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Full Length Research Paper

Biomass and nitrogen accumulation by green manure legumes as affected by phosphate rock in submerged soils

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Lowlands in West Africa are increasingly absorbing the growing pressure on land for food crop production. However, the current intensification of lowland cropping systems result in the mining of soil nutrient reserves, particularly of nitrogen (N) and phosphorus (P). While N can be supplied via biological N fixation (BNF) by legumes, P deficiency frequently limits both food crop growth as well as legume BNF, especially on acidic soils in the humid forest and savanna regions. Fortunately, large deposits of phosphate rocks (PR) exist in many African countries; and moreover, the PR efficacy can be enhanced when applied to submerged acidic soils. Thus the use of PR may not only supply the limiting P to soil and food crops, but also stimulate BNF-N by legumes including legumes used for green manuring in production systems. A screen house experiment was conducted to evaluate the effect of PR-P on the performance of N-fixing legume *Aeschynomene afraspera L.* during three successive cropping cycles under submerged conditions. BNF was estimated by the ^{15}N-isotope dilution technique, using rice (*Oryza sativa* L.) as a non-fixing plant. The experimental soil was Ultisol low in available P. Unprocessed PR from Mali was applied at 60 kg P ha^{-1}. Initial application of PR significantly increased legume above ground biomass and BNF over the unfertilized control by 1.4- and 3-fold respectively. This PR-induced growth stimulation further increased during the subsequent cropping period with a maximum biomass of 6.4 tons ha^{-1} and 80 kg of BNF-derived N ha^{-1} above the unamended control. The application of P significantly increased the percent N derived from the atmosphere (% N_{dfa}). However, compared to the first two cropping cycles, the efficacy of applied PR-P declined during the third cropping cycle, probably as a result of rise in soil pH following soil reduction. The results suggest that PR can be a useful P source for food crops in rotation with legumes.

Key words: *Aeschynomene afraspera*, phosphate rock, biological nitrogen fixation and flooded soils.

INTRODUCTION

According to estimates, lowlands and hydromorphic fringes occupy 22 million ha in West and Central Africa and the present time the resources is greatly underutilized (Andriesse and Fresco, 1991). With continued degradation of upland areas, lowlands have the potential to absorb the growing pressure on land for food crop production in the region. However, intensification of lowlands is leading to large scale nutrient depletion and the incidence of pests and diseases, which increasingly limit potential yield gains in the existing production systems under low-input management (Becker and Johnson, 1999). Thus, the intensification of lowlands in Côte d'Ivoire was associated with a decline in soil pH and extractable P and a negative N balance, resulting in declining rice grain yields (Becker and Johnson, 1999). To alleviate the constraint of nutrient mining in wetlands, the efficient use of resources

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Abbreviations: BNF, Biological N fixation (BNF); %N_{dfa}, Percent of N derived from the atmosphere; GM, Green manure legume; PR, Phosphate rock; TSP, Triple super phosphate.

Table 1. Physical and chemical properties of soil used in the concrete container in screen-house.

Characteristics	
Soil order	Ultisol (Aquults)
Texture	Loam
pH H_2O (1:2.5)	5.2
pH KCl	4.4
Organic C (%)	1.10
Total N (%)	0.08
Available P (Bray 1) mg kg^{-1}	4.0
Exch. Ca (cmol (+) kg^{-1})	1.26
CEC (cmol (+) kg^{-1})	3.86
Exch. Acidity (cmol (+) kg^{-1})	0.06
% (Al^{3+} + H^+) of CEC	1.55

internal to the production system (e.g. green manure legumes, GM) and the use of indigenous amendments (e.g. PR) may provide an affordable alternative to purchased inputs for resource-limited farmers (Somado et al., 2006).

