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SHORT COMMUNICATION

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Rock phosphate-P enhances biomass and nitrogen accumulation by legumes in upland crop production systems in humid West Africa

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Abstract Experiments were conducted during 1996–1998 in screen house and in the field in the humid forest zone of Côte d'Ivoire, to evaluate the effects of phosphorus (P) from phosphate rock (PR) on the performance of the root nodulating legume *Crotalaria micans* grown for 8 weeks. The experimental soils were acid Ultisols with <4 mg/kg extractable Bray-1 P. Tilemsi PR from Mali and triple superphosphate (TSP) were applied at 60 kg P ha^{-1} (screen house) and 90 kg P ha^{-1} (field) to the legume. Legume N-fixed (BNF) was estimated by the ^{15}N-isotope dilution and $\delta^{15}N$ natural abundance methods, using *Cassia obtusifolia* L. as a non-fixing legume reference plant. Without P supply, and under the field conditions, *C. micans* produced less than 1 tonne of biomass and accumulated 29 kg N/ha. The application of PR-P enhanced legume N by about fourfold over the unfertilised control. There was no significant difference between the effects of TSP and PR. Phosphorus application mainly affected the total amount of N accumulated rather than the percentage derived from the atmosphere (%N dfa) per se. Furthermore, the cumulative effects of PR-P on the performance of *C. micans* greatly improved with time in the screen house. This study confirms that Tilemsi PR is an agronomically effective source of P for short-duration legume green manure (GM) even in the first year of its application to acid P-deficient soils in the West African humid zone.

Keywords Acid soils · *Crotalaria* · Phosphate rock · Biological nitrogen fixation (BNF) · West Africa

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Introduction

Most traditional staple food crop-based production systems in West Africa are subsistence-oriented, with no or low use of purchased external inputs. They rely on extended periods of bush-fallow to regenerate soil fertility and prevent the build-up of weeds and other pests. However, this practice referred to as shifting cultivation, is no longer a viable option because of increasing pressure on land from growing populations in the region. Becker and Assigbé (1995) reported a reduction from 12–15 years of forest fallow in the 1980s to 3–7 years in the 1990s in upland rice-based systems of seven countries in West Africa. Multi-year trials in farmers' fields in upland rice-growing environments in Côte d'Ivoire indicated that this reduction in fallow length was associated with a 20–30% yield reduction and increased weed pressure (Becker and Johnson 1999). Resource-limited farm households are unable to purchase the inputs necessary to reverse yield declines related to land use intensification (e.g. N and P fertilisers). Therefore, identification of affordable cost technologies to improve soil fertility that result in an increase in crop productivity is likely to be attractive and readily adopted by the resource-limited farmers of the region.

The use of N-fixing legumes as preceding short-duration fallows offers the potential to sustain food crop yields under intensified land use. Becker and Johnson (1998 and 1999) showed that the N-fixing legume *C. micans* Link (Syn. *C. anagyroides* Kunth), grown as a preceding crop, increases upland rice productivity and suppresses weed growth under intensified land use in the humid forest zone of Côte d'Ivoire. Also, the study reported that rice yields after legume crops were correlated with the legume P uptake. Likewise, synergy between legume-fixed N and P supply has been reported (Cassman et al. 1993), and was suggested as a means to increase yields of crops grown in rotation with N-fixing legumes (Kirk et al. 1998). Tian et al. (1998) reported a 900% P-induced increase in N accumulation of tropical leguminous cover crops grown on Alfisols in Nigeria. Biomass and N accumulation of

Centrosema spp. increased by 193 and 259%, respectively, as P was added to an acidic P-deficient Oxisol (Cadisch et al. 1992).

Applying P from regionally available phosphate rock (PR) to N-fixing legumes may be an affordable technology to resource-limited farmers to produce a large amount of N-rich biomass, and at the same time enriches the soil through BNF. Large deposits of PR exist in West Africa (Buresh et al. 1997), and their use efficiency can be improved in P-deficient acid soils encountered in the West African humid forest zone (Mokwunye 1995).

The objective of this study was to evaluate the effect of indigenous PR application on the contribution of BNF to N accumulation and biomass production of short-duration (8 weeks) preceding cover crops, for a sustainable legume-food crop production system. The adoption of this practice by farmers in their farming calendar may be facilitated by the fact that preceding legumes are grown for green manuring in the absence of other major food or cash crops. Reluctance of farmers to entirely devote their fields to green manuring at the expense of cash or food crops is well-known (Becker et al. 1995).

