Output 2. Strategies developed to protect and improve soil quality

Activity 2.1 Develop a concept of, and strategies for, the establishment and maintenance of an "arable layer" for sustainable production

2.1.1 Determine the impact of disc harrowing intensity on soil properties and plant growth of agropastoral systems in the llanos of Colombia

Highlight:

- Showed that the maize and green manure cropping systems were better than the grass alone pasture system at separating the effect of increased number of disc harrow passes on soil physical and chemical characteristics.

Purpose:

To determine the impact of intensive disc harrowing on soil physical and chemical properties, soil phosphorus dynamics, plant growth and nutrient acquisition of contrasting agropastoral systems on an oxisol.

Rationale:

The sustainable management of savanna Oxisols is of high ecological and socioeconomic significance. Production systems in the most intensified areas of the savanna are characterized by continuous monocropping and continuous tillage with heavy machinery. While these systems are economically profitable, they result in soil erosion, compaction, reduced microbiological activity, declining quality of organic matter and deterioration of other soil physical properties. These soils are more susceptible to degradation than most soils, often degrading within 5 years of being opened up for agricultural production.

Soils in the 'Llanos' are characterized as highly acidic and infertile Oxisols and Ultisols, whose mineralogy is dominated by kaolinite and the oxides and hydrous oxides of iron and aluminum. Oxisols have a stable microstructure caused by strong aggregation of negatively charged kaolinite and positively charged gibbsite and goethite. However, these soils are susceptible to physical, chemical, and biological degradation once brought into cultivation.

Tillage practices with heavy machinery physically break macroaggregates into smaller units, leading to new surfaces. These changes in soil structure act on the pore-size distribution and thus influence drainage or plant-available water content. Pore-size distribution is one sensitive soil physical property that can be used to evaluate the influence of tillage on the physical condition of the soil because it regulates the rate of water entry into the soil. It also influences soil water fluxes, which affect plant nutrient availability and plant growth. Three important phenomena related to plant nutrition, which are negatively affected by reduction in macropores are: root growth, nutrient interception by roots, and soil drainage and aeration. Soil porosity of below 10 % will generally limit crop and pasture production. Reduced water infiltration encourages surface water run-off and, consequently, soil and plant nutrient losses brought about by soil erosion.

Phosphorus (P), which has a low mobility, particularly in Oxisols, is likely to be greatly affected by tillage practice. Soil disturbance during tillage operations may increase the degree of contact between fertilizer-derived P and soil particles, thereby, promoting the formation of stable insoluble P compounds. Oxisols of the 'Llanos' are characterized by low total and available P contents, and a relatively high P retention capacity. Phosphorus deficiency often limits crop and pasture productivity in these soils and is caused mainly by strong sorption of inorganic P (P_i) to Al and Fe oxyhydroxides. The bioavailability of these secondary Al and Fe phosphates is considered to be low because of specific adsorption caused by ligand exchange. Knowledge on phosphorus cycling in these soils is limited. In the past, only readily available P

was determined which may not effectively reflect plant-available P. This is because organic P (P_o) fractions are believed to contribute proportionately more with increasing P deficiency.

Soil compaction also hinders extensive root growth and reduces the soil volume from which plants can obtain P. Continuous cropping of a native soil also disrupts the peds (∅ > 2 mm), leading to loss of organic carbon and associated nutrients such as P. This implies that land preparation practices should be planned by taking into account the drastic reduction in soil aggregate size brought about by excessive use of machinery and it's resultant negative effect on physical, chemical and biological properties of the soil. A highly successful strategy for intensifying agricultural production sustainably and reversing problems of degradation involves the integration of crop/livestock systems, generally known as agropastoral systems.

In 1995 a field experiment was established in the 'Llanos' of Colombia to develop adequate soil tillage practices that could enhance the performance of agropastoral systems by improving plant growth, nutrient acquisition and nutrient cycling while minimizing the risk of soil degradation. The main objective of the present study was to determine the impact of intensive disc harrowing on: (i) soil physical and chemical properties; (ii) soil phosphorus dynamics; and (iii) plant growth and nutrient acquisition by contrasting agropastoral systems.

