Effect of low-phosphorus-containing legume residues on Bray-1 extractable phosphorus in reddish brown earth soil in dry zone of Sri Lanka

W. C. P. Egodawatta[1*] , R. L. Senanayake[2]

[1] Department of Plant Sciences, Faculty of Agriculture, Rajarata University of Sri Lanka.
[2] Agronomic Division, Field Crop Research and Development Institute - Mahailluppallama, Sri Lanka
*Corresponding author: *egowcp@gmail.com*

Abstract:
Experiments were conducted to study the dynamics of extractable P (P extracted by Bray-1-extracting solution) of reddish brown earth amended with or without green manure (GM) residues of contrasting P concentrations in the absence of growing plants. In two separate experiments, GM residues of *Crotalaria juncea* and of *Gliricidia sepium* with varying P concentrations were added to anacidic soil amended with Eppawala rock phosphate (ERP) or triple superphosphate(TSP). P concentration of the residues added varied, while lignin and C: N ratios were approximately similar. Changes in soil extractable Bray-1-P were measured at the end of the incubation period (60 or 80 days). In the aerobic soils, extractable P in the combined ERP+GM and TSP+GM treatments were significantly lower than in the ERP- or TSP- treated soils. The amendment with GM residues alone significantly increased Bray-1-P over the un-amended control in the case of the inorganic P-fertilized GM residues. The trend in extractable P was similar in the soils incubated under anaerobic conditions. However, in the case of ERP, concentrations of P extracted by Bray-1 solution did not significantly change in the presence or absence of GM. The results suggest that the incorporation of GM residues with low P concentration does not lead to a net P release in upland or lowland soils. These results have implications for phosphorus nutrient cycling, mainly in farming systems in dry zone of Sri Lanka, as most of the soils are poor and very low in available P.

Key words: Bray-1, *Crotalaria juncea*, Extractable phosphorus, Green manure, *Gliricidia sepium*

Introduction

Application of legume green manure (GM) is suggested to be effective in increasing the availability of native soil phosphorus (P) and the dissolution and utilization of rock phosphate (RP)-P by food crops.Resource poor farming is a major livelihood activity in rural regions of Sri Lanka, where many farmers seeking alternative for high cost mineral fertilizers. GM is a solution that they use widely, though without knowing the true scientific background. Indigenous knowledge of these subsistence farmers in the dry zone of Sri Lanka relies on organic inputs, including GM to sustain soil fertility. Studies have shown that GM residues incorporated into the soil are effective in increasing the availability of native soil phosphate and the dissolution and utilization of phosphate rock (PR-P) by subsequent crops[1-3]. It is hypothesized that organic acids produced during decomposition of the residues by the micro-faunal population prevent precipitation of phosphate by iron (Fe) and Aluminium(Al) oxides out of the soil solution[4], and as a result, phosphorus (P) concentration in the equilibrium solution increases. Competition for P-sorption sites between P and the released organic acids as well as complexation of Fe and Al oxides/hydroxides by organic acids have been suggested as the key factors controlling reduction of soil P-sorption capacity and P availability in soil solution[5]. The author reported that decomposition of *Tithonia diversifolia* residues reduced the P sorption and increased the available-P pools of an acid soil over a 16-week period. However, plant P availability does not always increase following GM incorporation. However, magnitude of the effect of GM on soil test P availability may depend on the organic residues quality, especially the C: P ratio[6].

This soil incubation study was undertaken to determine the effects of GM residues varying in P content on extractable P in the soil amended with plant residues under simulated aerobic upland and anaerobic flooded conditions.

Materials and Methods

This experiment was conducted in January 2012 in the laboratory of Field Crop Research and Development Institute Mahailluppallama.

Soil used and its characteristics
A subsample of Reddish Brown Earth (pH 5.2), deficient in P (4 mg kg-1 Bray-1 P) was used in the study (Table 1). A bulk surface sample (0-20 cm) from a natural fallow field was transported in sufficient quantity to laboratory; the soil was air-dried, sieved (<2mm), and homogenized.