Materials and methods

Experimental sites, soils, plant material and phosphorus sources

Experiments were conducted in 1996–1998 in Côte d'Ivoire at the main research centre of the Africa Rice Center (WARDA) at Mbé (screen-house trials, derived savannah zone, 7.5° N, 5.1° W, 280 m altitude), and at a field site in the humid forest zone in Danané (7.3° N, 8.2° N, 336 m altitude).

The experimental soils (Ultisols) were acidic (pH 4.6–5.2) and P-deficient (4 mg/kg Bray 1). They included: (1) a topsoil (0–20 cm) from a field under long-run natural fallow in Danané (soil 1) that was transported in sufficient quantity to Mbé, dried, sieved (<10 mm) and used for screen-house study; and (2) an upland soil in a rice field in Danané (soil 2). Some physical and chemical characteristics of the soils are presented in Table 1.

Table 1 Physical and chemical properties of soil used in the screen-house (1) and field experiments (2)

Characteristics	Screen-house (Mbé)	Field (Danané)
Soil order	Ultisol	Ultisol
Texture	Loam	Clay-loam
pH H_2O (1:2.5)	5.2	4.6
pH KCl	4.4	4.0
Organic C (%)	1.10	2.10
Total N (%)	0.08	0.14
Available P (Bray 1) mg kg^{-1}	4.0	3.8
Exch. Ca (cmol (+) kg^{-1})	1.26	0.41
CEC (cmol (+) kg^{-1})	3.86	5.53
Exch. Acidity (cmol (+) kg^{-1})	0.06	0.13

Crotalaria micans Link (*Syn*. *C*. *anagyroides* Kunth) was grown as N-fixing legume. This legume plant is native to Southeast Asia, and has been promoted as a promising weed-suppressing green manure in parts of West Africa (Becker and Johnson 1998). *Cassia obtusifolia* L. was used as a non-N_2-fixing reference plant for BNF estimation (Ladha et al. 1993).

Tilemsi PR from the neighbouring Mali was used as P source in the study. This PR has been shown in certain environmental conditions to be as effective and economically profitable as the imported water-soluble triple superphosphate (Bationo et al. 1997). The total P content of the phosphate sample used in the experiments was 13.7% P (30% P_2O_5), with an available P of 4.2% P_2O_5 as measured in neutral ammonium citrate. The molar PO_4/CO_3 ratio was 4.8. Water-soluble triple super phosphate (TSP) was used as a reference P source in the study, and it is manufactured by treating PR with phosphoric acid (H_3PO_4) and is characterised by a content of 19.6% water-soluble P (44% P_2O_5).

Plant culture

Legume seeds were scarified for 30 min in concentrated sulphuric acid (commercial grade) to break dormancy and achieve a high and even germination rate. They were then rinsed with tap water and air-dried. To inoculate the legume plant, fresh nodules of *C*. *micans* were collected in a nearby field, and squashed in distilled water. The scarified seeds were soaked overnight in the rhizobial suspension before planting. Pre-treated seeds were dibble-seeded at a density of 100 seeds m^{-2} (0.10×0.10 m) in both the screen-house and the field.

Fixing and non-fixing legumes were grown for 8 weeks at the same spacing. Eight weeks correspond to the time available for a short-duration preceding green manure crop grown in the transition period between the dry and rainy seasons, in the absence of food or cash crops in the field.

BNF determination

In the screen house, ^{15}N-labeled ammonium sulphate fertiliser (10% atom excess) was used to estimate the proportion of legume-N accumulated that was derived from BNF. Due to insufficient ^{15}N-labeled fertiliser material, BNF-N in the field was measured, using the $\delta^{15}N$ natural abundance method.