Grown for a short period (45 - 60 days), many GMs such as the flood-tolerant N_2-fixing legume *Aeschynomene afraspera* have been reported to accumulate a significant amount of N to provide much of the N required by rice (Somado et al., 2003). Further, P application to GMs enhances biomass and N accumulation through biological N fixation (BNF) (Becker et al., 1990; Somado et al., 2003). Becker et al. (1991) reported that P application to semi-aquatic legume, *Sesbania rostrata* doubled N accumulation by the legume. In addition, Shinde et al. (1978) observed that PR applied to a continuously flooded acid soil 2 - 3 weeks prior to submergence solubilized enough PR to meet P requirements of the rice crop. The objective of this study was to further explore the efficacy of PR as a source of P to enhance nitrogen supply via BNF in lowland rice-legume production systems. A screen house experiment was carried out to study the efficacy of PR from Mali on biomass production and N accumulation by a short-duration, flood-tolerant legume under submerged conditions. Clearly, P supply is essential for optimizing BNF and its contribution to nitrogen supply in the succeeding cereals such as rice (Somado et al., 2003).

MATERIALS AND METHODS

Experimental site, soil, plant material and phosphorus sources

Experiments were conducted in concrete containers in the screen house at the main research station of the Africa Rice Center (WARDA) at M'bé (derived savannah zone, 7.5° N, 5° W, 280 m altitude) in Côte d'Ivoire. Mean temperature and photosynthetically active radiation (PAR) during the screen house experiments varied between 27 and 31°C and between 15 and 19 MJ, respectively.

The experimental soil was an Ultisol acidic in pH (pH 5.2) and low in P (4 mg/kg Bray 1 extractable P). The soil in bulk was collected from the surface layer (0 - 20 cm) Danané field site in the humid forest zone of Côte d'Ivoire, air-dried, sieved (< 10 mm), and mixed in the concrete mixer. The field site sampled did not have any past record of fertilization. Selected physical and chemical characteristics of the soil are in Table 1.

A. afraspera J. Leonard was grown for evaluating its N_2-fixing potential. The legume originates from tropical Africa (Becker et al., 1990) and is widely distributed in lowlands, and temporarily flooded soil niches. The ability of *A. afraspera* for extensive nodulation (both on stem and root), high N_2-fixation and tolerance to flooding as well as rapid growth determine its value as a promising nitrogen source for crop grown in rotation under submerged conditions. Rice (*Oryza sativa* L.) was used as a non N_2-fixing reference plant for BNF estimation (Pareek et al., 1990).

Tilemsi PR from Mali was used as P source in the study. This PR had shown potential as an effective and economically profitable P source as compared to imported triple super phosphate (TSP) fertilizer (Bationo et al., 1997). Total P content of the phosphate rock sample used in the experiment 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 for the PR was 4.8. Water-soluble triple super phosphate (TSP) was used as a reference P source in the study. TSP is manufactured by treating PR with phosphoric acid (H_3PO_4) and contains 19.6 % water-soluble P (44% P_2O_5).

Plant culture

The legume seeds were scarified for 30 min in concentrated sulphuric acid (commercial grade) to break dormancy and achieve a high and even germination rate. The seeds were then rinsed with tap water and air-dried. To inoculate the legume plant, fresh nodules of *A. afraspera* were collected from 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 x 0.10 m) in the concrete containers. *A. afraspera* (N_2-fixing) and rice (non N_2-fixing) were grown for 8 weeks at the same spacing. Eight weeks correspond to the time available for a short-duration preceding GM grown in the transition period between the dry and rainy seasons, in the absence of food or cash crops in the field.

BNF estimation

^{15}N-labeled ammonium sulphate fertilizer (10% atom excess) was used to estimate the proportion of legume-N accumulated that was derived via BNF. Each concrete container sown to *A. afraspera,* contained two open-ended PVC-pipes (20 cm-diameter and 40 cm long each) where the soil had been ^{15}N- labeled. Prior to P and K application, ^{15}N-labeled ammonium sulphate (10 atom % excess) was added to the soil. The ^{15}N labeled fertilizer was applied, by dissolving in 380 ml distilled water, at a rate of 1 kg ^{15}N ha^{-1} (container) (Hardarson and Danso, 1990). Ten ml of this solution was pipetted into a beaker and the volume made to the 20 ml mark with distilled water.. The upper 15-cm soil layer 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 once at the start of the experiment. In the subsequent cropping cycles, it was expected that some of the ^{15}N would remain in the available soil N pool, and that the soil N system will be sufficiently stabilized for reliable BNF estimation

