. At each sampling event, some weedy plants were randomly sampled from each fallow plot (for BNF assessment). When plants were ready for harvest (Dec 2010–Jan 2011, date depending on the genotype), all plants within a net plot of 6.5 × 5 m (excluding the outer 0.5 m borders of the plots) were cut and the pods were separated from the haulms. The total amount of pods was weighed and a sub sample was taken. Whole haulms were evenly spread across the plots and left on the field (without incorporating). Soybean grains were shelled from the pods of the sub sample, and the grains dried at 65 °C to constant weight. Total dry grain yield was calculated based on the total fresh weight of the harvested pods, the fresh weight of the pods from the sub sample, and the dried grains from the sub sample.

Second season: trial installation and management

The rotation effects of the different soybean genotypes amended with different P rates on a subsequent maize crop were evaluated during the long rains of 2011. In order to distinguish between the ‘N’ and the ‘P effect’ of soybean on maize, sub plots were split into two sub–sub plots. P was applied in one sub–sub plot (P deficiency eliminated to assess the ‘N effect’), and N was applied in the other sub–sub plot (N deficiency eliminated to assess the ‘P effect’). The maize crop was sown in March 2011 at an interline spacing of 0.75 m and an intraline spacing of 25 cm. Sub–sub plots measured 7.5 × 3 m and hence consisted of 11 lines of 3 m long. All plots received again a basal application of 50 kg K ha⁻¹ as MOP. P was applied as TSP at a rate of 44 kg P ha⁻¹ and N was applied as Calcium Ammonium Nitrate (CAN) at a rate of 120 kg N ha⁻¹. N was split-applied, with 1/3th applied at sowing, and 2/3th applied at 6 weeks after sowing. At sowing, MOP and TSP or CAN were applied in furrows of about 10 cm depth, and covered with little soil. IR (imidazolinone-resistant) maize seeds (coated with imidazolinone for protection against Striga, variety W303, Western Seed Company Ltd.) were planted in the furrows. Three weeks after sowing, maize plants were thinned to 1 plant per

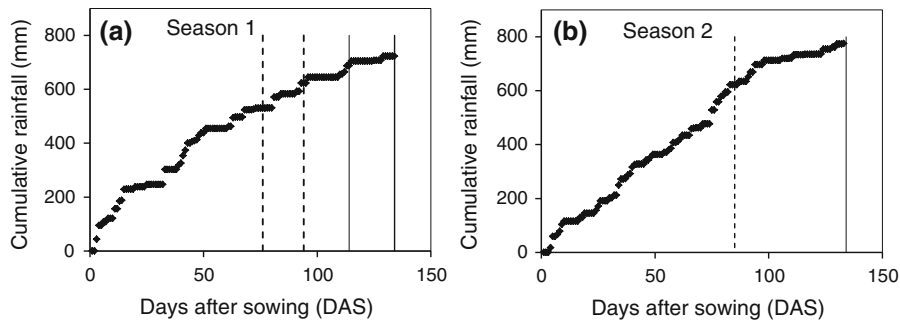


Fig. 1 Cumulative rainfall in Nyabeda during **a** season 1 (Sep 2010–Jan 2011) and **b** season 2 (March–Aug 2011). *Dashed lines* indicate the time of biomass sampling for soybean (at mid-

25 cm. For the second application of N, CAN was applied near the lines and slightly incorporated. The fields were weeded twice by a team of farmers. Rainfall during the second season was adequate with a cumulative rainfall of 799 mm (Fig. 1b).

Second season: plant sampling and harvest

At silking (when silks were clearly visible), 3–5 ear leaves (leaves below the ears) were sampled in each sub-sub plot (+N and +P) of the plot and dried at 65 °C. At harvest, all maize plants within the net sub-sub plot (6.5 × 2 m, excluding the outer 0.5 m borders of the sub-sub plots) were cut, and the cobs were separated from the stovers. The total amount of cobs and the stovers were weighed and a sub sample of both was taken. Maize grains were removed from the cobs, and stover and grain samples were dried at 65 °C and weighed. Maize dry grain and stover yield was calculated based on the total fresh weight of the cobs or stovers, the fresh weight of the sub samples, and the dry weight of the grains or the stovers from the sub sample.

Plant analyses and calculations

Soybean shoots, soybean grains, maize ear leaves, maize stovers and maize cobs (rachis + grains) were ground and digested in hot H₂SO₄ and H₂O₂, according to the method of Parkinson and Allen (1975). P and N concentrations in the digests were determined colorimetrically (Okalebo et al. 1993).

Levels of N fixation were estimated according to the N natural abundance method. N concentrations and N isotope ratios in the ground biomass samples were determined by mass spectrometry (Thermo Scientific,

podding, date dependent on genotype), or of ear leaf sampling for maize. *Full lines* indicate the time of harvest (dependent on genotype for soybean)

Delta V Advantage Isotope Ratio MS coupled through ConFlo IV to Thermo Scientific Flash EA/HT). The percentage of N derived from the atmosphere was calculated as follows (Högberg 1997; Unkovich et al. 2008):

$$\%N_{dfa} = (\delta^{15}N_{ref} - \delta^{15}N_{fix}) / (\delta^{15}N_{ref} - B) \times 100 \quad (1)$$

where $\delta^{15}N_{ref}$ and $\delta^{15}N_{fix}$ are the ¹⁵N natural abundances (in parts per thousand deviations, ‰, from the ¹⁵N:¹⁴N ratio in atmospheric N₂, which is 0.0036765 and corresponds to 0.3663 atom % N) in the non-fixing reference species (here, a sample of non-leguminous weedy species from a fallow plot) and the fixing species (here, soybean) respectively, and *B* the ¹⁵N natural abundance in the shoot of the fixing species totally relying on N₂ fixation (to adjust for isotopic fractionation between root and shoot within the legume). A *B* value of −0.982 was used, which is the mean *B* value observed among a range of soybean genotypes by Hounnandan et al. (2008). The amount of N fixed was estimated by multiplying the total amount of N in the aboveground biomass at mid-podding and the %N_{dfa} measured in the plant shoot samples.