In the screen house, each micro-plot sown to *C*. *micans* contained two open-ended PVC pipes (20 cm diameter and 40 cm long each) where the soil had been previously ^{15}N-labelled. The ^{15}N-labelled fertiliser was applied after dissolution in 380 ml distilled water at a rate of 100 mg ^{15}N m^{-2} (i.e. 1 kg ^{15}N ha^{-1}) (Hardarson and Danso 1990). Ten milliliters of this solution were pipetted into a beaker and filled with distilled water up to the 20-ml mark. The upper 15 cm of each PVC-contained soil was removed and mixed manually with the 20 ml ^{15}N solution before

returning the soil to the PVC pipe. The enriched ammonium sulphate was added only once at the beginning of the screen-house experiment. In the following cropping cycles, we expected some of the ^{15}N to remain in the available soil N pool for BNF estimation (Pareek et al., 1990). One legume plant was grown in each ^{15}N-isotope-labelled PVC-pipe.

Under field conditions, the same reference plant was grown along with *C. micans* in the unlabelled soil of the planted plot. Both legumes were harvested 8 weeks after sowing at the onset of flowering.

Experimental set-up and measurement

In the screen house, concrete boxes measuring 2×1×0.6 m were filled with soil 1 (Table 1) to a depth of 40 cm. A 10-cm gravel layer underneath the soil and a lateral outlet to the drainage canal allowed for drainage and avoid water stagnation. Each of the nine concrete-based micro-plots was kept constantly aerobic at field capacity.

Local farmers had repeatedly planted the field to rice. No record of previous fertilisation was known. During the rainy season preceding the experiment, the field was uniformly planted to an unfertilised rice crop. At the beginning of the following experiment, land was hand-cleared by slashing the vegetation, which was then removed from the field. Each plot in the field measured 5×4 m.

Basal P was applied upon planting of the legumes. Finely ground PR (<0.3 mm) was applied at 60 kg P ha^{-1} $cycle^{-1}$ (screen house) and 90 kg P ha^{-1} (field), and TSP (in granular form) was broadcast at 60 kg P ha^{-1} (both screen house and field), and manually incorporated into the upper 10 cm of the soil. All treatments received at the beginning of the various experiments included a uniform dose of 100 kg K ha^{-1} as potassium chloride (50% K). No mineral N fertiliser was supplied. The three treatments (no P, PR and TSP) were replicated three times and arranged in a randomized complete block design.

At the onset of flowering, legume shoots were cut at the soil level from an area of 0.8 m^2 (screen house) or 12 m^2 (field), and weighed to determine the fresh matter yield of the legume plants. Dry weight was then determined after oven drying at 70°C for 3 days. Nodulation of six (screen house), or 15 (field) randomly selected hills within each replication was evaluated at harvest of the legume crop. The nodulated roots were removed by excavating a circular hole around each hill. Nodules were then counted. Legume plants were cleared of adhering soil, separated into stem plus leaves and root samples and separately weighed. The samples were oven-dried and then sub-samples were taken for total N and ^{15}N analysis.

Yield and nutrient contents (N and P) of the legume aboveground biomass were determined at harvest. The legume shoot biomass samples were analysed for P by digesting the samples with a 2:1 (v/v) mixture of concentrated nitric and perchloric acid. The P concentration in the digests was analysed by colorimetry following the vanado-molybdate yellow colour method (Okalebo et al. 1993). The oven-dried material was ground further into a fine powder using a ball-mill. Five-milligram sub-samples were weighed into small tin capsules, which were then closed and rolled into a ball. These samples were then analysed for total N and ^{15}N using an elemental N analyser connected to a mass spectrometer (Reineking et al. 1993).

Soil samples were collected at the initiation of the trials and their analyses were carried out in three replicates using sub-samples from composite samples of five (screen house) and ten (field) randomly taken samples per plot. Each composite sample of 500-g moist soil was air-dried and analysed for different parameters. These included particle size (Gee and Bauder 1986), pH (soil–KCl solution ratio of 1:2.5), CEC (Chapman 1965), sum of bases (Jackson 1967), organic C (Okalebo et al. 1993), total Kjeldahl N, extractable Bray-1 P and exchangeable acidity (Okalebo et al. 1993).

Calculations and statistical analysis

The proportion of plant N, which was derived from atmosphere (% N_{dfa}), was calculated using the ^{15}N dilution and the $\delta^{15}N$ natural abundance methods, as appropriate.

^{15}N-isotope dilution technique The following, Eq. 1 of Hardarson and Danso (1990), was used to compute $\%N_{dfa.}$

$$\%N_{dfa} = \left[1 - \left(N_{fix}/N_{ref}\right) \times 100\right], \quad (1)$$

where: N_{fix} is the ^{15}N atom % excess of the N-fixing plant, and N_{ref} the ^{15}N atom % excess of the non-fixing reference plant.