Materials and Methods:

Site description and experimental design: The experiment was carried out at Matazul farm (4º 9′ 4.9″ N, 72º 38′ 23″ W and 260 m.a.s.l.) located in the Eastern Plains (Llanos) near Puerto Lopez, Colombia. The area has two distinct climatic seasons, a wet season from the beginning of March to December and a dry season from December to the first week of March and has an annual average temperature of 26.2 ºC. The area has mean annual rainfall of 2719 mm, potential evapotranspiration of 1623 mm and relative humidity of 81 % (data from the nearby Santa Rosa weather station, located at the Piedmont of the Llanos of Colombia). Prior to treatment application, the area was under a native savanna pasture consisting of native grasses. The land is generally flat (slope < 5 %), the soil is deep, well structured and has a textural distribution in the first 10 cm of about 40 % clay, 30 % silt and 30 % sand (loam texture). The bulk density in the native savanna is 1.30 g cm^{-3} in the top 0-5 cm soil layer, followed by lower values of 1.27 and 1.23 g cm^{-3} at the 5-10 and 10-20 cm soil layers, respectively. The soil has low fertility and the availability of P in the soil is low because of the soil's high P fixation capacity. The soil is classified as Isohyperthermic Kaolinitic Typic Haplustox in the USDA soil classification system.

The native savanna pasture (unimproved grassland) was opened in the third week of April 1995 and upland rice (cv. Oryzica Sabana-6) was planted with different intensities of tillage (2, 4 or 8 disc harrow passes) to a depth of 8 to 10 cm. Each tillage treatment had a plot size of 54 x 20 m. These treatments continued for 2 years with upland rice cultivation. At the beginning of the third year (third week of April 1997), each tillage main plot was used to introduce the following 3 cropping systems: (i) Grass alone pasture (*Brachiaria dictyoneura* CIAT 6133 cv. Llanero), (ii) Green manure (*Crotalaria juncea* cv. Common), and (ii) Maize (*Zea mays* cv. Sikuani 110). Native savanna was also included as a control to study changes in the soil conditions without tillage.

The treatments were arranged in a split-plot design (tillage intensity as main plots and cropping systems as sub-plots) and replicated four times. The size of each main plot was 42 x 10 m and sub-plot was 10 x 10 m leaving a border of 6 m between plots. Dolomitic lime (28% Ca and 10% Mg) was applied (Mg ha^{-1}) 1.0 for maize, 0.5 for Crotalaria and 0.5 for grass-alone pasture. Maize received (kg ha^{-1}) 80 N as urea; 50 P as TSP; 100 K as KCl; 8 Zn as $ZnSO_4$; 4 S as $ZnSO_4$; and 9 B as borax. *Crotalaria* received 22.5 N; 40P and 50 K. Grass-alone pasture received 20 P, 45 K and 4 Zn. Native savanna treatment received no fertilizer application as commonly practiced by farmers in the region.

Soil and plant sampling and analytical procedures: In the last week of June 1997 (2 months after establishment of agropastoral systems), soil samples from different agropastoral systems including native savanna were collected. The pore-size distribution was determined from the moisture characteristic curves. Undisturbed soil cores (50 x 25 mm) in four replicates per depth in each treatment were taken from: 0-5, 5-10 and 10-20 cm soil layers. Saturated soil cores were weighed and then subjected to different suctions (5, 10, 100, 300 and 1500 KPa). Pore-size distribution was calculated using Kelvin Equation. Pores were divided into macropores (> 50 µm; drained at a suction of less than or equal to 6 KPa), mesopores (50 to 0.2 µm; water retained between 6 – 1500 KPa), and micropores (< 0.2 µm; water retained over 1500 KPa. A composite soil sample, consisting of 50 cores, was also collected in a grid pattern from the whole plot. These samples were air-dried, and then visible plant roots were removed before they were gently crushed to pass through a 2-mm sieve. The < 2-mm fraction was used for subsequent chemical analysis. Other measurements that were made included: bulk density, soil nutrient availability, shoot biomass, plant nutrient composition, and shoot nutrient uptake.