P fertilizer use efficiency in the soybean–maize rotation, where P was applied to the soybean crop only, was evaluated by calculating total P recovery for the sub-sub plots where no P was applied to the maize, as follows:

$$\begin{aligned} \text{Total P recovery (\% of applied P)} \\ = \frac{\text{maize + soybean P uptake}}{\text{amount of applied P}} \times 100 \end{aligned} \quad (2)$$

Soybean P uptake was estimated as shoot P uptake observed at mid-podding, while maize P uptake was

estimated as the total amount of P in the aboveground plant parts (stovers and grains) at harvest. As a part of the P taken up by the maize could have been previously taken up by the soybean (and recycled through residue decomposition), total P recovery calculated by equation (2) \leq recovery of fertilizer P.

Greenhouse study

The rotation effect of the same soybean genotypes (TGx 1740-2F, TGx 1895-33F and Namsoy 4M) amended with different rates of P on a subsequent maize crop, relative to a maize crop following a maize crop, was evaluated in a pot trial. In a first 'season', maize and the three soybean genotypes were grown in soil from Nyabeda amended with two rates of P, resulting in optimal (FPR) and suboptimal (50 % FPR) P supply. In a second 'season', maize plants were grown in the same pots, either amended with N (to evaluate the 'P effect'), a fresh application of P (to evaluate the 'N effect'), or both N and P (to evaluate potential rotation effects other than improved N or P nutrition).

First season

Top soil (0–20 cm) was sampled at the border of the field in Nyabeda where the field trial was established. The soil was sun-dried and passed through a 5 mm sieve. P was added through a KH_2PO_4 solution at two rates: 300 and 150 mg P kg^{-1} soil (FPR and 50 % FPR respectively), with the first rate expected to result in optimal P availability (based on P adsorption isotherms, not shown). Further, the soil was amended with nutrient solutions containing MgSO_4 , MgCl_2 , KCl, CaCl_2 , ZnCl_2 , CuSO_4 , CoCl_2 , $\text{Na}_2\text{B}_4\text{O}_7$ and Na_2MoO_4 at rates of 53 mg Mg kg^{-1} , 29 mg S kg^{-1} , 380 mg K kg^{-1} , 86 mg Ca kg^{-1} , 0.48 mg Zn kg^{-1} , 0.19 mg Cu kg^{-1} , 0.05 mg Co kg^{-1} , 0.29 mg B kg^{-1} , and 0.05 mg Mo kg^{-1} soil. N was not applied. The experiment was carried out in 12 replicates. Pots made from PVC tubes (height 50 cm and diameter 15 cm) were filled with an equivalent of 7.5 kg of dry soil, and placed in a randomized complete block design in a greenhouse at TSBF-CIAT in Nairobi, Kenya. Before sowing, the moisture content of the pots was increased to 28 % w/w. Soybean seeds were inoculated with a peat-based rhizobial inoculum (Legumefix Ltd.), and in each pot three soybean or two maize seeds were sown. Five days after emergence, plants were thinned to 1 plant per pot.

The moisture content of the pots was monitored by weighing the pots on a balance and plants were watered daily. Average minimum and maximum temperatures in the greenhouse during the first season were 20 and 33 °C respectively. Maize was harvested 38 days after sowing (DAS). Soybean plants were harvested at flowering, and the period of growth to reach this physiological stage differed among genotypes: TGx 1740-2F and Namsoy 4M were harvested at 46 days after sowing while TGx 1895-33F was harvested at 52 days after sowing. Plant shoots were cut, dried at 65 °C and weighed. The shoots were chopped into small pieces (0.5–1 cm), and a sub sample of 5 % of the plant dry weight was taken for plant analysis. After harvest, the pots were left to stand on the greenhouse benches without addition of water.

Second season

Two weeks after the harvest of the last soybean genotype, the top layer (0–10 cm) of the soil in the pots was loosened with a stick, the plant residues corresponding to each pot incorporated, and the top soil layer rewetted. Fresh P and N were added by pouring nutrient solutions over the soil surface in the pots. For N application, a solution was used containing $\text{Ca}(\text{NO}_3)_2$ and NH_4NO_3 with 60 % of the N in the form of nitrate and 40 % of the N in the form of ammonium. N was added at a rate of 400 mg N kg^{-1} soil, but was split-applied: 1/3th was added before sowing (3 days after residue incorporation), and 2/3th was added 16 DAS. A solution containing CaCl_2 was used to add equal amounts of Ca to pots that did not receive N. P was added 4 days after residue incorporation with a KH_2PO_4 solution at a rate of 300 mg P kg^{-1} soil, and a solution with KCl was used to add equal amounts of K to pots where no P was applied. No other nutrients were re-applied. Seven days after residue incorporation, the moisture content of the pots was increased to 28 % w/w, and two maize seeds were sown per pot. Plants were thinned to 1 plant per pot 5 days after emergence. Average minimum and maximum temperatures in the greenhouse during the second season were 22 and 37 °C respectively. Maize plants were harvested 37 DAS. The shoots were cut, dried at 65 °C and weighed.