$\delta^{15}N$ natural abundance method The following, Eq. 2 of Shearer and Kohl (1986), was used to calculate $\%N_{dfa}$.

$$\%N_{dfa} = \left[1 - \left(\delta^{15}N_{ref} - \delta^{15}N_{fix}\right)/\left(\delta^{15}N_{ref} - B\right)\right] \times 100, \quad (2)$$

where $\delta^{15}N_{ref}$ is the $\delta^{15}N$ of the non-fixing reference plant, δ^{15}Nfix is the $\delta^{15}N$ of the fixing legume plant and B the $\delta^{15}N$ of the fixing plant grown hydroponically on N-free media. *B* value for *Crotalaria* spp. was −0.73% (Ladha et al. 1993)

$$\delta^{15}N = 1000\left(R_{sample} - R_{airN_2}\right)/R_{airN_2}$$

$$R = mass29/mass28 = {}^{15}N^{14}N/{}^{14}N_2$$

The amount of total N fixed from atmosphere was estimated using the equation:

$$N(fixed) = \left(\%N_{dfa}/100\right) \times TNY \quad (3)$$

Total N yield (TNY) was calculated from the N concentration of the legume shoot and dry matter yield:

$$TNY = (LSN/100) \times DM \quad (4)$$

where: TNY is the total N yield (kg ha^{-1}), LSN is the legume shoot N concentration (%) and DM is the dry matter yield (kg ha^{-1}) of legume.

Data were analysed using an analysis of variance (ANOVA) procedure of the SAS program (SAS 2001). Unless otherwise indicated, the probability level of 5% was considered statistically significant.

Results

Phosphorus applied in the screen house had a significant positive effect on total P uptake by *C. micans*. The increase was 2.3 (TSP) and 1.4-fold (PR) above the unfertilised control (Table 2). Phosphorus accumulation by the legume was even more impressive in the field when P was applied as TSP (8.6-fold) or PR (4.6-fold) was applied (Table 2).

Enhanced P uptake by *C. micans* resulted in a significant increase in legume biomass yield as P fertiliser was supplied in the field. The increase was in the range of 5.3-fold with TSP and 4.3-fold with PR above the no-P treatment (Table 2). No significant difference was observed between the two P sources.

The application of PR and TSP enhanced total N accumulation by *C. micans* grown in the screen house by 51 and 84%, respectively, over the unfertilised control treatment (Fig. 1). In the field, the increase ($p<0.01$) in legume total N accumulation was 5- (TSP) and 3.6-fold (PR) over the unfertilised treatment. There was no significant difference between the two P fertiliser sources (Fig. 1).

Table 2 *C. micans* shoot dry weight and P uptake as affected by P sources in the screen-house (1st cropping cycle) and field experiments

P sources	Legume shoot dry weight (kg ha^{-1})		Legume P uptake (kg ha^{-1})	
	Screen-house	Field	Screen-house	Field
0 P	2,000	802	3.1	1.3
PR	2,410	3,478	4.4	6.2
TSP	3,219	4,280	7.3	11.5
Mean	2,543	2,853	4.9	6.3
$LSD_{0.05}$	761	1,379	1.29	2.8
Probability	0.027	0.005	0.002	0.001

