Phosphorus status and cycling in native savanna and improved pastures on an acid low-P Colombian Oxisol

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Abstract

On acid low-phosphorus (P) Colombian Oxisols, improved pastures with acid-soil-tolerant grass and legume varieties have increased beef production by a factor of 10 to 15 with only modest P fertilizer inputs. This indicates that the efficiency of P fertilization could be greater than is commonly expected on such strongly P-sorbing soils. To understand the effect of improved pastures on P cycling and availability, we estimated P budgets, and characterized soil P by sequential fractionation, isotopic exchange and biological activity measurements on soil samples from unfertilized native savanna, and fertilized improved grass-only (*Brachiaria decumbens* cv. Basilisk) and grass-legume (*B. decumbens + Pueraria phaseoloides*, Kudzu) pastures established in 1978 on a medium-textured isohyperthermic, tropeptic haplustox. Comparison of calculated P budgets, based on inputs and exports, with total soil P contents showed that fertilization, as part of the improved pasture management, had resulted in a measurable increase of total P in the surface 0–20 cm soil layer of nearly 30 $mg\,kg^{-1}$ or about 20% over the savanna level. Sequential soil P fractionation of different seasonal samplings indicated that grass-legume maintained higher organic and available inorganic P levels with less temporal variation than the two other types. The linkage of organic P and available P was also reflected in soil biological activity. Estimates of P in microbial biomass and phosphatase activity were significantly higher in grass-legume than grass-only and savanna. The improvement in soil P availability, as measured by solution P concentration, P sorption and exchangeable P, was much greater in grass-legume than in grass-only. With comparable fertilizer inputs and greater product exports, improved P availability in grass-legume cannot be due to differences in budgets but can be attributed to changes in the overall biological activity in the soil-plant system caused by the presence of legumes in the vegetation cover. Total C, organic P content and macrofaunal activity were all significantly higher in grass-legume soils. Greater turnover of organic litter in grass-legume may provide for steadier organic P inputs and, therefore, higher P cycling and availability.

Introduction

Savannas cover about 45% of the land, or 243 million hectares, in Latin America. They have traditionally been used for extensive cow-calf and feeder grazing with low management and almost no purchased inputs. Productivity was correspondingly low. The main chemical constraints on agricultural production on savanna soils are low nutrient reserves, acidity, aluminum toxicity, and high phosphorus (P) fixation (Sánchez and Logan, 1992). Phosphorus is among the nutrients that most limits plant production on acid savanna soils. Livestock that graze native savanna often suffer P deficiency, which affects especially

their reproductive performance (Fisher et al., 1992). Amending these soils with large amounts of fertilizers (von Uexküll and Mutert, 1993) is uneconomical, logistically impractical and ecologically questionable. Consequently, improved use efficiency of low amounts of fertilizer and the cycling of soil P are important for a resource-conserving and environmentally safe agricultural system. Unfertilized soils have insufficient P available to permit significant cycling of soil P stocks, and thus may not meet the P requirement of improved forage plants (Thomas et al., 1995) and the grazing animals (Fisher et al., 1992). Therefore, a combination of fertilization and pasture improvement is the most promising strategy for improving P availability and grassland productivity.

Field experiments to evaluate the long-term performance, composition and production of introduced exotic pastures under set stocking were initiated at Carimagua on the Eastern Plains of Colombia in 1978 (Lascano and Estrada, 1979) and maintained for 20 years. Improved pastures, using acid-soil-tolerant grass and legume varieties with high P use efficiency, were established and maintained using small P fertilizer applications aimed at activating the soil's nutrient cycles. These improved pastures have increased beef production by a factor of 10 to 15 over extensive native savanna systems (Lascano and Estrada, 1989; Thomas et al., 1995). This dramatic production increase, obtained with only modest P fertilizer inputs, indicates that the efficiency of P fertilization on such highly weathered, strongly P-sorbing soils can be greater than commonly expected provided the savanna vegetation is improved to increase P cycling and availability.

P availability can be assessed by examining the partitioning of applied and native P among different organic (P_o) and inorganic (P_i) forms (Tiessen et al., 1994) or by measuring isotopically exchangeable pools (Fardeau, 1993; Frossard et al., 1994). Several studies have shown the importance of P_o mineralization in providing available P (Friesen and Blair, 1988; Tiessen et al., 1992; Ball-Coelho et al., 1993; Umrit and Friesen, 1994). Although methods to assess soil P_o availability are not yet satisfactory (Tiessen et al., 1994), phosphatase activity and microbial P may serve as indicators of potential P_o availability (Stewart and Sharpley, 1987; Tiessen et al., 1994).

The objective of this study is to estimate P budgets of an unfertilized native savanna and two fertilized improved pastures based on grass-only and grass-legume mixtures, and to evaluate the effect of the introduced pastures on P cycling and availability using fractionation, isotopic exchange and biological activity measurements.

Materials and methods

The field experiment at Carimagua

Soil samples were taken in a long-term pasture experiment established in 1978 at the ICA-CIAT (Instituto Colombiano Agropecuario; Centro Internacional de Agricultura Tropical), Carimagua research station, Meta, Colombia (4°30 N, 71°19 W, 150 m above sea level). The area is representative of well-drained savannas. Mean annual temperature is 26°C and mean annual rainfall is 2200 mm with a distinct dry season from December through March. The soils are Oxisols (tropeptic haplustox, isohyperthermic) with a silty clay loam texture (39% clay, 42% silt, 19% sand) and a bulk density of 1.3 g cm^{-3} (Gijsman and Thomas, 1995). Soil chemical characteristics are summarized in Table 1.