Plant analyses

The sub samples of the soybean and maize plants of the first season, and the maize shoots of the second

season, were ground, digested in hot HNO_3 and P concentrations in the digests determined with ICP-OES (Perkin-Elmer Optima 3300 DV).

Statistical analysis

Differences between nutrient treatments and genotypes were tested for significance with a mixed model ANOVA using PROC MIXED in SAS (SAS Institute Inc 2012). For analysis of data from the field trial in the first season, genotype and P rate were tested as fixed effects, and replicate block was added as a random factor. For analysis of data from the second season, effects of the treatments imposed during the first season were tested within the treatments of the second season (+N and +P) separately, and the same fixed and random factors were added to the model. For analysis of data from the greenhouse trial, crop/genotype and P rate in the first season and N and P treatment in the second season were tested as fixed effects, and replicate block was added as a random factor. The LSMEANS statement in SAS was used to evaluate differences between the means by calculating the standard errors of the difference (SED).

Results

Field trial

Soybean biomass accumulation, N fixation and grain yield in the first season

Application of P did not significantly enhance biomass production (Fig. 2a). For TGx 1740-2F and Namsoy 4M (early- to medium-maturing genotypes), plants reached the mid-podding stage at 75 DAS, while plants of TGx 1895-33F (late-maturing) reached the mid-podding stage at 95 DAS. As a result of its longer growth period, biomass accumulation at mid-podding of TGx 1895-33F was larger than that of TGx 1470-2F and Namsoy 4M. Differences in P uptake among genotypes followed similar patterns and no significant effect of P rate on P uptake was observed (data not shown).

The $\%N_{\text{dfa}}$ was, on average, 35 % and was unaffected by genotype or P rate (data not shown). The amount of N fixed was not affected by P application but differed among genotypes (Fig. 2b). TGx

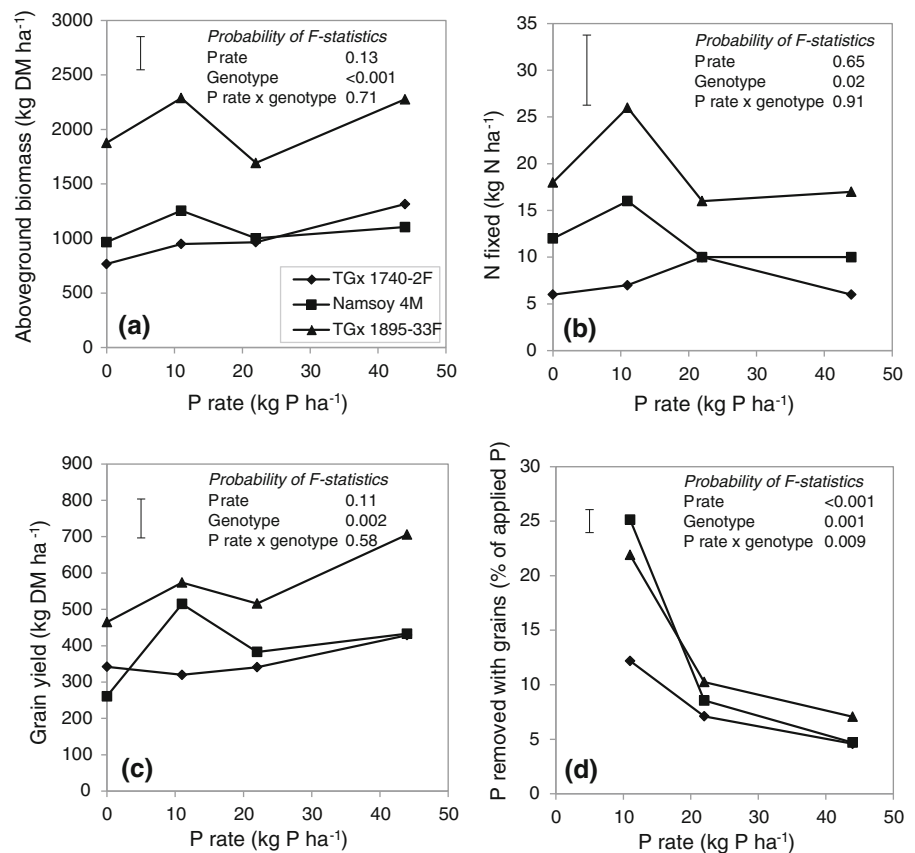
1895-33F fixed a larger amount of N (19 kg N ha^{-1}) than Namsoy 4M (12 kg N ha^{-1}) and TGx 1740-2F (8 kg N ha^{-1}), while the latter fixed a lower amount of N than the other two genotypes. As the $\%N_{\text{dfa}}$ did not significantly differ among genotypes, differences in the amount of N fixed among genotypes were explained by differences in total N content rather than by differences in $\%N_{\text{dfa}}$.