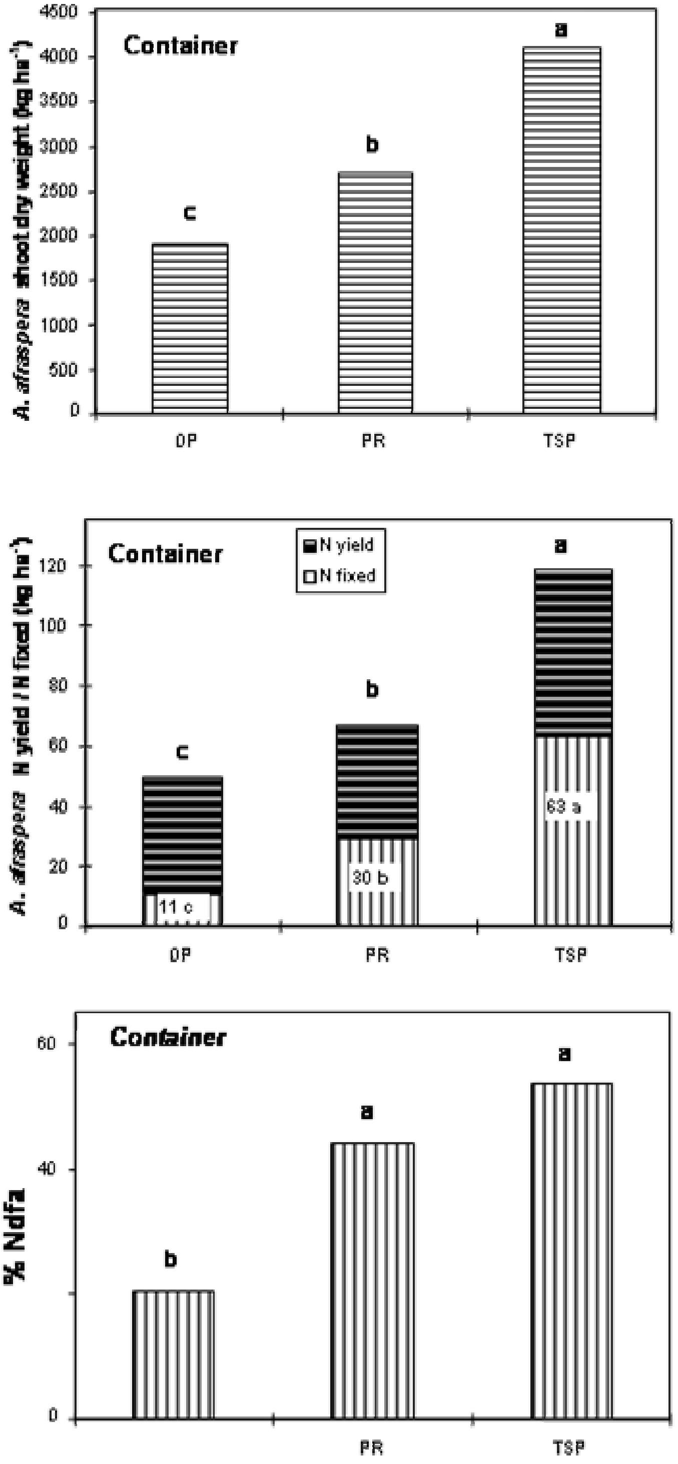

Figure 1. Legume shoot dry weight, N yield, amounts of N fixed and proportion of N derived from atmosphere (% N_{dfa}) as affected by P sources in containers under submerged conditions. Bars capped with the same letters are not significantly different ($LSD_{0.05}$, $p<0.05$).

(Pareek et al., 1990). Rice *(O. sativa* L.) was used as a non-fixing reference plant (Pareek et al., 1990). The soil was saturated with water and puddled manually after application of P and K. Scarified and inoculated seeds of *A. afraspera* were then planted and thinned to 2 seedlings per stand after emergence. The soil was submerged under a 3 - 5 cm water layer and the flooded conditions were maintained throughout the growing period. Both the N-fixing legume and the non-fixing reference rice crops were harvested 8 weeks after sowing.