The following treatments were included in the study:

1. Native savanna: unimproved native grassland pasture, burned every 16 months and grazed with 0.25 steers ha^{-1}.
2. Grass-only: improved pasture, containing *Brachiaria decumbens* Stapf cv. Basilisk, grazed with 1 steer ha^{-1} during the dry and 2 steers ha^{-1} during the rainy season.
3. Grass-legume: improved pasture, containing *B. decumbens* cv. Basilisk with *Pueraria phaseoloides* (Roxb.) Benth. CIAT 9900 (Kudzu), grazed at rates equal to the grass-only treatment. The average legume biomass content during 1979 to 1993 was 40% (C.E. Lascano, personal communication).

Experiment plot sizes were 2 ha (Figure 1) and were laid out on the broad flat crest of a wide interfluvial landscape having little visual variability. Although originally replicated in two blocks, one block was given over to a new experiment 10 years after establishment. Earlier data from the experiment (animal live weight gains, biomass production, etc.) showed no significant block effects that would suggest a spatial gradient in the site (C.E. Lascano, personal communication). Nevertheless, additional evaluation of spatial variability was carried out as described below to identify any pre-existing gradients that may not have been revealed in the earlier results of the original trial.

Table 1. Soil pH, total carbon, total nitrogen and exchangeable cations in the 0–10 cm soil layer of native savanna, and grass-only and grass-legume improved pastures

Treatment	pH(H$_2$O)	Total C	Total N	Exchangeable cations				
				Al	Ca	Mg	K	H
		mg g^{-1}	mg g^{-1}	- - - - - - - - - - - cmol$^+$ kg^{-1} - - - - - - - - - - -				
Savanna[a]	4.80 b	23.5 ab	1.45 b	2.42 a	0.26 c	0.11 b	0.08	0.27 b
Grass-only[a]	4.85 b	23.2 b	1.56 ab	2.05 b	0.42 b	0.20 a	0.10	0.39 a
Grass-legume[a]	4.96 a	24.9 a	1.67	1.90 b	0.89 a	0.23 a	0.09	0.26 b
F-test[b]	**	*	***	***	***	***	ns	***

[a] Means of 8 samples per treatment. Means within a column followed by the same letter are not significantly different ($p = 0.05$) by Tukey's multiple range test. [b] *F*-test: ***$p < 0.001$, **$p = 0.001$–0.01, *$p = 0.01$–0.05, ns = not significant.
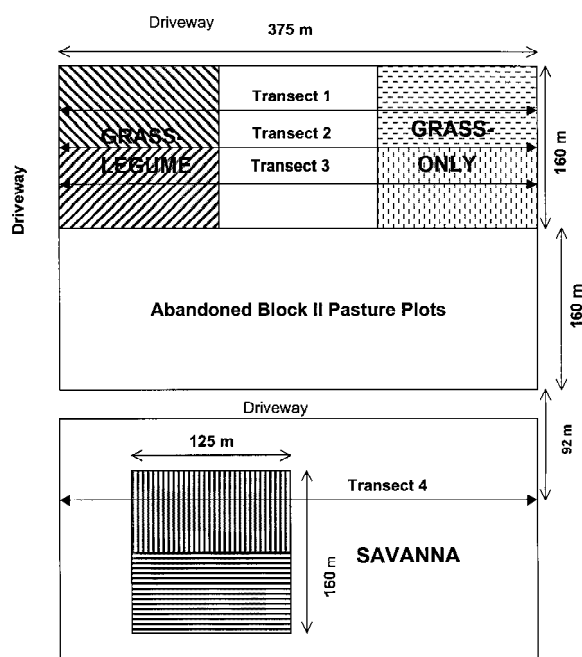

Figure 1. Plan of the experimental site showing treatment plots with two pseudo-replicates each and the transects.

More detailed information about pasture management is presented in Tergas et al. (1984) and Lascano and Estrada (1989). Total nutrient input through fertilizers, applied from 1978 to 1995, is presented in Table 2 (C.E. Lascano, personal communication). The native savanna was never fertilized.

Soil sampling

Since plots were no longer replicated in the field, considerable effort went into studying spatial variability in order to assess the significance of treatment effects on a sound basis.

Table 2. Fertilizer input over 17 years (1978-1995) in long-term pastures.

Treatment	N	P	K	Ca	Mg	S
	- - - - - - - - - - kg ha^{-1} - - - - - - - - -					
Grass-only	0	113	72	741	64	88
Grass-legume	0	116	89	741	71	101

In order to access seasonal variability in P fractions, pseudo-replicates were obtained by dividing each treatment plot into two 1 ha sub-plots and delimiting two 1 ha areas in native savanna (Figure 1). From each sub-plot, 16 soil samples (0–10 cm) were obtained, and randomly chosen groups of four such samples were bulked for the measurements. This resulted in a total of 8 bulked samples, four for each sub-plot within each treatment. This sampling scheme was applied in 1993 during the late rainy season (September 16), in the middle of the 1994 dry season (January 26) and at the beginning of the next rainy season after the first good rain (April 13, 1994). Soil samples were cooled immediately in the field and brought back to the laboratory in ice-chests where they were stored at 4°C in plastic bags until microbial P analysis was carried out. A part of each sample was immediately air-dried and sieved to pass a 2-mm mesh before chemical analysis. Sequential P fractionation, determination of total carbon and phosphatase activity were done on 1-mm sieved samples.