Soybean grains were harvested at 114, 121 and 134 DAS for TGx 1740-2F, Namsoy 4M and TGx 1895-33F respectively. Average grain yield was low ($444 \text{ kg DM ha}^{-1}$), and grain yields did not increase significantly with the application of P (Fig. 2c). The average grain yield of TGx 1895-33F was significantly higher than that of Namsoy 4M and TGx 1740-2E. By exporting the grains from the field, only 19, 9 and 6 % of the amount of applied P were, on average, removed from the field for the 25 % FPR, 50 % FPR and FPR treatments respectively (Fig. 2d). The percentage of applied P removed with the grains was significantly lower for TGx 1740-2E compared to TGx 1895-33F and Namsoy 4M in the 25 % FPR treatment and compared to TGx 1895-33F in the FPR treatment. Whenever P was applied, P balances at the end of the soybean cropping season (amount of P applied minus the amount of P removed with the grains, i.e. kg P ha^{-1} remaining) were positive irrespective of the rate of applied P or the soybean genotype cultivated.

Maize P and N uptake and grain yield in the second season

P and N concentrations in the ear leaves of maize grown in the second season in Nyabeda are presented in Fig. 3. Ear leaf P concentrations reached higher levels in the sub-sub plots that received N, but no P, at the onset of the 2nd season than ear leaf P concentrations in sub-sub plots that received a fresh application of P, but no N (on average $1,900$ and $1,500 \text{ mg P kg}^{-1} \text{ DM}$ respectively, $P < 0.001$). Irrespective of P and N treatments, ear leaf P concentrations were not significantly affected by the preceding soybean genotypes or the P rate applied to the soybean crop (Fig. 3a, b). Application of N in the second season significantly increased N concentrations in the ear leaves (on average $32,000$ and $23,000 \text{ mg N kg}^{-1} \text{ DM}$ with and without N respectively, $P < 0.001$), but no effect of the preceding soybean genotype or 1st-season P rate on ear leaf N concentrations was observed (Fig. 3c, d).

Fig. 2 **a** Aboveground biomass, **b** amount of N fixed in the aboveground biomass at mid-podding, **c** grain yield at harvest and **d** the amount of P removed from the field by exporting the grains, for three soybean genotypes grown in Nyabeda, Nyanza province in Kenya. Four P rates were applied: 44 kg P ha⁻¹, expected to result in optimal P supply (FPR), 1/2nd of the full rate (50 % FPR), 1/4th of the full rate (25 % FPR) and a control treatment without P (No P). Error bars represent maximum standard errors of the difference for comparison of genotype × P rate means



When P was applied to the maize crop, maize yielded on average 3.7 t of grains ha⁻¹ and maize yields were not affected by the P rate applied in the first season (Fig. 4b). When N (but no P) was applied to the maize, maize grain yields were significantly affected by P application in the first season ($P = 0.02$), and increased from 2,300 kg DM ha⁻¹ in the control treatment to 4,300 kg DM ha⁻¹ for maize relying on residual P with 44 kg P ha⁻¹ applied to the previous soybean crop (Fig. 4a). In none of the treatments, maize grain yield (Fig. 4), total maize P uptake (not shown) or total maize N uptake (not shown) was affected by the preceding soybean genotypes.

P fertilizer use efficiency in the soybean–maize rotation

Total P recovery in the soybean–maize rotation (Eq. 2) was determined for sub-sub plots where N, but no P, was applied at the start of the second season (Fig. 5). The total P uptake of the maize crop was

several times higher than that of the soybean crops (on average 13 kg P ha⁻¹ for maize compared to 2 kg P ha⁻¹ for soybean), and therefore total P recovery was mainly determined by maize P uptake. The latter remained unaffected by the soybean genotype preceding the maize. Total P recovery significantly increased with decreasing 1st-season P rates ($P = 0.002$). For the 25 % FPR treatment, the total P recovery exceeded the amount of P applied. At the highest P rate (FPR), less than half of the amount of P applied was recovered in the soybean and the maize crops.

Greenhouse study

Shoot P uptake in the first season

Shoot P uptake increased with increasing P rate ($P < 0.001$) and differed among maize and soybean genotypes ($P < 0.001$), but no interaction effect between crop/genotype and P rate on shoot P uptake was observed (Table 2). Shoot P uptake increased with, on average, 30 % at the highest P rate compared

Fig. 3 P and N concentrations in the ear leaves of maize grown in the second season of a soybean–maize rotation in the trial field in Nyabeda, as affected by the soybean genotype planted in the first season and the rate of P applied to the soybean crop (FPR = 44 kg P ha⁻¹, 1/2nd of the FPR, 1/4th of the FPR or no P), for sub-sub plots where the maize crop (second season) did not receive additional P but received N at an optimal rate (a, c), and sub-sub plots where maize did not receive N but received P at an optimal rate (b, d). Error bars represent maximum standard errors of the difference for comparison of genotype × P rate means