0P Unfertilised control, *PR* phosphate rock, *TSP* triple superphosphate

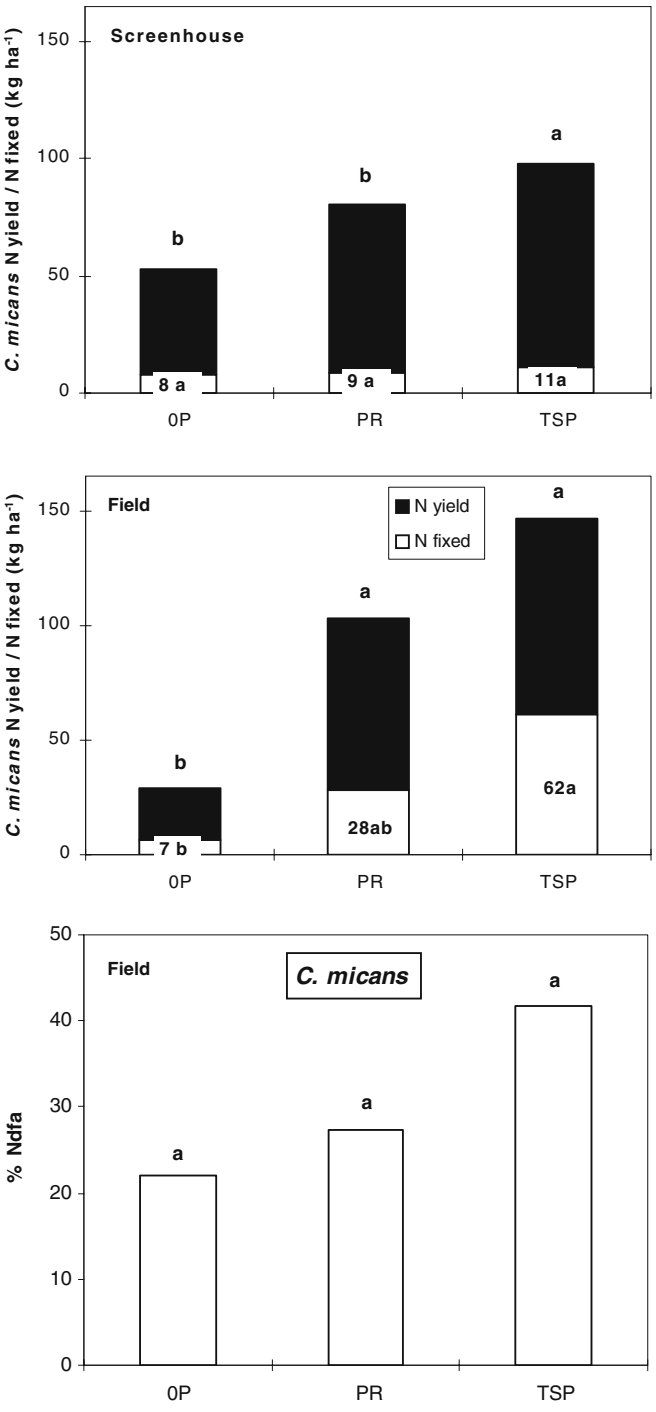

Fig. 1 Legume N yield, amounts of N fixed and proportion of N derived from atmosphere (%N_{dfa}) as affected by P sources in screen house (1st cropping, ^{15}N isotope dilution) and field ($\delta^{15}N$ method). *Bars* capped with the same letters are not significantly different (LSD, $p<0.05$)

The amount and percent of N fixed (N_{dfa}) were estimated by the ^{15}N-isotope dilution and the $\delta^{15}N$ natural abundance methods in the screen house and the field experiments, respectively. Therefore, caution is required in the compar-

ison of the BNF-N estimates under the various experimental conditions.

BNF-N measured in the first cropping cycle in the screen house was inconsistent, low and not significantly affected by P fertilisation, irrespective of the P source (Fig. 1). An attempt was made, therefore, to compare treatment effects on N_2 fixation by examining the ^{15}N atom % excess differences in *C. micans* as an indicator of the relative differences in N_2 fixation by the N-fixing plant induced by P fertilisation. This approach has the advantage of excluding any imprecision associated with the choice of an inappropriate non-fixing reference plant. The differences in N_2 fixation then only depend on the relative ^{15}N enrichments in the N-fixing crop as influenced by the imposed treatments (P fertilisation). The non-obligatory requirement for a reference crop in comparing treatment effects may be justifiable when the precise quantification of N_2 fixation is not compelling (Danso et al. 1993). Equation 1 gives a valid basis for such methodology. Again, data showed no significant differences in ^{15}N enrichments in the aboveground biomass of the N-fixing legume (*C. micans*) as a result of P fertilisation. The ^{15}N atom % excess in *C. micans* was 2.839, 1.570 and 1.817 for the unfertilised control, PR and TSP treatments, respectively, with a $LSD_{0.05}$ value of 1,117. For the non-fixing legume (*C. obtusifolia*), these values were 2.429, 1.776, 1.991 and 1.084, respectively.

In contrast, in the field, the amount of N_2 fixed (kg ha^{-1}) was about ninefold ($p<0.05$) enhanced by TSP and fourfold by application of PR, as compared to the unfertilised control (Fig. 1). Again, no significant difference was observed between the two P sources. However, phosphorus application did not significantly affect the percentage of N derived from atmosphere ($\%N_{dfa}$) per se, the value of which was <50% (Fig. 1).