For a more detailed assessment of spatial variability, three transects were laid out to pass over both the grass-only and the grass-legume pastures, and a fourth transect within savanna (Figure 1) in November 1995, at the end of the rainy season. Along each transect, 36 intervals of 10 m were marked, and soil was sampled

down to 10 cm by a 8 cm diameter auger at a random location in each interval. This resulted in 144 soil samples.

Additional samples down to 100 cm depth were taken along 6 other transects in each treatment. For each transect, 6 random locations were determined by random numbers, and a 5 cm diameter core was obtained at each location. In order to reduce measurement efforts, the resulting 36 cores of each treatment were randomly assigned to four groups of nine cores each. These nine cores were cut into layers (0–10, 10–20, 20–40, 40–60, 60–80, and 80–100 cm), and the corresponding segments were mixed. This resulted in four replications for each layer in each treatment.

All soil samples were air-dried and sieved (<2 mm) before total P determination.

Soil analyses

Soil P was fractionated sequentially following a reduced method of Hedley et al. (1982) using the following extractants: H_2O with anion exchange resin, 0.5 M $NaHCO_3$ and 0.1 M NaOH (Tiessen and Moir, 1993). Inorganic P in extracts was determined by the molybdate-ascorbic acid method of Murphy and Riley (1962), as described in Tiessen and Moir (1993). Total P in the $NaHCO_3$ and NaOH was measured after digestion with potassium persulfate ($K_2S_2O_8$) (Bowman, 1989). Organic P was calculated as the differences between total P and P_i in the $NaHCO_3$ and NaOH extracts, respectively. Total soil P was determined by perchloric acid ($HClO_4$) digestion (Olsen and Sommers, 1982).

Phosphate sorption and desorption were assessed on two samples per grassland system using the method described in Tiessen et al. (1993): subsamples of 2 g soil were equilibrated for 72 h with 20 mL of 0.01 MKCl solutions containing P concentrations between 0 and 200 mg L^{-1}. After centrifugation, P sorption was calculated by analyzing P remaining in the supernatant. Adsorption isotherms were described using the van Bemmelen-Freundlich equation ($A = a\,c^b$) to calculate the P addition required to reach a P concentration of 0.2 mg L^{-1} in the equilibrating solution. This is comparable to the standard P requirement (Fox and Kamprath, 1970) except for the background electrolyte ($CaCl_2$).

Total C was determined by combustion in a LECO CR-12 furnace with infrared detection of CO_2. Total N was determined by autoanalyzer following Kjeldahl digestion. Exchangeable Al and H were extracted with 1 MKCl and determined by titration; exchangeable cations were extracted with neutral 1 MNH$_4$-acetate and determined by atomic absorption spectrometry using standard methods.

The P concentration in the soil solution (c_P) was determined after equilibrating a soil suspension of 10 g and 100 mL deionized water for 16 h (Fardeau, 1993). About 10 mL of suspension were taken, centrifuged at 5000 rpm for 15 min and filtered through a 0.025 μm pore-size membrane under negative pressure to obtain the soil solution. The c_P values were obtained using the colorimetric method of Murphy and Riley (1962), as described in Tiessen and Moir (1993). A cuvette with 10 cm path length was used.

Acid soil-phosphatase activity at pH 6.5 was measured according to the method of Tabatabai (1982). Microbial biomass P was estimated by the procedure described by Oberson et al. (1997a) using field moist soil samples taken during the late rainy season. Phosphorus was extracted by Bray II (Olsen and Sommers, 1982) from $CHCl_3$-fumigated and unfumigated samples after a short fumigation period. The P released by fumigation (P_{Chl}) was calculated using a mathematical description of P added vs. P extracted (Morel et al., 1996) by Bray II determined independently (Oberson et al., 1997a).

Isotopic exchange of P

The isotopic exchange kinetics of P_i between soil solution and solid phase were assessed by injecting carrier-free $^{32}PO_4$ into a soil-solution system at steady state (Fardeau, 1993; Frossard et al., 1994; Morel et al., 1994).

Following the equilibration of 10 g of soil in 99 mL deionized water for 16 hours, the ^{32}P tracer, 1 mL of carrier-free solution of $^{32}PO_4$ ions having a radioactivity (R) of about 1 MBq, was added at time zero and mixed with a magnetic stirrer. After 4, 10, 40 and 100 min, 8 mL of suspension were removed with a polyethylene syringe and the solution was immediately separated from soil particles using a micropore filter (0.01 μm pore size, 47-mm dia.). The ^{32}P-radioactivity remaining in the solution (r_t) after the four periods of exchange (t) was measured by liquid scintillation.