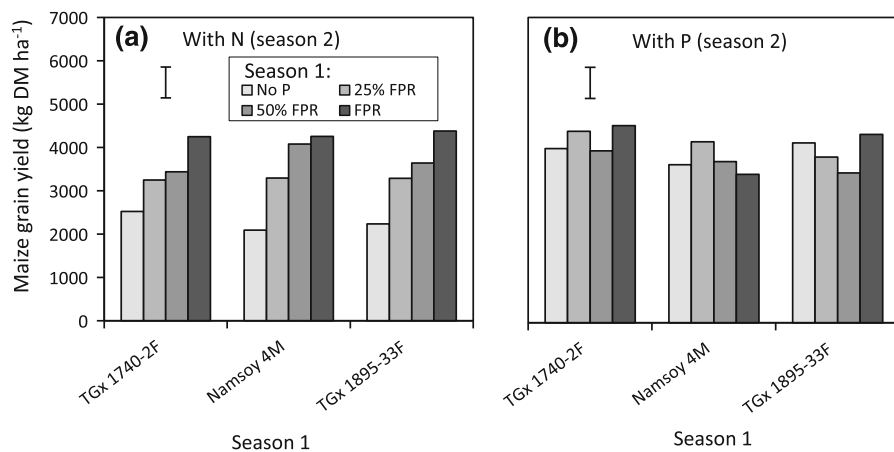
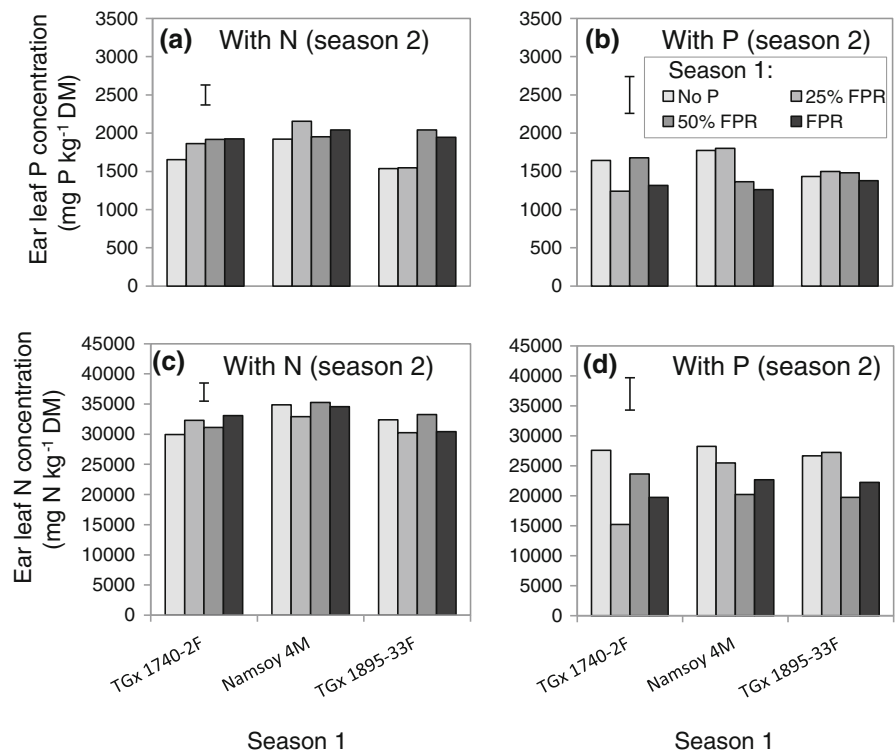


Fig. 4 Maize grain yields in the second season of a soybean–maize rotation in the trial field in Nyabeda as affected by the soybean genotype planted in the first season and the rate of P applied to the soybean crop (FPR = 44 kg P ha⁻¹, 1/2nd of the FPR, 1/4th of the FPR or no P), for sub-sub plots where the

maize crop (second season) did not receive additional P but received N at an optimal rate (a) and sub-sub plots were maize did not receive N but received P at an optimal rate (b). Error bars represent maximum standard errors of the difference for comparison of genotype × P rate means

to shoot P uptake at the reduced P rate (21 and 17 mg P plant⁻¹ respectively). Shoot P uptake by the early- to medium-maturing TGx 1740-2F and Namsoy 4M was significantly lower than shoot P uptake by the late-maturing TGx 1895-33F and that of maize.

Maize growth in the second season

Maize shoot biomass and shoot P concentration were highest in the treatment where N and P were freshly applied in the second season, lowest when only N, but

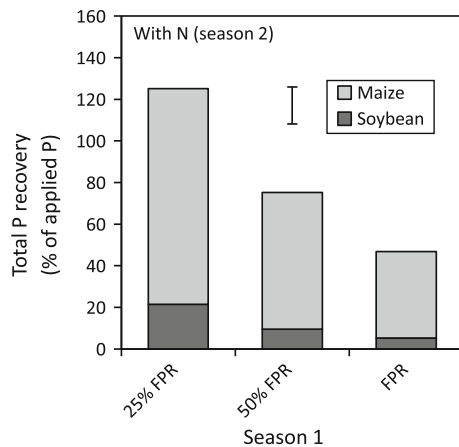


Fig. 5 Total P recovery, i.e. total amount of P taken up by maize and soybean divided by the amount of applied P in a soybean–maize rotation in the trial field in Nyabeda where P was applied to the soybean crop at three rates (FPR = 44 kg P ha⁻¹, 1/2nd of the FPR, 1/4th of the FPR or no P), in sub–sub plots where the maize crop (second season) did not receive additional P but received N at an optimal rate. The error bar represents the maximum standard error of the difference. Means across soybean genotype–maize rotations are presented

Table 2 Shoot P uptake of maize and three soybean genotypes (grown until flowering) in a pot trial with Nyabeda soil amended with P resulting in optimal P supply (FPR) and 1/2nd of this rate (50 % FPR)