Sub sample of the soil was analysed for pH in water or 1N KCl (1:2.5), Bray-1-P (0.03 M NH_4F + 0.025 M HCl) in a 1:7 soil to solution ratio[7], organic C (Walkey-Black method)[8] and total N (Kjeldahl distillation)[9], cation-exchange capacity (CEC) and exchangeable base cations (1N NH_4OAc, pH 7)[10], and total P[11](colourimetrically after digestion of soil with nitric and perchloric acid). Exchangeable acidity (Al^{3+} + H^+) was measured by the titration method using non buffered, neutral salt (KCl). Some physico-chemical characteristics of the soil are summarized in Table 1.

Table 1. Physico-chemical properties of soils used in the study.

Soil parameters	
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

Fertilizer P and green manures
The Eppawala Rock Phosphate (ERP) (finely ground <100 µm, 14.1% P) was used in the experiments. The chemical composition of theERP is given in Table. 2. Triple superphosphate (TSP) (45% P_2O_5) in the granular form was used as a reference for comparison.

The GM residues used in the study were shoot biomass (stem + leaves) of *Gliricidia sepium*(upland tree legume) and *Crotalaria juncea* (annual fodder legume); before use, the residues were cut into small pieces (<0.25mm).

Chemical analyses of GM residues
Sub samples of the biomass were oven dried (700C) for 72 h, ground, and analysed for total N (Kjeldahl procedure)[9], total 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 colourimetry following the vanado-molybdate yellow-colour method[12]. Lignin content was measured using the acid detergent fibre-permanganate method[13]. The residues varied in P content, but lignin and C:N ratios were approximately similar (Table 3).

Table 2. Selected characteristics of Eppawala Rock Phosphate used in the study.

Neutral ammonium citrate solubility (P_2O_5)	As a fraction of total (%)						
	P	CaO	MgO	CO_2	SiO_2	Al_2O_3	Fe_2O_3
2.1	14.1 (33% P_2O_5)	43	0.19	2.2	0.80	2.20	12.2

Experimental procedure: Incubation study
Green manure legume residues (air-dried, <0.25mm) with varying P contents were added at a rate of 5% (w/w) to 25g sample of air-dried soil in 100ml plastic bottles. Phosphorus fertilizer as TSP (granular) or ground (<100 µm) ERP was added to the mixture at a rate of 5% of soil (w/w). Soil moisture in the plastic bottles was adjusted to field capacity of the soil (Experiment 1) or saturated with distilled water (Experiment 2) to simulate aerobic upland and anaerobic flooded conditions, respectively. The plastic bottles in the simulated upland condition were loosely covered, whereas those in the anaerobic system were capped airtight. The bottles were regularly weighed to maintain constant moisture condition throughout the incubation period. Distilled water was added as required, to maintain the moisture levels.

Treatments in either experiment included: Treatment 1: control (no GM and no P added to soil in plastic bottles); Treatment 2: GM1 grown in soil (fertilized with ERP) added alone to soil in plastic bottle (no P added); Treatment 3: GM2 grown in soil (fertilized with TSP) added alone to soil in plastic bottle (no P added); Treatment 4: GM1 grown in soil (fertilized with ERP) + ERP added to soil in plastic bottle; Treatment 5: ERP added to soil in plastic bottle (no GM added); Treatment 6: GM2 grown in soil (fertilized with TSP) + TSP added to soil in plastic bottle; Treatment 7: TSP added to soil in plastic bottle (no GM added).

In either experiment, all plastic bottles replicated three times and containing each of the seven treatments above, were placed in an incubator at constant temperature (25°C) using a randomized complete block design. The incubation period was 60 and 80 days under aerobic and flooded anaerobic conditions, respectively, and coincided in each case with the average time to maximum tillering in rice crop, corresponding to the peak in nutrient uptake.