The isotopically exchanged P (E_t) was calculated assuming that, at any given exchange time, the specific activity of phosphate in solution ($^{32}PO_4/^{31}PO_4$) is equal to the specific activity of the exchangeable phosphate on the solid phase (Fardeau, 1993; Frossard
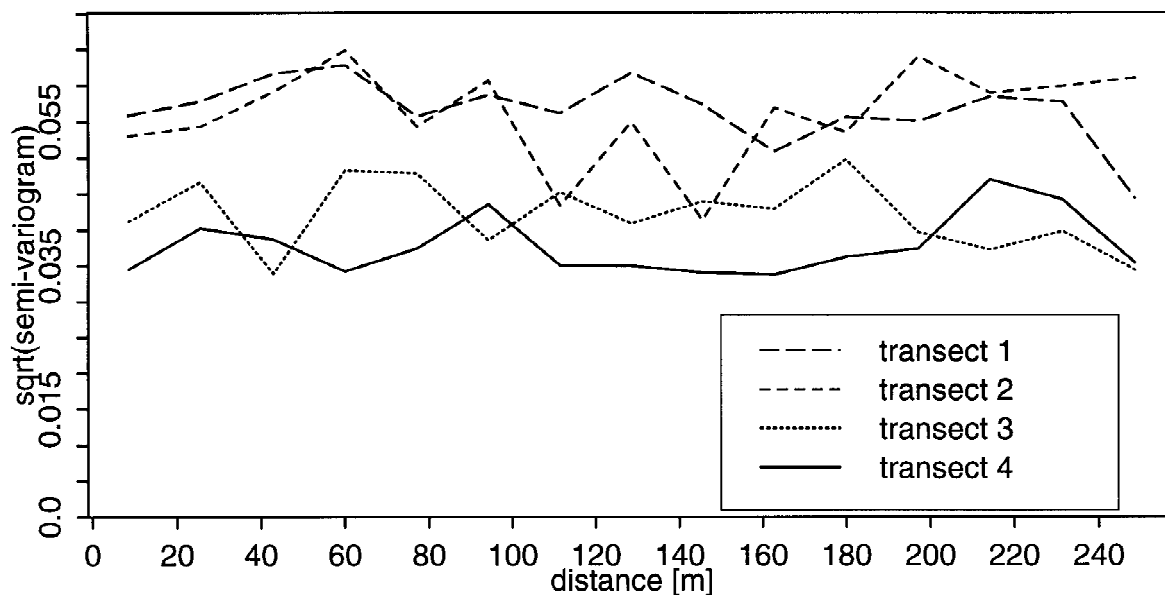
Figure 2. Semivariograms of the logarithm of total P content along the four transects.

et al., 1995):

$$r_t/10c_P = R/E_t \tag{1}$$

Available soil P_i was then described by the intensity factor, c_P, the instantaneous buffering capacity, r_1/R, and the quantity factor, E_1 calculated for 1 min of exchange using the equation (Fardeau, 1993; Frossard et al., 1995):

$$E_t = 10c_P/[\ m(t + m^{1/n})^{-n} + r_{eq}/R] \tag{2}$$

where m and n are parameters obtained from the isotopic exchange experiment, and r_{eq} is estimated with Equation (1) by assuming that all P_i has been exchanged (Fardeau, 1993; Frossard et al., 1994).

The relationship between quantities of isotopically exchangeable P and P concentration in the soil solution was evaluated by adding four P levels (50, 100, 200 and 300 mg P-KH_2PO_4 kg^{-1} soil) to 1:1 soil:water suspensions in duplicate. All soil suspensions were mixed gently for 5 min and equilibrated for 1 day at about 25^oC without shaking. The equilibrated soil suspensions were diluted with 89 mL of deionized water (giving a final soil to solution ratio of 1:10) and gently mixed for an additional 16 h period. Then, isotopic exchange kinetics were carried out as described above. The relationship between E_1 and c_P was described using a Freundlich equation with an asymptotic term (Morel et al., 1994) using non-linear,

least-squares parameter-optimization:

$$E_1 = vc_P^w + A \tag{3}$$

where v is the P exchanged after 1 min at $c_P = 1$ mg P L^{-1}, and w is a constant which describes the nonlinear increase in E_1 with c_P. Parameter estimates were compared for the different grassland systems using the maximum likelihood test (Huet *et al.*, 1992).

Statistical analysis

Statistical analysis was carried out using log transformed data in order to meet the assumptions of analysis of variance (i.e. additive effects, symmetric errors with equal variance).

Semivariograms (Cressie, 1993, Chapter 2.2.2) for the data obtained along the four transects were drawn to assess if spatial correlation existed between residuals of total P values (i.e. total P values minus mean of treatment within the transect). To determine for grass-only and grass-legume pastures if transects gave raise to a block effect, means obtained for the different transects within each grassland system (Figure 1) were compared by testing transect effects against the transect*treatment interaction term.

Since no spatial correlation existed (Figure 2), the effect of grassland system and soil depth on total P values was tested by two-way analysis of variance (ANOVA) assuming independent errors. As the interaction between treatment and depth was significant,
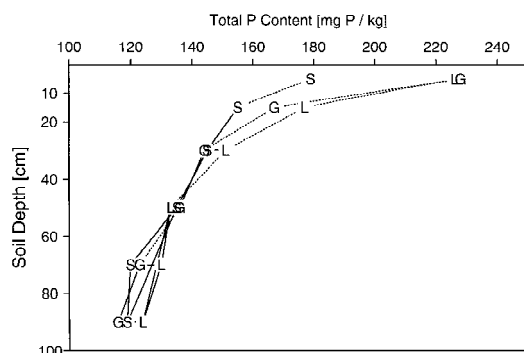
Total P Content [mg P / kg]

Figure 3. Total P content in different soil layers of improved pastures and native savanna soils sampled in 1995. L = Grass-legume, G = Grass-only, S = Savanna; means of 4 samples per treatment and layer.

the effect of treatment within a given soil depth was analyzed using one-way ANOVA. If the *F* test was significant ($p < 0.05$), the means were compared using Tukey's multiple range test. The same procedure was applied to compare the soil depths within a given treatment (Figure 3).