The cumulative effect of P on the performance of *C. micans* is showed in Fig. 2. The patterns of biomass yield, N_{dfa} and N and P accumulation during the second and third cropping cycles showed a steadily increasing trend. By the third cropping, legume biomass yield and N accumulation in the plots treated with PR steadily caught up with soluble P as a P source. Nitrogen fixation in the second cropping was not determined because cross-contamination of plant materials during sub-sampling for ^{15}N analyses was suspected. Estimates of the amounts of N fixed (kg ha^{-1}) by *C. micans* showed an ascendancy ($p<0.05$) over the study period (cropping cycles effect). The pattern in legume total P accumulation was similar to that for the amount of N fixed. The performance of the unfertilised *C. micans* improved over the same period of study, though at a less strong rate as compared with the application of P fertilisers (Fig. 2).

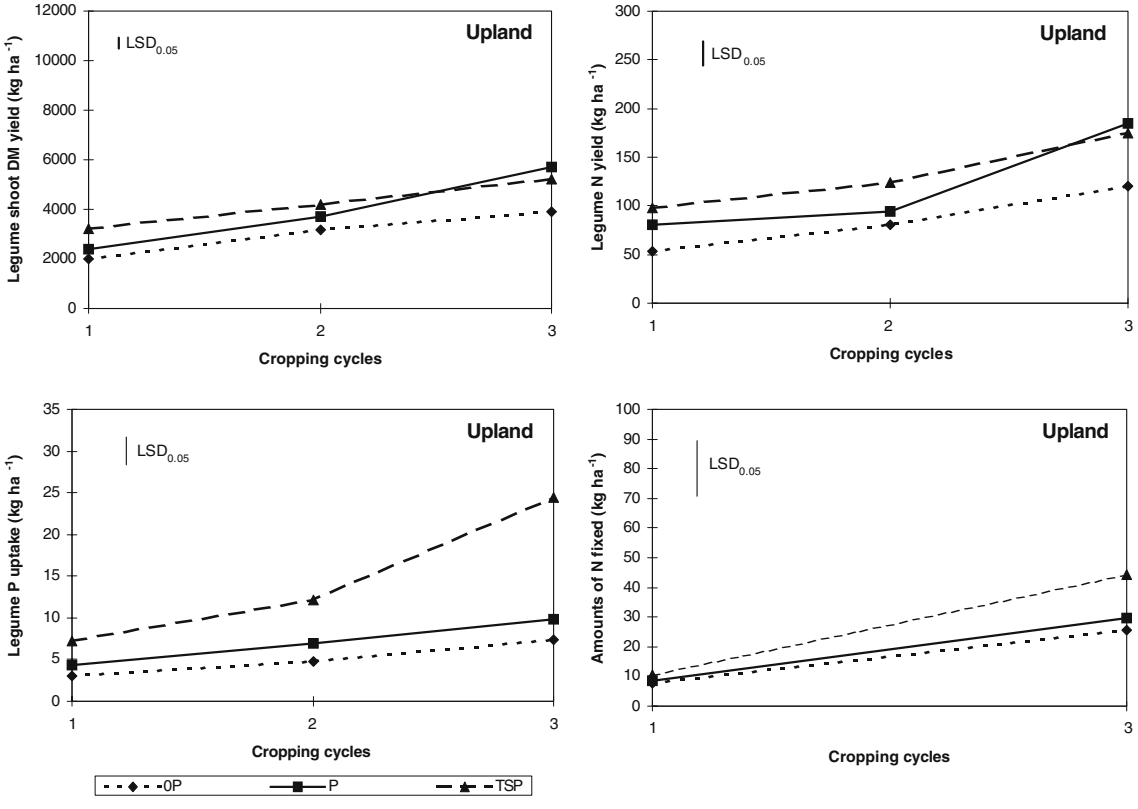

Fig. 2 Effects of repeated P additions on legume biomass yield, P and N accumulation over three cropping cycles under upland conditions in the screen house. $LSD_{0.05}$ values are meant to compare P sources over cropping cycles

Discussion

The 8-week legume growth duration was chosen to coincide with the dry-to-wet season transition period before the cropping season in the humid forest zone of Côte d'Ivoire. Adoption of legume-food crop rotations by farmers may be facilitated by the fact that the short (8 weeks) cropping cycle of the preceding cover legume would be completed in the absence of other major food crops in the farming calendar. Reluctance of farmers to devote their fields to green manuring at the expense of cash or food crops has been reported (Becker et al. 1995).