Experimental set-up and measurements

The soil (Table 1) was filled to a depth of 40 cm into concrete boxes measuring 2 m x 1m x 0.6 m. A 10 cm gravel layer underneath the soil and a lateral outlet to the drainage canal allowed for drainage. Each of the 9 containers was kept constantly flooded. Basal P was applied at planting of the legume crop. Finely ground PR (< 0.3 mm) and TSP (in granular form) were applied at 60 kg P ha^{-1} $cycle^{-1}$ and manually incorporated into the upper 10 cm of the soil. All treatments received at the beginning of the experiment a uniform dose of 100 kg K ha^{-1} as potassium chloride (50% K). No mineral N fertilizer was supplied. The three treatments (no P, PR and TSP) were replicated three times and arranged in a randomized complete block design.

Eight weeks after sowing, legume shoots were cut at the soil surface level from an area of 0.8 m^2, and weighed to determine the fresh matter yield of the legume plants. Dry weight was then determined after oven drying at 70°C for three days.

Aboveground and belowground plant parts were oven-dried, and sub-samples were then taken for total N and ^{15}N analysis. Biomass yield and nutrient content (N and P) of the legume aboveground biomass were determined at harvest. The legume shoot biomass samples were analyzed 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 analyzed following the vanado-molybdate yellow color method (Okalebo et al., 1993). The oven-dried material was finely ground using a ball-mill. Five-mg sub-samples were weighed into small tin capsules, which were then closed and rolled into a ball. These samples were then analyzed for total N and ^{15}N using an Elemental N analyzer connected to a mass-spectrometer (Reineking et al., 1993).

The experimental soil samples were collected at the initiation of the experiment and the onset of flowering and analyses carried out in three replicates using sub-samples from randomly selected composite samples per treatment. Each composite sample of 500 g moist soil was air-dried and analyzed 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 (Okalegbo et al., 1993), total Kjeldahl N, extractable Bray-1 P and exchangeable acidity (Okalegbo et al., 1993).

Calculations and statistical analysis

The proportion of plant N derived from atmosphere (% N_{dfa}), was computed using the following equation (1) from Hardarson and Danso, (1990)

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

where, N_{fix} is the mean % ^{15}N atom excess in stems, leaves and roots of *A. afraspera* (), and N_{ref} the mean % ^{15}N atom excess in stems, leaves and roots of the non-fixing rice plant.

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

$$N\ (fixed) = (\%Ndfa/100) \times TNY \quad (2)$$

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

$$TNY = (LSN/100) \times DM \quad (3)$$

Where: TNY= Total N yield (kg ha^{-1})
LSN = Legume Shoot N concentration (%)
DM = dry matter yield (kg ha^{-1}) of legume.

Data were analyzed 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

Total P accumulation in the legume biomass significantly ($p<0.01$) increased over the control by 1.9 (PR) and 3.8-fold (TSP) to about 6 and 12 $kgha^{-1}$, respectively. The increase in total P accumulation in response to P application was associated with a significant increase in the legume shoot P concentration. Legume shoot P concentration was 1.13, 1.23 and 2.56 g kg^{-1} DM for the unfertilized control, PR and TSP treatments respectively, with a .$LSD_{0.05}$ value of 0.07 g kg^{-1} DM.

Nitrogen fixation in *A. afraspera* as affected by P nutrition using TSP or PR was measured using the ^{15}N-isotope dilution technique. n the first cropping cycle, PR-P application significantly ($p<0.01$) increased the proportion of plant N-derived from fixation (% N_{dfa}) by a factor of 2.2-fold to 44% over the unfertilized legume. There were no significant differences between PR and TSP (Figure 1). Likewise, the application of P in the concrete container in the first cycle increased ($p<0.001$) total N accumulation in *A. afraspera* from 49 kg N ha^{-1} in the unfertilized control plot to about 119 kg N in TSP and 69 kg N ha^{-1} in the PR treatments. Further, P fertilization enhanced the contribution of BNF to legume-N accumulation over the control without P by as much as 6- (TSP) and 3-fold (PR) (Figure 1).

The amounts of N2 fixed per plant were significantly correlated with legume P uptake (r=0.99***). Significant linear correlations were also found between amounts of N2 fixed per plant and legume P uptake (r = 0.97***) and shoot P content (r = 0.87**), and with soil extractable P-Bray1 at the onset of legume flowering (r=0.77*).