	Shoot P uptake (mg P plant ⁻¹)	
	50 % FPR	FPR
Maize	19	25
TGx 1740-2F	12	17
Namsoy 4M	12	18
TGx 1895-33F	17	21
	Probability of F-statistics	SED ^a
Crop/genotype	<0.001	1.6
P rate	<0.001	1.5
Crop/genotype × P rate	0.63	1.7

Means of 12 replicates are presented

^a ‘SED’ is the standard error of the difference for comparison of treatment means

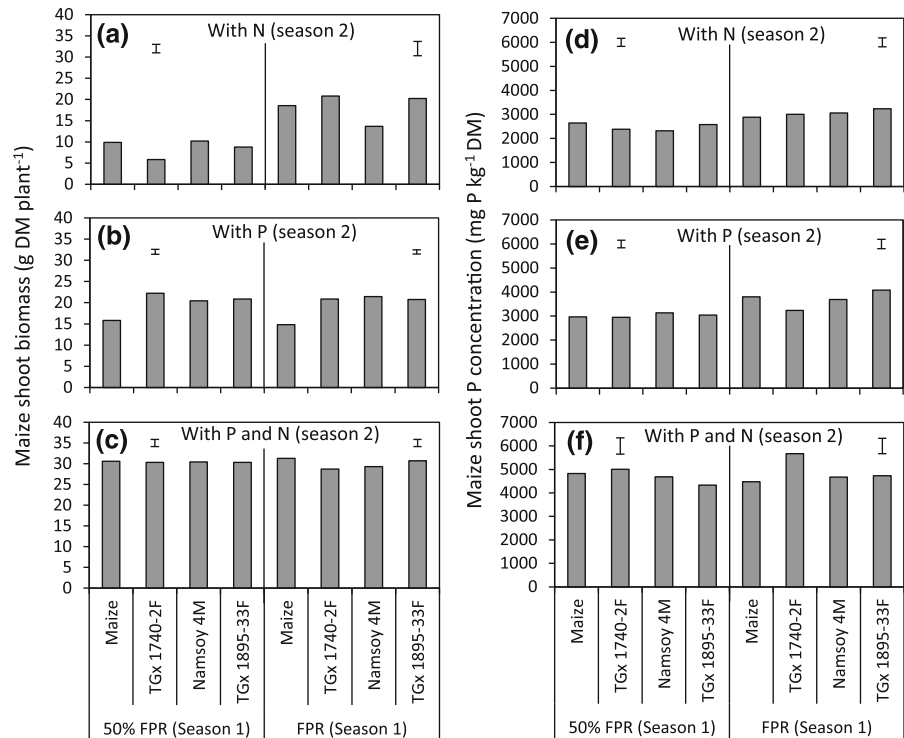
no fresh P was applied, and intermediate when only P, but no N was freshly applied ($P < 0.001$) (Fig. 6). Shoot biomass of maize plants following soybean differed from that of maize plants following maize only when no N was applied. In none of the P and N treatments, shoot P concentrations differed between

maize following maize and maize following soybean. Furthermore, in none of the treatments, the soybean genotype preceding the maize significantly affected maize shoot biomass or shoot P concentration. When both N and P were freshly applied in the second season, the P rate applied in the first season did not affect maize shoot biomass or shoot P concentrations. In this treatment, maize plants produced, on average, 30 g of shoot DM (Fig. 6c) and shoot P concentrations were optimal for growth (on average, 4,800 mg P kg⁻¹ DM) (Fig. 6f). When P, but no N, was applied to the maize, maize shoot biomass was about 33 % larger for maize following soybean (on average 20 g DM plant⁻¹, i.e. 65 % of the biomass produced at optimal nutrient supply) than maize following maize (15 g DM plant⁻¹, i.e. 50 % of the biomass produced at optimal nutrient supply) ($P < 0.001$) (Fig. 6b). With fresh P application, the P rate applied to the previous crop did not affect shoot biomass but affected shoot P concentrations (on average, 3,000 mg P kg⁻¹ DM when 50 % FPR had been applied and 3,700 mg P kg⁻¹ DM when the FPR had been applied, $P < 0.001$) (Fig. 6e). When N, but no P was applied, maize shoot biomass was largely affected by the P rate applied in the previous season ($P < 0.001$) (Fig. 6a). When 50 % of the FPR had been applied to the previous crop, maize plants produced, on average, 9 g of shoot DM, i.e. only 29 % of the shoot biomass produced at optimal N and P supply. When the FPR had been applied to the previous crop, maize shoot biomass was, on average, 18 g DM plant⁻¹, i.e. 58 % of the shoot biomass produced at optimal N and P supply. Similarly, shoot P concentrations were significantly higher ($P = 0.01$) when the FPR had been applied to the previous crop compared to those when 50 % FPR had been applied (on average, 3,000 mg P kg⁻¹ DM and 2,500 mg P kg⁻¹ DM respectively) (Fig. 6d).