Phosphorus fractionation and analysis: A shortened and modified sequential P fractionation procedure was used on 0.5-g sieved (< 2-mm) soil sample. In brief, a sequence of extractants with increasing strength was applied to subdivide the total soil-P into inorganic (P_i) and organic (P_o) fractions. The following fractions were determined. (1) resin P_i, anion exchange resin membranes (in bicarbonate form) were used to extract freely exchangeable P_i. The remaining P_o in the H_2O of the resin extraction step was digested with potassium persulfate ($K_2S_2O_8$). (2) Sodium bicarbonate (0.5 *M* $NaHCO_3$, pH = 8.5) was then used to remove labile P_i and P_o sorbed to the soil surface, plus a small amount of microbial P. (3) Sodium hydroxide (0.1 *M* NaOH) was next used to remove P_i, which is more strongly bound to Fe and Al compounds and associated with humic compounds. (4) The residue containing insoluble P_i and more stable P_o forms ('residual P') was digested with perchloric acid ($HClO_4$). To determine total P in the $NaHCO_3$ and NaOH extracts, an aliquot of the extracts was digested with $K_2S_2O_8$ in H_2SO_4 at >150 °C to oxidize organic matter. Organic P was calculated as the difference between total-P and P_i in the $NaHCO_3$ and NaOH extracts, respectively. Inorganic P concentrations in all the digests and extracts were measured calorimetrically by the molybdate-ascorbic acid method. All laboratory analyses were conducted in duplicate determinations and the results are expressed on an oven-dry weight basis.

Statistical analysis: Analyses of variances were conducted to determine the significance of the effects of the planted fallows and the crop rotation system on soil parameters. Planned F ratio was calculated as TMS/EMS, where TMS is the treatment mean square and EMS is the error mean square. Where significant F-values (at the 5 % level) occurred, mean separation was performed. Unless otherwise stated, mention of statistical significance refers to $P < 0.05$.

Results and Discussion:

Soil properties: Changes in total porosity and macroporosity as influenced by the intensity of disc harrowing are shown in Figure 17. Intensive disc harrowing improved macroporosity values of 0-5 cm soil layer up to 59 % for grass alone pasture system compared to native savanna. In general, disc harrowing improved macroporosity values of different agropastoral systems compared to native savanna. Intensive disc harrowing (8 passes per year) increased macroporosity values of 0-5 and 10-20 cm soil depth layers of the maize system. At 10-20 cm soil depth, while total porosity was not affected by the intensity of disc harrowing for each system, macroporosity significantly increased for pasture system. Mesoporosity and microporosity values were not much affected by the intensity of disc harrowing for different agropastoral systems (results not shown).

Results on the influence of the number of disc harrow passes on soil bulk density are shown in Table 25. One important aspect to note with regards to bulk density in native savanna is the presence of a high value (1.26 $g.cm^{-3}$) in the 0-5 cm soil layer. Disc harrowing at 2, 4, and 8 passes per year significantly reduced bulk densities for pasture compared to the native savanna in the 0-5 cm soil layer (Table 25). Bulk density

values were relatively unaffected by disc harrowing intensity for the green manure and maize systems. For 5 to 10 cm soil depth, disc harrowing resulted in a small decrease of bulk density for pasture system. Intensive disc harrowing (8 passes per year) significantly increased bulk density values of the green manure system at 5-10 cm soil depth. Volumetric moisture content values of the native savanna system were greater than those of the other systems at 0-5 cm soil depth (Table 25). Intensive disc harrowing (8 passes per year) significantly improved volumetric moisture content of green manure and maize systems at 5-10 cm soil depth compared with 2 passes per year. Grass alone pasture system showed no marked changes in volumetric moisture content across soil layers.