The effects of grassland system on P availability were tested by ANOVA using a nested classification (Steel et al., 1997) in which treatment effects were tested against the combined terms of pseudo-replicate within treatment and bulked sample within pseudo-replicate within treatments (since pseudo-replicate effects were not significant). (As discussed below, results of spatial variability analysis justify this treatment of the data.) If the *F* test was significant ($p < 0.05$), the means were compared using Tukey's multiple range test. The effect of grassland system and season (sampling time) and their interaction were evaluated with two-way ANOVA. As no significant interactions were found, the effect of seasons and treatments on P fractions was tested using Tukey's multiple range over all treatments and over all seasons (Figure 4).

Results and discussion

Calculated P budgets

We estimated P budgets to determine the relation between P input in improved pastures and P output via beef cattle, and to better understand the modifications in soil P status of the improved pastures. P budgets were calculated for the period until 1995 by subtracting the P removed from the system by animals from the P applied in mineral fertilizers (Table 3). P expor-

Table 3. Estimated P budget over 17 years (1978-1995) in native savanna and improved pastures.

Treatment	Estimated P budget		
	Input	Export	Balance
	------ kg P ha^{-1} ------		
Savanna	–	2.4	-2.4
Grass-only	113	27	+86
Grass-legume	116	41	+75

ted with the animals was assumed to be 8 g P per kg of live weight gain (NRC, 1984). Live weight gains in the improved pastures were 200 kg ha^{-1} yr^{-1} for grass-only and 300 kg ha^{-1} yr^{-1} for grass-legume pastures (Lascano and Estrada, 1989), whereas, in savanna, live weight gains were 15–20 kg ha^{-1}yr^{-1} (Fisher et al., 1992).

Positive P budgets were obtained for both improved pastures, whereas a small negative budget was found for the unfertilized savanna (Table 3). Until 1995 the calculated gains in total P above the savanna level due to pasture improvement were 86 kg P ha^{-1} for grass-only and 75 kg P ha^{-1} for grass-legume. The effect of pasture improvement was therefore an increase in total P stock of the plots and an increase of the nutrient flux through improved pasture and animal production. This should be reflected in the measurable P budgets and the distribution of P among active and passive fractions.

Total soil P

The semivariograms of the total P contents show no spatial correlation of total P contents for any of the 4 transects (Figure 2). No significant block effect was found in any of the three paddocks (including the intervening degraded pasture paddock), in agreement with the absence of a pseudo-replicate effect in the stratified sampling analysis. The absence of spatial trends or correlations indicates that the three plots can be statistically compared assuming independent errors even though the remaining field design did not provide blocks of randomized replicated treatments.

Total P content in the soil decreased with soil depth (Figure 3). Total P contents of both improved pasture treatments were significantly higher than those of the native savanna soil in the 0–10 cm and 10–20 cm layers but were not significantly different in deeper layers (Figure 3). Significantly more P accumulated
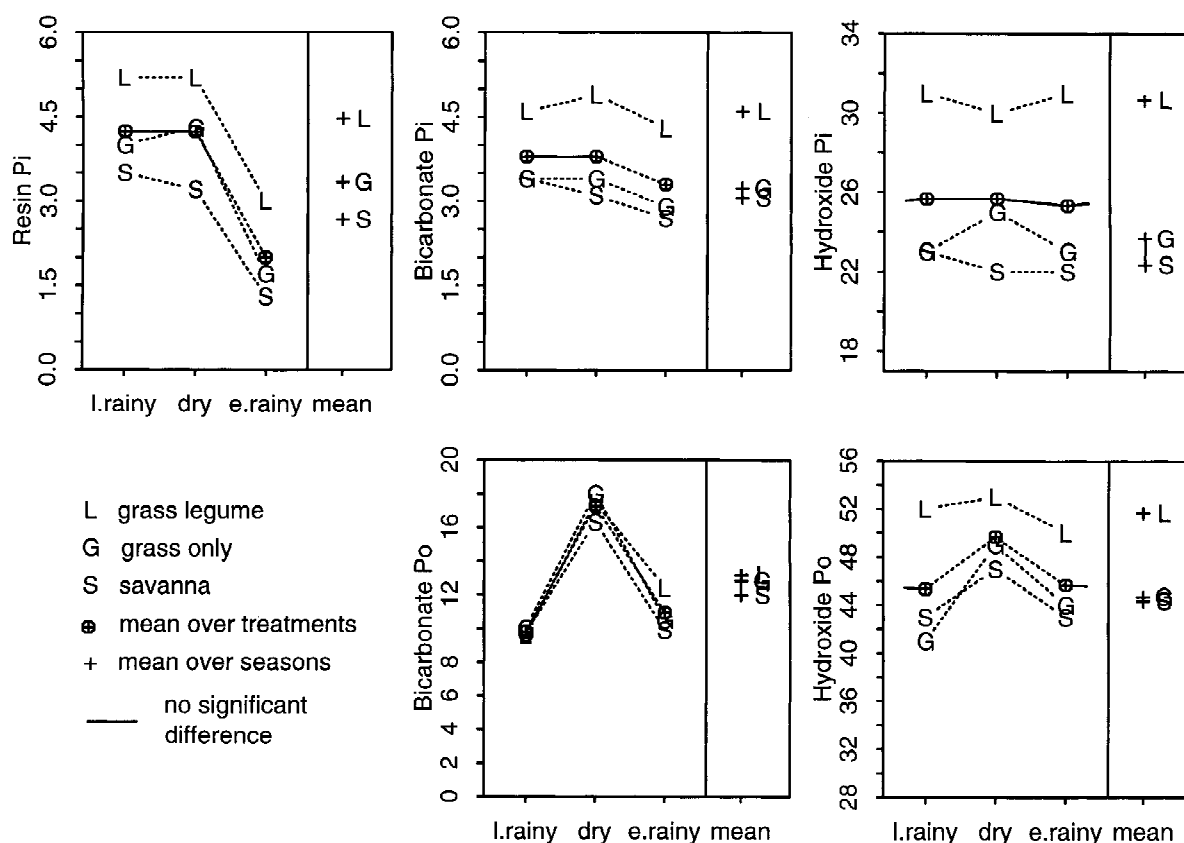
Figure 4. Contents of resin P_i, bicarbonate and hydroxide extractable inorganic and organic P (mg kg^{-1} soil) in the three grasslands at three different sampling dates. Each point is the mean of 8 replicate samples. Treatment and season means are also shown. Means which are not significantly different are connected by solid lines.