Production of GM biomass with varying P contents
Green manure biomass with different P contents was previously obtained (Table 3) by growing separately *Gliricidia sepium* (upland tree legume) and *Crotalaria juncea* (lowland field conditions), using various P-fertilizer sources. In the upland setup, *G. sepium* plants were fertilized with three P sources (no P and TSP, ERP applied at 60 kg P ha–1) to obtain GM biomass with different P contents. In pots, *C. juncea* was grown using the same sources and rates of P fertilizers. The shoots of the *C. juncea* plants grown in lowland soils were harvested separately 8 weeks after sowing, at the onset of flowering and *G. sepium* was harvested 8 weeks after fertilizer application and 4 weeks after lopping.

Statistical analysis
Normality and homogeneity of variances were tested before detailed statistical analysis. An ANOVA was performed using General Linear Model (GLM) (SAS institute, 2003). Contrast means were calculated to discriminate means, and means were declared as significantly different at $p < 0.05$.

Results and Discussion

At the end of each incubation period, soil was extracted by a solution where the composition was 0.03 M in NH_4F and0.025 M HCl. This extraction technique is referred to as the Bray-1 method and most suitable to assess P availability in acidic soils.

Table 3. Chemical composition of legume residues used in the incubation.

Legume residue characteristic	N ($g\ kg^{-1}$)	Total P ($g\ kg^{-1}$ plant)	C:N	Lignin (%)
Crotalaria juncea(Lowland)				
No P fertilizer applied	26.2	1.4	17.0	8.0
Phosphate rock	33.3	1.7	16.8	7.9
Triple super phosphate	30.4	2.2	16.8	8.3
Gliricidia sepium (Upland)				
No P fertilizer applied	25.5	1.5	14.2	12.5
Phosphate rock	25.5	2.3	13.9	11.8
Triple super phosphate	28.0	2.5	13.9	11.5

The soil used in the present study was acidic (Table 1). Significant treatment effects were observed in the extractable Bray-1 P at the end of each incubation period (60 or 80 d of incubation) (Table4). The results indicated a significant reduction in extractable Bray-1 P, when P fertilizers regardless of the source, were combined with GM residue of low P contents. The changes in extractable Bray-1 P measured at the end of each incubation period are shown in Table 5.

Under the aerobic upland soil condition, soil Bray-1-extractable P in the combined ERP+GM or TSP+GM was significantly lower than that in treatments with ERP or TSP applied alone. Further, soil amendment with GM residues alone significantly increased Bray-1 P over the unamended control, only in the case of TSP fertilized GM residues (Table 5). Similarly, in the flooded anaerobic conditions, addition of GM in combination with TSP significantly decreased extractable P, as compared with sole application of P. In the case of ERP, concentrations of P extracted by Bray-1 solution did not significantly change in the presence or absence of GM. In addition, soil amended only with residues from ERP- or TSP-fertilized GM had a higher Bray-1 P than the unamended control. However, no significant differences were observed between the two sources of P.

Table 4. Single degree of freedom contrast of means of P (mg kg soil^{-1}) extracted by Bray-1 solution in soil amended with P fertilizers and green manure legume (GM) under aerobic and anaerobic incubation conditions.

Treatment contrast	Associated probabilities	
	Aerobic (upland)	Anaerobic (lowland)
TSP vs ERP	<0.001	<0.001
GM1 vs GM2	0.03	0.16
ERP+GM vs ERP	<0.001	0.055
TSP+GM vs TSP	0.027	0.02
GM main effect	0.22	0.41
P main effect	0.59	0.28
GM x P interaction	0.035	0.056

TSP, triple superphosphate ERP, Eppawala rock phosphate
GM1 and GM2, green-manure legume fertilized with ERP or TSP, respectively

In general, extractable Bray-1 P was higher in the upland than in the lowland soil (Figure 1), reflecting differences in P behaviour under aerobic and anaerobic conditions[14] However, assessment of the P status of the reduced soil by a chemical test conducted on air-dried soil, may not provide a reliable estimate of soil P availability after submergence Nonetheless, it is noteworthy that though the availability of P is influenced by flooding[15], yet most of the practical aspects of P availability are done in air dried state for practical application of the results.