Discussion

A residual effect of P applied to the soybean crop on the subsequent maize crop was observed when no fresh P was applied to the maize, both in the greenhouse and the field trial. Hence, even on strongly P-fixing soils such as the Nyabeda soil, maize can still benefit from P applied to a preceding soybean crop. Residual effects of P applied to a soybean crop on a subsequent maize crop have been observed by several

Fig. 6 Maize shoot biomass and shoot P concentration of maize grown in the second season of a pot trial in Nyabeda soil where in the first season maize and three soybean genotypes had been planted in combination with a P rate resulting in optimal P supply (FPR) and 1/2nd of this rate (50 % FPR). In the second season, maize plants were supplied with either N at an optimal rate (**a, d**), P at an optimal rate (**b, e**) or N and P at optimal rates (**c, f**). Means of four replicates are presented. *Error bars* represent standard errors of comparison of maize grown after different crops (maize and soybean genotypes) within a specific combination of 1st and 2nd season P and/or N treatments



authors (Abaidoo et al. 2007; Zingore et al. 2008; Kihara et al. 2010). In the pot trial, P deficiency was not fully overcome by residual P effects. Maize relying on residual P produced only 29 % and 58 % of the shoot biomass produced with fresh P application when 50 % FPR and the FPR had been applied in the previous season respectively (Fig. 6). When 50 % FPR had been applied in the first season, the average plant P concentration was 2,700 mg P kg⁻¹ DM which is below the sufficiency range for maize in early growth stages (3,500–5,000 mg P kg⁻¹ DM, Lockman 1969). However, in the field, ear leaf P concentrations of maize grown without P application in the second season were below the sufficiency range (2,000–4,000 mg P kg⁻¹ DM, Bennett 1993) only when no P or 25 % FPR had been applied in the previous season (Fig. 3). When the FPR or 50 % FPR had been applied to the previous soybean crop, P concentrations in the ear leaves were around 2,000 mg P kg⁻¹ DM indicating that P nutrition of the maize, relying on residual P, was likely close to optimal. The results indicate that, if applied in sufficient quantities, residual P may largely, although not fully, overcome P limitations to maize.

Nevertheless, the residual P effect was likely enhanced by N application. The application of N may lead to improved root development which, in turn, results in enhanced P uptake under conditions of suboptimal P availability. Contrary to what is expected, P concentrations in the maize grown in the field were significantly lower when P, but no N, was applied in the second season than when N, but no fresh P was applied (Fig. 3). Hence, it seems that although P was supplied at a high rate, N deficiency limited P uptake in the first treatment. Indeed, ear leaf N concentrations were below the sufficiency range (27,000–35,000 mg N kg⁻¹ DM, Bennett 1993) when no N was applied. In addition to the findings in the field, P concentrations in the maize grown in the pot trial were significantly lower when only P was applied to the maize than when N and P were both applied (Fig. 6). This demonstrates that P fertilizer use efficiency is largely affected by N nutrition. Similarly, the study of Kihara et al. (2010) showed that the residual effect of P applied to the previous soybean crop on maize was larger when N was applied to the maize. Such interactions between N and P may have important implications for P fertilizer use efficiency in

soybean–maize rotations: a soybean crop with a positive N balance may not only result in a benefit to the subsequent maize crop in terms of N nutrition, but also improve benefits from residual P.

Several authors have reported that the amount of N fixed by legumes on P-deficient soils increases with increasing P application (Sanginga et al. 2000; Jemo et al. 2010). This increase in N fixation may be the consequence of enhanced growth of the legume plants leading to an increased N demand and an improved capacity to provide carbon to the rhizobia. Further, enhanced P supply may directly improve nodulation and N fixation as the process of N fixation requires a considerable amount of P. In this study, 4 P rates were applied to three different soybean genotypes, but none of the soybean genotypes significantly responded to increasing P rates, neither in terms of biomass accumulation or N fixation nor in terms of grain yield (Fig. 2). Biomass and grain yield tended to increase with increasing P rates but differences remained non-significant. The disease which affected soybean plants may have played a role in the poor P response of the soybean crop. Promiscuous soybean genotypes selected by IITA (TGx 1740-2F and TGx 1895-33F) for their high N fixation potential and dual-purpose nature were used. However, nodulation was poor and levels of N fixation remained low. Possibly, the acid pH (5.1, see Table 1) and the high levels of exchangeable aluminium and especially manganese ($>3,000$ mg Mn kg⁻¹ soil of extractable Mn, see Table 1) hindered nodulation and N fixation. It is known that low pH and high levels of exchangeable aluminium and manganese restrict rhizobium growth, nodulation and the growth of the host plant, resulting in low levels of N fixation (Zahran 1999; Liao et al. 2006). As expected from the lack of response to P application of the soybean crop in terms of N fixation, maize growth in the second season was not affected by the level of P applied to the soybean when P was freshly applied to the maize. Hence, the ‘N effect’ of soybean was not increased by applying larger rates of P.

Although soybean did not respond to P, variation in P uptake and N fixation was observed among soybean genotypes (Fig. 2). On the one hand, the late-maturing genotype TGx 1895-33F took up more P and fixed more N than the early- to medium-maturing genotypes TGx 1740-2F and Namsoy 4M. On the other hand, a significantly larger portion of the applied P and a larger amount of N was exported from the field for