In the first cropping cycle legume DM was significantly increased over the unfertilized treatment. The mean increase in DM was 1.4 and 2.2 times in response to PR and TSP application, respectively (Figure 1).

On the other hand, the repeated applications of P significantly increased the legume biomass and N accumulation during the first two cropping cycles only, and the legume biomass and N accumulation significantly decreased in the third cropping cycle (Figure 2). The effect of PR on the amount of N_2 fixed (kg ha^{-1}) was beneficial ($p<0.01$) to *A. afraspera* during the first two cropping periods only (30 and then 78 kg N fixed ha^{-1}, respectively).

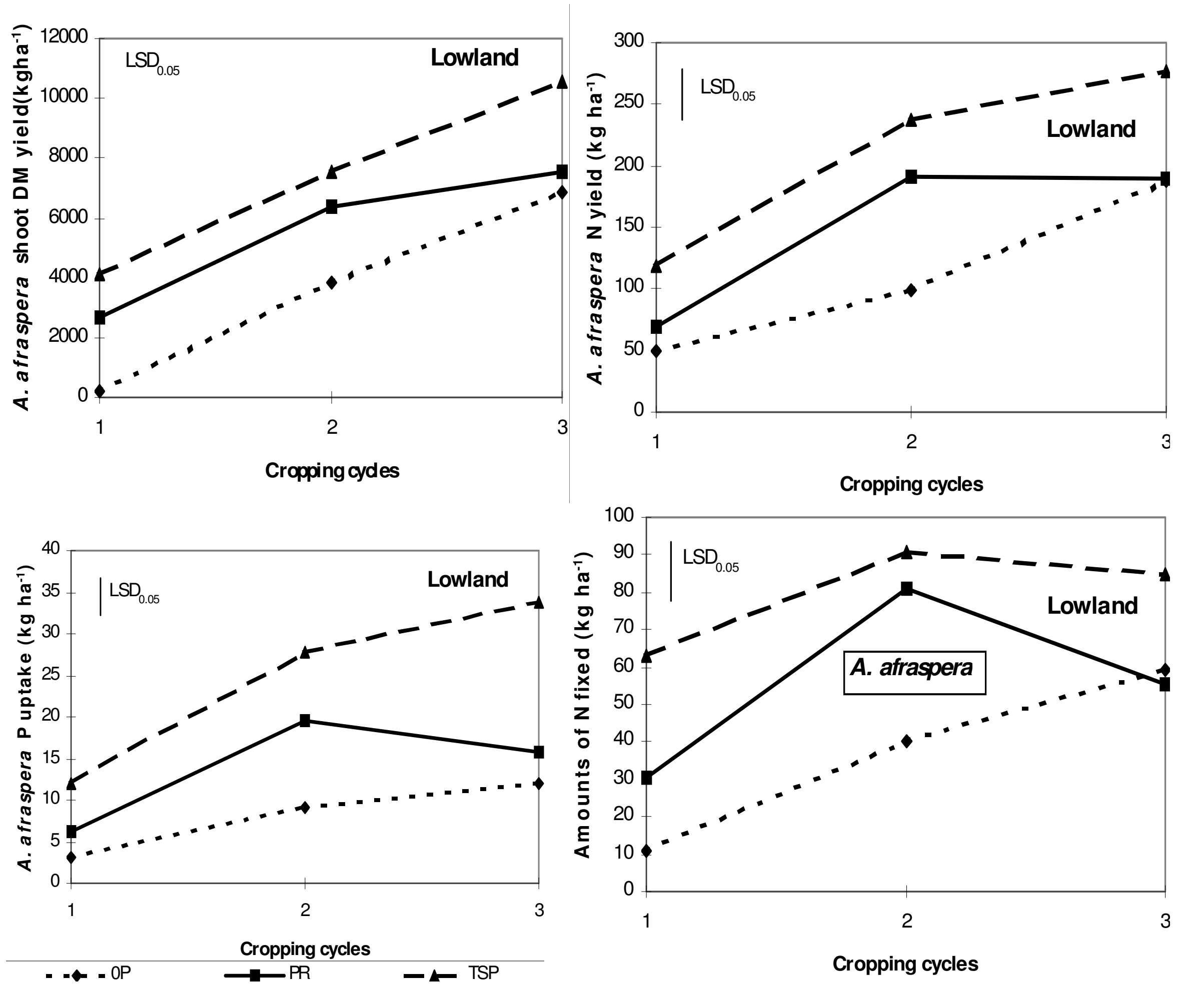

Figure 2. Effects of repeated P additions on legume performance during three subsequent cropping cycles under submerged conditions in the concrete container. $LSD_{0.05}$ values are meant to compare the same P source over cropping cycles.