Neither nodulation nor the percent of N derived from atmosphere ($\%N_{dfa}$) per se by *C. micans* responded to P fertilisation, regardless of the P source. This result supports suggestions made by Ankomah et al. (1996) and Sanginga et al. (1996) that the effect of P on N_2 fixation of root-nodulating legume species was mainly in the total amount of N fixed rather than on the percent of N derived from the atmosphere ($\%N_{dfa}$) per se. On the other hand, total N accumulation of *C. micans* was enhanced as a result of improved biomass yield as P was added. A relatively high shoot N concentration in both the fixing and the non-fixing legumes (mean N content in the aboveground matter was 3 and 3.3%, respectively) suggests that soil N supply was probably adequate to meet legumes N requirements. For energetic reasons (Graham and Rosas 1979), uptake of soil mineral N substituted for or may even have repressed N_2 fixation (Becker et al. 1986). The translocation and sieving of the experimental soil might have stimulated soil microbial activity and N-mineralization processes.

However, no attempt was made to confirm this hypothesis on the experimental soil. Data (not shown) on mineral N performed on in situ soil samples could not corroborate this suggestion. It is possible that the suspected enhanced microbial activity as a result of the soil translocation and sieving might not have taken place in situ. Furthermore, it cannot be excluded that rhizobial seed inoculation with indigenous isolates from West Africa was inefficient, as *C. micans* originates from Southeast Asia (Becker, personal communication) and has so far not been cultivated in West Africa (no efficient rhizobial strains were present in the soil). It is noteworthy that by the third consecutive legume cropping, N_2 fixation increased on average from 9 to 36 kg ha^{-1} or 12 to 33% N_{dfa} (Fig. 2). This may be related to the adaptation or the build-up of the rhizobial population in the soil with time.

Results (Fig. 2) suggest that the effect of PR on the performance of *C. micans* was improved with time and PR effectiveness was equal to the soluble P source by the third cropping cycle. A similar high effectiveness of applied PR-P in relation to soluble P was reported from mucuna trials in Vietnam (Ng Thai Tsiung 1993) and with soybeans in Indonesia (Wajananawat 1993). Our results could be explained by examining the chemical status of the soil in which the legume crop was grown. The exchangeable acidity ($Al^{3+} + H^+$) of the soil was low and less than 2.5% of CEC (Table 1). Thus, P-fixation may not have been an important mechanism affecting PR-P availability in this soil. The importance of P-fixation in most West African acid Ultisols may have been largely overestimated (Mokwunye and Hammond 1992). In fact, these soils have low capacity for P adsorption and, frequently, a rather low Al saturation (Buresh et al. 1997).

The improvement in the performance of the unfertilised legume crop with the successive cropping cycles (Fig. 2) might be the result of additional phosphate ions released from the decomposition of incorporated legume residues.

De Swart and van Diest (1987) demonstrated that in acid soil, solubilization of Tilemsi PR (the same material was used in the present study) proceeds rapidly enough to supply sufficient P to young plants of *Pueraria javanica*. This may explain the observed positive response of the legume biomass yield to applied PR-P, which was statistically similar to that of TSP (Table 2). Although the $\%N_{dfa}$ by *C. micans*, as measured in the field (Fig. 2) was not significantly affected by application of PR-P, it was similar (28%) to the 36% reported by Becker and Johnson (1998) using the same measurement method in the humid forest zone in Côte d'Ivoire.

Conclusion

Under P-deficient acid soil conditions in the West African humid forest zone of Côte d'Ivoire, significant positive response to rock-P application was observed for biomass and N accumulation by the root-nodulating N-fixing *C. micans*. The significant increase in the total amount of N accumulated by *C. micans* was the effect of higher biomass yield response to PR-P application. This study suggests that *C. micans*, when grown as a preceding legume crop, can meet the N demand of the subsequent food crop, provided it is fertilised with phosphorus. The indigenous unprocessed Tilemsi rock-P from Mali appears to be an effective source in this regard.

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