Soil chemical characteristics at different soil depth layers as influenced by the intensity of disc harrowing and agropastoral systems are shown in Table 26. Native savanna system without tillage and fertilizer application has shown low values of soil pH, available P and exgangeable K, Ca and Mg. The amount of available P (Bray II) decreased sharply below the 5-10 cm soil layer under all the systems tested. The amount of P was largest under the green manure and this was followed by the maize treatment (Table 26). At the 0-5 and 5-10 cm soil layers under the green manure and the maize cropping systems, the amount of P increased as the number of disc harrow passes per year were increased. However, for the maize treatment this increase was significant only at the 5-10 cm soil layer (Table 26). The grass alone pasture had the least amount of P and was, on average, 56 % lower at the 0-5 cm soil layer than the green manure treatment, which had the largest amount. The largest amount of exchangeable K was observed under the maize treatment and the second largest amount was observed under the green manure treatment. Most of K was found at the 0.5 cm soil layer in all the cropping systems and decreased rapidly after this soil layer, especially in the grass alone pasture system. Under the maize and grass alone pasture systems, the amount of K tended to decrease as the number of disc harrow passes were increased from 2, 4 to 8 per year (Table 26). The amounts of exchangeable Ca and Mg were largest in the maize treatment followed by the green manure cropping system. The number of disc harrow passes did not significantly affect the amount of Ca or Mg at all soil layers under the grass alone pasture and the green manure cropping systems. The amount of exchangeable Al was larger under the grass alone pasture compared to the green manure or the maize cropping system and it was not significantly affected by the number of disc harrow passes.

Soil P pools: The amount of extractable biologically available P was generally concentrated in the 0-5 and 5-10 cm soil layers and differed with the cropping system used (Figure 18). The largest amount of this fraction was obtained under the green manure followed by maize and then grass alone pasture cropping system, which, on average, represented respectively 19 %, 13 % and 12 % of the total P at the 0-5 cm soil layer. However, this fraction showed decreasing trend with increasing soil depth under all three systems at 2, 4, and 8 disc harrow passes per year (Figure 18). Eight disc harrow passes per year resulted in the highest amount of biologically available P under green manure and maize at the 0-5 and 5-10 cm soil layers. The high amount of available P at 8 disc harrow passes per year resulted in high P uptake of maize under this treatment (Table 27). Under grass alone pasture the biologically available P was less affected by tillage practices than under the green manure and maize cropping systems. The biologically available P under the grass alone pasture treatment was affected significantly by number of disc harrow passes only at the 0-5 cm soil layer, where 2 disc harrow passes per year had the largest amount. The number of disc harrow passes had little effect on the biologically available P at the 20-40 cm soil layer (Figure 18), and the amount was the same under all three cropping systems.

Similar to the biologically available P, moderately available P also showed different trends under green manure, maize and grass alone pasture and decreased with increasing soil depth. This fraction accounted for 37, 34 and 30 % of the total P for green manure, maize and grass pasture, respectively. Thirty-three, 45 and 49 % of the extracted NaOH-P_t was in the organic fraction (NaOH-P_o) for green manure, maize and grass pasture, respectively, at the 0-5 cm soil layer (results not shown). The number of disc harrow passes resulted in larger differences of moderately available P at the 0-5, 5-10 and 10-20 cm soil layers under the green manure followed by the maize cropping system. Under the grass alone pasture treatment, the

number of disc harrow passes had little effect on moderately available P for all soil layers (Figure 18). Under the green manure the largest amount of the moderately available P was obtained when 8 disc harrow passes per year were used followed by 2 disc harrow passes per year at the 0-5 cm soil layer. Under maize, on average, the largest amount of moderately available P was obtained with 8 disc harrow passes per year (Figure 18).

The largest amount of P was obtained in sparingly available P fraction and, on average, accounted for 46 %, 51 % and 57 % of the total P for the green manure, maize and grass alone pasture cropping systems, respectively, for all soil layers. The largest amount of the sparingly available P was extracted at 8 disc harrow passes per year (Figure 18). However, this fraction was highly variable under the green manure treatment and was not significantly affected by disc harrowing. Treatment effects were better separated under maize than under the grass alone pasture system (Figure 18).