The BNF by the legume declined significantly ($p<0.01$) to 55 kg ha^{-1} in the third cropping cycle (Figure 2). The effect of TSP had a trend similar to that of PR. In contrast, the performance of the legume in the P-unfertilized treatment improved over time, though at a slower rate, compared to treatments with applied P.

DISCUSSIONS

The rationale for using GM in crop production systems is to meet a cereal crop's N requirement, and reduce farmers' dependency on external mineral N inputs for sustainable food production (Ladha et al., 1992). The present study was undertaken with the objective of improving the contribution of BNF to N accumulation and biomass production of a preceding flood-tolerant GM (*A. afraspera*) through application of PR. The initial application of P, especially in the case of PR was largely beneficial to the flooded-tolerant legume grown in P-deficient acid soil under continuously flooded conditions; and the applied P increased nitrogen and biomass accumulation by *A. afraspera*. Shinde et al. (1978) observed that application of PR to an acidic soil, 2 - 3 weeks prior to submergence, solubilized PR-P to meet P-requirements for rice growth. Similarly, we observed that a 2 week unflooded period between PR-P application (at legume planting) and the start of continuous flooding in the present study improved plant establishment after legume sowing. Enhanced biomass and N accumulation with P application has also been reported for the semi-aquatic *S. rostrata* (Becker et al., 1990). A strong linear correlation between P uptake and BNF by *A. afraspera* was observed. Similar results were reported by Cassman et al. (1993), who suggested a synergy between legume fixed-N and P supply. This synergy needs to be exploited to maximize yields of cereals grown in rotation with N-fixing legumes.

In the present experiments P deficiency limited legume crop performance. As in previous studies (Somado et al., 2003), alleviating this deficiency with PR increased the accumulation of biomass and nitrogen in *A. afraspera*. However, it cannot be excluded that the limited soil volume in containers led to an overestimation of PR effects due to high root density and lessened impact of phosphate ions diffusion problem (Junk and Claassen, 1997). Also, results in the concrete container experiment showed the positive effect that PR potentially has on the performance of the flood-tolerant legume (Figures 1 and 2). Furthermore, *A. afraspera* is indigenous to West Africa (Becker et al., 1990) and effective rhizobia have been shown to be abundant in a wide range of soils of the sub-region (Alazard and Duhoux, 1987). Efficient indigenous rhizobial populations may have likely accounted for a good P response of *A. afraspera.* The results in Figure 2 suggest that repeated sequences of flooding and drying of the submerged soil in the course of the experiments resulted in decreased effectiveness of PR with time. Similarly, Chien (1977) reported decreased PR dissolution in a flooded acid soil with time. It is possible that a flood-induced rise in soil pH may have been responsible for the observed decline in PR effectiveness in submerged soil and the resulting performance decline in *A. afraspera*. However, no attempt was made to measure pH *in-situ* during the experiment. The improvement in the performance of the unfertilized legume crop with the subsequent cropping cycles (Figure 2) might be due to the increasing availability of native soil P as a result of submergence in the concrete container (Narteh and Sahrawat, 1999).

Conclusion

Significant response to PR application was observed for biomass and nitrogen accumulation in *A. afraspera* grown for 8 weeks under submerged conditions in concrete containers. These results confirm the potential for enhanced N contribution of *A. afraspera* grown as a short-duration preceding legume crop with P fertilization. Even under submerged soil conditions, the Tilemsi PR was an efficient source of P. Furthermore, the study shows a synergy between legume fixed-N and P from PR. This synergy can be exploited to maximize yields of a cereal crop grown in rotation with the flood-tolerant legume

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