in the 10-20 cm soil layer under grass-legume than grass-only. In contrast, Beck and Sánchez (1996) observed significant P movement into deeper soil layers of cropped Ultisols in a long term field experiment at Yurimaguas, Peru, where much higher P fertilizer doses were applied.

To assess the original P content of the plots and evaluate the effects of grassland system on surface soil P enrichment, we compared P contents above with P contents below 20 cm depth where no treatment effects were observed. The mean total P contents were 167, 198 and 202 mg kg^{-1} for the top 20 cm of savanna, grass-only and grass-legume, respectively. Between 20 to 100 cm mean total P contents were 130, 130 and 135 mg kg^{-1} for savanna, grass-only and grass-legume, respectively, i.e. show no difference. Comparing these layers one can conclude that the top soil of the savanna was enriched over the subsoil P content by about 37 mg kg^{-1} through processes such as biocycling over the time of soil formation (Letkeman et al.,

1996). Both improved pastures showed an enrichment of about 67 mg kg^{-1} in the topsoil over their respective subsoils, indicating that they were enriched by approximately 30 mg kg^{-1} with fertilizer P in addition to the biocycling that affected all three sites similarly. When the calculated net gain of approximately 81 kg P ha^{-1} for the two improved pastures (Table 3) is converted to units of concentration using a bulk density of 1.3 g cm^{-3}, an increase of 31 mg kg^{-1} in the top 20 cm layer is obtained, in agreement with the measured value. Thus, fertilization as part of the pasture improvement has resulted in a measurable increase of total P near 30 mg kg^{-1} or about 20% over the original savanna levels. The greater movement of P to depth under grass-legume compared to grass-only pastures (significant [$p < 0.001$] at 10–20 cm; marginal at 20–40 cm) can be related to greater root biomass production in subsoil when legumes are present. In an adjacent long-term experiment on the same soil, greater C accumulation in subsoil was measured in *B.*

humidicola pasture with legumes than without (Fisher et al., 1994).

Phosphorus fractions and availability

Seasonal dynamics of P fractions

The seasonal dynamics of P fractions in the three pastures were examined by sampling successively during the late rainy season, mid dry season and the subsequent early rainy season (Figure 4). Resin extractable P_i levels generally indicated highest P availability in grass-legume followed by grass-only and savanna. In all grasslands, resin P_i levels were highest during the late rainy season through to the mid dry season, but declined abruptly with the beginning of the next rainy season. Compared to late rainy/mid dry season mean values, these declines amounted to 61%, 59% and 42%, respectively, for savanna, grass-only and grass-legume systems, although absolute changes (2.1, 2.5 and 2.2 mg P kg^{-1} soil) were similar for all three. Bicarbonate extractable P_i reacted similarly to resin P_i to changing seasons although changes were not as dramatic (47%, 15%, and a non-significant 9% decline in savanna, grass-only and grass-legume, respectively). Bicarbonate P_i and hydroxide extractable P_i levels were again higher under grass-legume than savanna and grass-only, but hydroxide P_i did not change with changing seasonal conditions. The relative seasonal changes in resin P_i, bicarbonate P_i and hydroxide P_i in soil under the three grassland systems reflect, therefore, increasing stability in these fractions, a stability which also appeared to be influenced (in relative terms at least) by pasture composition in the order savanna < grass-only < grass-legume.