The sum of organic P (Sum-P_o) was quite stable through out the profile with a small decrease in the amount with increasing soil depth (Figure 18). The amount of H_2O-P_o, $NaHCO_3$-P_o and NaOH-P_o were uniform throughout the soil profile and under all cropping systems and were 3-5, 17-23, and 73-78 % of the Sum-P_o, respectively. Treatment effects on Sum-P_o were more pronounced at the 0-5 and 5-10 cm soil layers. Under green manure the largest amount of Sum-P_o was obtained at 2 disc harrow passes per year under green manure and at 4 disc harrow passes per year under maize at the 0-5 cm soil layer. The Sum-P_o profile distribution was uniform under grass alone pasture with no significant differences among the intensity of disc harrow treatments.

Plant growth and nutrient acquisition: The effects of the intensity of disc harrowing on leaf biomass, stem biomass and total shoot biomass production and nutrient uptake of different agropastoral systems are shown in Table 27. Two passes of disc harrow per year (6 passes in 3 years) are sufficient for best performance of grass alone pasture in terms of both biomass production and nutrient acquisition. Additional disc harrowing resulted in a decreased leaf biomass production and reduced nutrient uptake (Table 27). Maize showed greater leaf biomass production and nutrient acquisition with 8 passes of disc harrow per year (Table 27). The green manure cropping system had greater leaf biomass production and nutrient acquisition, particularly Ca, with 4 disc harrow passes per year.

Land preparation by machinery leads to a constant breakdown and reduction in soil aggregate size. The action of rainfall and gravity results in a re-packing of these aggregates and, consequently, the total soil porosity and pore-sizes are reduced. The resulting changes in macroporosity affect water flow, which in turn affects nutrient availability and thus impact negatively on the productive capacity of the soil. Considering the low pore space at plant-available matrix potentials in Oxisols, the low amount of mesopores could make the soil prone to drought during dry spells in the rainy season. But we found no marked changes in mesopores of different agropastoral systems.

Results on total porosity suggest that the real rooting depth promoted by tillage was limited only down to 10 cm soil depth. Results on porosity also indicate that tillage of savanna soils could increase the volume of desired pore sizes (macropores) especially in the 0-10 cm soil depth. Good tillage practices that stimulate root growth could also contribute to better soil conditions.

Bulk density values of the native savanna soils suggest that the surface layer, which regulates the entry of water and the flux of air into the profile, exhibits less total porosity than the other layers. Therefore, for crop and pasture production, this constraint at topsoil depth must be alleviated by adequate tillage practices that maintain lower values of bulk density and reduce the risk of soil compaction. Disc harrowing significantly reduced bulk density values of the pasture system compared to the native savanna. Below 10 cm of soil depth, disc harrowing had relatively small effects on different agropastoral systems. This implies that disc harrowing reduced bulk density in the vicinity of the action of discs. Improvement

in volumetric moisture content in the 5-10 cm soil layer with intensive disc harrowing observed with green manure and maize systems might have contributed to superior leaf growth and nutrient acquisition.

Although separating total P into seven fractions helps to elucidate the differences in size of various P fractions, the P fractions are of greater practical value when divided into fewer functional pools of similar availability with management implications. These pools can then be used to improve soil P management and serve as decision-making tools. In this study the P fractions are divided into three groups: (1) biologically available P; (2) moderately available P; and (3) sparingly available P. The biologically available P pool (H_2O-P_o, resin-P_i, and $NaHCO_3$-P_i and -P_o) is the first to be removed by plant roots and mycorrhizal fungi from the soil and is considered to be available to plants in a short time (from days to a few weeks). The resin P_i is 'readily available' for plant uptake. The bicarbonate-P_i is highly related to P uptake by plants. The H_2O-P_o and bicarbonate-P_o are considered 'readily mineralizable' and highly related to P uptake by plants. A close relationship is known to exist between resin P_i and P_o on weathered soils. The major component of labile P_o is a diester PO_4, which prevents it from binding strongly to soil minerals and makes it susceptible to rapid mineralization.