Conversely, in the case of ERP, a rise in pH upon submergence was possibly an additional factor that did not favour dissolution of ERP under reduced soil conditions. Nonetheless, the similarity of the responses under upland and lowland soil conditions clearly indicated that application of GM residues adversely affected soil extractable P regardless of P source and soil water regime.

Little evidence of beneficial effects are avaable on resin-P of combined application of T with GM residues, as compared with sole aplication of TSP[5]. Using the 32P-isotope diluti technique,depressing effects of GM residues P derived from the more reactive rock phospha (RP) and the water-soluble TSP were illustrate Using a broad range of RPs of variable reactiity characteristics and GM residues of contrasti chemical properties, these workers showed th the extent of the influence of GM in improving t solubility of RP and soil P availability was assciated with the GM quality, especially its P cocentration. Thus, whether net mineralization of occurs in legume plant residues depends at lea partly on the P content of the incorporated pla material.

Results of the present study could also be explaine by examining the chemical composition of the G residue used in the incubation study (Table 3). Th P concentration in the residues was less than th critical level of 2.5 g kg^{-1} dry matter (DM)[16] or th critical P concentration of 3 g kg^{-1} DM[2], for net release in soils amended with GM. It is notewothy that the P concentration of GM residues use in the experiments[6] ranging between 0.9 and 2 kg^{-1} DM indicated the possibility of assessing mieralization of P. However, net P immobilization i soils amended with organic materials of P concentration as low as 0.9 g kg^{-1} has been reported[17].O the contrary, increase in extractable soil Bray-1 in potted soil planted for rice, under flooded coditions, with the combined application of P fertiizer and residues of *Aschynomenea fraspera* floo tolerant legume[2]. In the present study, Bray-1 was used to monitor the changes in available without effects of plants. InUltisols, soil-solution concentrations in soil solution were too low[15] unless fertilized with high P. It is then easy to pick-u small changes in Bray-1-extractable P.

The results obtained in present study (Figure 1 was contradicted[2], however in lined with a stud on tropical Ultisols in 2007[14]. The discrepanc might be attributed to the P-mobilizing capacity of the rice plants grown under anaerobic soi conditions in the former experiment. In fact, tw studies[18,19] suggested that rice plants growing i reduced soil, acidify their rhizosphere through th following mechanisms: (1) production of H^+

during oxidation of Fe^{2+} by root-released O_2 and (2) direct root release of H^+ to balance excess uptake of cations due to the predominantly NH_4 supply in N nutrition.

Table 5.Extractable soil Bray-1 P in seven treatments following incubation of the soil with GM under upland (60 d) and lowland (80 d)conditions.

Treatment	Extractable soil Bray-1 P	
	Upland	Lowland
Control	0.6f	0.8d
GM 1	1.6f	4.1 d
GM 2	27.2e	4.5 d
ERP+GM	53.4d	35.5 c
ERP	91.7c	39.9 c
TSP+GM	203.0b	158.7b
TSP	212.0a	172.3a
p	< 0.001	< 0.001
S.E.	18.6	15.4

Means followed with the same letters are not significantlydifferent (Tukey'sat 0.05). GM1 and GM2: GM fertilized with ERP and TSP,respectively, and solely applied. Control: no GM and no P were added to soil in plastic bottle. S.E. is standard error of the mean.

Acidification through production of H+ during oxidation of Fe^{2+} would then mobilize further ERP-P and bring about additional P into the soil solution in flooded soils. Likewise, rice grown in P-deficient aerobic soil is efficient in mobilizing P from soil through a different mechanism: excretion of organic anions by the roots[20]. The organic anion is considered to chelate metal ions (Fe, Mn, and Al), which would otherwise immobilize P by precipitation of soluble P.

Conclusion

Under upland or flooded soil conditions, addition of GM residues to soil amended with ERP and TSP did not increase extractable Bray-1 P. This was attributed to the low P concentration of the incorporated residues. The general contention that GM application increases the availability of native soil P and promotes the dissolution and utilization of ERP, should be taken with caution. This study suggests that the soil P cycling is determined by the quality of the incorporated plant residues, especially their P concentration.

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