In contrast to resin P_i, P_o was highest in the dry season when decomposition processes are curtailed. While bicarbonate extractable P_o was hardly affected by the grassland system, it significantly increased during the dry season and was at the same level for both rainy season samplings. Hydroxide extractable P_o followed a similar trend to resin extractable P in that it was higher in grass-legume than in the two other pastures at all times, and showed the lowest variation over the seasons in grass-legume (Figure 4). Guggenberger et al. (1996) similarly found that amounts of NaOH-extractable phosphonates and diester P followed the order grass-legume > grass-only > savanna. The increase of NaOH extractable P_o in the grass-legume pasture soil was paralleled by total carbon and nitrogen contents (Table 1). Rao et al. (1994) repor-

ted similar changes in soil organic matter composition after legumes were introduced.

The fractionations on different parts of the seasonal cycle therefore indicated that grass-legume maintained higher organic and available P levels more consistently than the savanna or grass-only. The close relationship between available resin P and P_o has also been observed by Tiessen et al. (1984) on weathered soils.

Biological activity in relation to P dynamics

The linkage of P_o and available P should be reflected in soil biological activity. Estimates of P in the soil microbial biomass (P released by chloroform fumigation, P_{Chl}) were significantly higher in grass-legume than grass-only and savanna whereas acid phosphatase activity was higher in improved pasture than in native savanna soils (Table 4). In all soils, acid phosphatase activity was somewhat higher than in similar Brazilian soils with C contents bracketing the C content of the soils included in this study (examined by Feller et al., 1994, using the same method). These results further reflect the beneficial effects of the grass-legume system on soil biological activity already reported for soil macrofaunal activity (Decaëns et al., 1994). Macrofaunal biomass, mainly earthworms, in grass-legume was twice that in grass-only which was also twice that in native savanna, each increment being a reflection of greater C inputs through increased pasture productivity, and the first increment additionally reflecting higher quality plant biomass of grass-legume (Lascano and Estrada, 1989). P_{Chl} measurements performed on the mid dry and early rainy season samplings (Oberson et al., 1997b) showed declining microbial P contents during the dry season and increasing P_{Chl} values with the onset of the rainy season, in counterpoint with extracted P_o fluctuations.

P sorption and isotopic exchange parameters

The raised P status of improved pastures is visible in the P concentration in the soil solution (c_P, Table 4). Although c_P was very low (< 8 μg P L^{-1}), it increased by 2 and 6 fold between savanna and grass-only and between savanna and grass-legume, respectively. Both sorption and isotopic exchange parameters (Table 4) support the overall improvement of P availability. The amounts of sorbed P to reach 0.2 mg P L^{-1} decreased when c_P values increase in agreement with previous results (Fox and Kamprath, 1970; Morel et al., 1994).

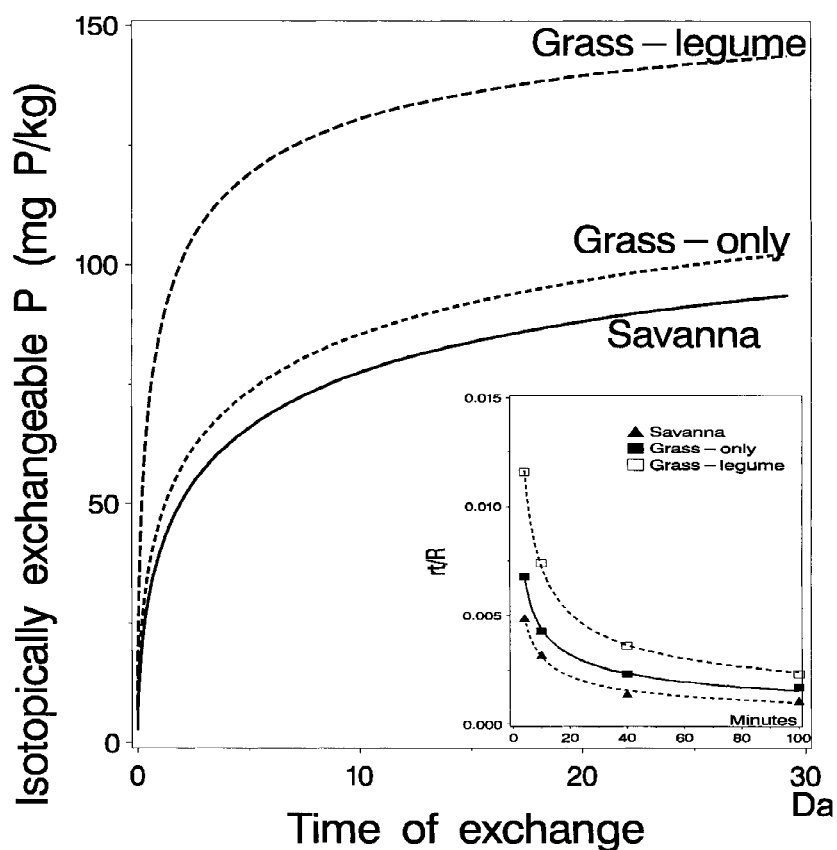The isotopically exchangeable P (E_t) (Figure 5) was greater in grass-legume than grass-only and sa-

Figure 5. Increase of isotopically exchangeable P (E_t) with exchange time for the savanna grass only and grass-legume soils. Calculation of E_t [= 10 $c_P/(r_t/R)$] is based on experimentally derived parameters of isotopic exchange kinetics, shown in the insert, obtained during 100 minutes. Parameters for Equation (2) were: n = 0.52, 0.48 and 0.55; m = 0.010, 0.013 and 0.024; r_{eq}/R = 0.0001, 0.00018, 0.00048 for savanna, grass-only and grass-legume, respectively.