TGx 1895-33F compared to the lower-yielding TGx 1740-2E. Several authors have reported that growing early-maturing soybean genotypes result in significantly lower or even negative N balances (Ogoke et al. 2003; Sanginga et al. 2003; Singh et al. 2003). However, late-maturing genotypes may produce higher grain yields, leading to higher levels of P and N exported from the field at harvest. Nevertheless, the observed differences in P and N balances among genotypes did not affect P and N nutrition of maize in the second season. The residual effect of the P applied in the first season did not depend on how much P had been exported with the soybean grains (Fig. 4). Further, none of the genotypes was able to increase P availability to maize relative to other genotypes. Ear leaf P and N concentrations, total maize P and N uptake and maize grain yields were affected by fertilizer treatments but not by the soybean genotypes preceding the maize (Fig. 3). In addition, maize grown in the pot trial following soybean was not affected by the genotype preceding the maize, neither in terms of P nutrition nor in terms of N nutrition or other rotation effects (Fig. 6). Similarly, Abaidoo et al. (2007) could not demonstrate differences among soybean genotypes in rotation effects on maize under different modes of P nutrition. To the contrary, Sanginga et al. (2002) reported maize yield increases relative to maize grown after maize ranging from 20 to 130 %, depending on the soybean genotype incorporated in the rotation. Differences in rotation effects among soybean genotypes on maize were also observed by Jemo et al. (2006). In our field trial, levels of soybean production and N fixation were low which limited rotation effects and genotype-related differences in effects on maize.

Total P recovery in the soybean–maize rotation was heavily determined by P uptake of the maize crop, which was several times higher than that of the soybean crop. Hence, the proportion of P taken up by the maize that was previously taken up by the soybean and released to the maize through decomposition of soybean residues was likely small. When 25 % FPR (11 kg P ha⁻¹) was applied, the total P recovery exceeded the amount of P applied (Fig. 5), and when the highest P rate (44 kg P ha⁻¹) was applied, less than half of the amount of P applied was recovered. Application of 22 kg P ha⁻¹ seemed to be the most optimal rate as the P balance at the end of the rotation cycle remained positive while P fertilizer use

efficiency was high with up to 75 % of the applied P recovered in the first two seasons and P supply to the maize was adequate.

In the field, we did not include a maize crop in the first season and the rotation effects of soybean on maize relative to maize following maize could therefore not be determined. The results of the pot trial allowed us to evaluate whether N, P and/or other rotation effects of soybean on maize occur. Maize growth was higher for maize following soybean than for maize following maize only when no N was applied in the second season (Fig. 6). This shows that, at least under greenhouse conditions, soybean can affect a subsequent maize crop only in terms of N nutrition, and that soybean preceding a maize crop does neither result in a 'P effect' nor in other rotation effects. P nutrition was not improved when soybean preceded maize compared to when maize preceded maize. It therefore seems unlikely that soybean features P-mobilizing strategies as observed for other legume crops by Kamh et al. (2002). In a field trial with mucuna (*Mucuna pruriens*) grown in rotation with maize, Vanlauwe et al. (2000a, b) found that the incorporation of mucuna into the rotation resulted in improved availability of rock phosphate and that this led to enhanced P uptake by a subsequent maize crop. The observed increases in availability of rock phosphate were attributed to enhanced P solubilisation by mucuna through rhizosphere processes. Rhizosphere processes such as acidification and the release of organic anions may be more important when sparingly soluble P sources such as rock phosphate are used. Apart from N and P effects, no other rotation effects were observed. Our results contradict the results of Sanginga et al. (2002), who found that the increase in maize yield of maize grown after five soybean genotypes relative to maize grown after maize could not be fully explained by an increase in N uptake, and concluded that other rotation effects had played a role. Likely, such other rotation effects are more important under field conditions.

The amount of soybean residues added to each maize plant in the second season was lower in the pot trial (6–11 g DM maize plant⁻¹) than in the field trial (15–43 g DM maize plant⁻¹, considering 53,333 plants ha⁻¹) but in the pot trial the added residues were more concentrated (on a soil weight or area basis). Despite the fact that the conditions in the field and the pot trial were profoundly different, similar

treatment effects were observed in both trials. Firstly, the residual P effect of P applied to the soybean on a subsequent maize crop was observed in both trials. Secondly, both in the pot and the field trial, it was found that application of P to the soybean did not lead to larger N effects on the maize. Lastly, in the field, P concentrations were higher in maize plants receiving N but no P in the second season compared to maize plants receiving freshly applied P but no N, and this was likely due to the importance of N for root development and subsequently P uptake. This finding was not confirmed in the same treatments in the pot trial, possibly because P deficiency in the pots was more severe than in the field. However, in the pot trial, the P concentration in the maize plants grown with both N and P freshly applied was higher than that in the maize plants receiving only fresh P. Hence, the results of the pot trial confirmed the occurrence of important interactions between N and P as observed in the field trial.

Conclusions

As levels of N fixation remained unaffected by P application, the N effect of soybean on maize was not enhanced by increasing P supply to soybean. Nevertheless, the results demonstrate that even on strongly P-fixing soils, P can be applied to a soybean crop instead of the subsequent maize crop, without compromising P supply to the maize. While P nutrition of maize was improved by residual P effects, no indications were found that soybean further increases P availability to maize. Further, no variation in rotation effects on maize was observed among soybean genotypes, and variation in P uptake among soybean genotypes did not result in differences in residual P effects on maize. It seems that further gains in P fertilizer use efficiency in soybean–maize rotations can only be obtained by increasing P fertilizer use efficiency by the soybean and maize crop individually, for instance by using P-efficient soybean or maize genotypes, and by optimizing the rates of P used. Interactions between P and N observed in the maize crop further indicated that P uptake may be largely determined by N nutrition. Problems with P and N deficiency in low-input cropping systems should therefore be tackled in an integrated manner.

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