Table 4. Indicators of inorganic soil P availability and biological activity in the 0-10 cm soil layer in native savanna and improved pastures.

Indicator[a]	Savanna[b]	Grass-only[b]	Grass-legume[b]
c_p (μg P L^{-1})	1.3 (0.3)	2.6 (1.1)	7.9 (1.9)
r_1/R	0.010 (0.001)	0.013 (0.002)	0.022 (0.002)
E_1 (mg kg^{-1})	1.4 (0.3)	1.8 (0.7)	3.4 (0.6)
Bray II P (mg kg^{-1})	1.3 (0.1)	1.4 (0.2)	2.2 (0.3)
Preq$_{0.2}$ (mg kg^{-1})	302 (2)	275 (15)	253 (6)
P_{chl} (mg P kg^{-1})	5.2 (0.8)	5.9 (1.0)	7.3 (2.2)
APA (mg ρ-nitrophenol kg^{-1} h^{-1})	181 (7)	213 (11)	239 (16)

[a] c_P, P_i concentration in the soil solution obtained at a 1:10 soil:water ratio (values at the detection limit were replaced by 1 μg P L^{-1}(Sinaj et al., 1998)); r_1/R, ratio of radioactivity remaining in soil solution after one minute of isotopic exchange to total amount of radioactivity added; E_1, amount of isotopically exchangeable P after one minute of exchange calculated using Equation (2) with the actual c_P; Preq$_{0.2}$, amount of P required to give a solution concentration of 0.2 mg P L^{-1}; P_{Chl}, chloroform-released P; and APA, soil acid phosphatase activity.
[b] Mean and SE for 8 samples per treatment for all data except the standard P requirement (2 samples per treatment); results obtained on samples taken during the late rainy season.

vanna soils. Calculated E_1 values (quantity factor) using Equation (5) for the same intensity level, c_P, of $1\,\mu g\,P\,L^{-1}$ were 5.3, 3.1 and 2.1 mg kg^{-1} for savanna, grass-only and grass-legume, respectively. This indicates that the number of P-reacting sites was reduced in grass-only and grass-legume soils by about 41% and 61%, respectively. Grass-legume was reduced 34% relative to grass-only.

The very low r_1/R (buffer capacity) values (Table 4) indicate that the soils are highly P sorbing (Frossard et al., 1992). Using the method of Fox and Kamprath (1970) which uses CaCl$_2$ as a background electrolyte, the native savanna soil (results not shown) had a standard P requirement of near 500 mg P kg^{-1} soil, which classifies the soil as medium to highly P sorbing. In KCl, the calculated P requirement was 300 mg kg^{-1} (Table 4).

The P requirement and r_1/R values indicate that the sorption capacity decreased under improved pastures, especially for grass-legume (Table 4). Fertilizer inputs to the improved pastures (Table 2) have been relatively low, resulting in an increase in total P levels of nearly 20%. P availability as measured by solution P concentrations, P sorption and exchangeable P has been improved in both managed grassland systems relative to savanna. This improvement was much greater in grass-legume than in grass-only, indicating that, at comparable fertilizer inputs and greater product exports, an improved P availability in grass-legume has been achieved by changes in biological activity caused by the vegetation cover. As P availability was improved more in grass-legume than in grass-only pastures, legumes appear to affect P availability more than grasses alone. Differences in available P and P sorption parameters between the grass-only and grass-legume pastures (Table 4) were clearly much greater than differences in budgets (Table 3) and must be attributed to a beneficial effect of the grass-legume mix. Total C content, macrofaunal activity (Decaëns et al., 1994), organic P content (Guggenberger et al., 1996) were all significantly higher in grass-legume soils. Although plant biomass and resulting litter production are not necessarily higher in grass-legume than grass-only pastures, their higher litter quality results in increased mineralization and nutrient turnover (Thomas et al., 1993; Rao et al., 1994). Because of greater litter turnover induced by the legume-grass mixture, greater amounts of organic compounds may provide higher levels of actively cycling organic and labile P. In addition some organic compounds may compete for P sorption sites and improve P availability, as ob-

served by various in vitro experiments for soils with well crystallized minerals (see literature compilation in Frossard et al., 1995). Thus, although less of the applied P remains in the grass-legume system, soil solution P was higher there than in the grass-only system.

Conclusions

Fertilization as part of the pasture improvement has resulted in a measurable increase of total P near 30 mg kg^{-1} or about 20% over the original savanna levels. A greater movement of P to 20-cm depth under grass-legume compared to grass-only pastures could be related to greater root biomass production in that layer when legumes are present.

Sequential soil P fractionation of different seasonal samplings indicated that grass-legume maintained higher organic and available inorganic P levels with less temporal variation than the two other types. The improvement in soil P availability, as measured by solution P concentration, P sorption and exchangeable P, was much greater in grass-legume than in grass-only. With comparable fertilizer inputs and greater product exports, improved P availability in grass-legume cannot be explained by the differences in budgets but can be attributed to changes in the overall biological activity in the soil-plant system caused by the presence of legumes in the vegetation cover. Estimates of P in microbial biomass, phosphatase activity, total C and organic P content and macrofaunal activity were all significantly higher in grass-legume than grass-only and savanna soils. Because of greater turnover of organic litter, the grass-legume mix may provide for steadier organic P inputs and therefore higher P cycling and availability.

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