The amount of biologically available P was markedly greater with the green manure treatment followed by maize and grass alone pasture system. Eight disc harrow passes per year resulted in the highest amount of biologically available P under green manure and maize that could contribute to high uptake of P by maize. Under grass alone pasture the biologically available P was less affected by tillage practices than under the green manure and maize systems. This could be explained by the fact that only the soil within the vicinity of the disc harrow action (0-20 cm) was disturbed.

Moderately available P pool consists of NaOH extractable Pi and P_o, which is assumed to be plant available for the medium term, i.e., from months to a few years. This fraction denotes the soil P reserve that is plant available when converted to readily available P through biological and physico-chemical transformations. This fraction is thought to be associated with humic compounds, and with amorphous and some crystalline Al and Fe phosphates. The sodium hydroxide (0.1 *M*, pH = 8.5) used to extract moderately available P is known to completely solubilize the synthetic iron and aluminium phosphate and labile-P_o. Similar to the biologically available P, moderately available P also showed different trends under green manure, maize and grass alone pasture and decreased with increasing soil depth. Since the moderately available P is plant available in the medium term as outlined above, the high amount of this fraction at 8 disc harrow passes per year also could have contributed to the high P uptake of maize.

Sparingly available P as used in this study is different from the residual P, because it includes the HCl and the hot concentrated HCl fractions. The sparingly available P contains insoluble P_i and more stable P_o forms and is not available on a short time scale such as one or more crop cycles. However, a small fraction of this pool may become available during long-term soil P transformations. The largest amount of the sparingly available P was extracted at 8 disc harrow passes per year and treatment effects were better separated under maize than under the grass alone pasture system.

Since P loss from systems occurs mainly through processes in the soil, minimizing P interaction with soil is an important management tool for increasing P cycling. Phosphorus maintained in organic pools may be better protected from loss through fixation than P flowing through inorganic pools in soil. Orthophosphate monoesters fractions dominate the P_o fraction and are less easily hydrolyzable, and thus less plant available, than the orthophosphate diester fraction. Systems that retain more of P_o are expected to cycle P better. We found that the sum of organic P (Sum-P_o) was quite stable throughout the profile with a small decrease in the amount with increasing soil depth. This shows that 24 % of the total soil organic P (Sum-P_o) is in the 'easily mineralisable' form and can contribute to plant available P, and the remaining 76 % is in more stable forms of P_o that are involved in the long term transformation of P.

Two passes of disc harrow per year (6 passes in 3 years) were found to be sufficient for best performance of grass alone pasture. Additional disc harrowing resulted in a decreased shoot biomass production and reduced nutrient uptake. A high number of disc harrow passes is likely to create a marked reduction in soil pore volume and affect nutrient uptake by plants. Results from a greenhouse experiment indicated that *Brachiaria* grass growth and N uptake were greatly influenced by the size of soil aggregates. This study also showed that N uptake from soil was a function of aggregate size, indicating that any excess preparation of soil could negatively affect the uptake of this nutrient. It is possible that excessive tillage might have reduced moisture content in the upper soil layer that could decrease the ability to acquire nutrients by the introduced pasture grass.

Soil organic matter is an important component of Oxisols because it carries the majority of exchange sites and also participates in the formation of stable microaggregates and controls the degree of clay dispersion. More than two disc harrow passes per year for the grass alone pasture treatment could decrease the amount of soil organic matter. This is because of the physical breakdown of aggregates during ploughing and the subsequent higher organic carbon mineralization, which may have resulted in N and P losses through leaching and fixation by soil, respectively. This could have resulted in reduced nutrient uptake, particularly N, and thereby growth of the pasture.

Maize showed greater aboveground production and nutrient acquisition with 8 passes of disc harrow per year. This result, especially for maize, is unexpected considering the negative attributes of reduced soil moisture content and soil compaction resulting from increased disc harrowing, as mentioned earlier. The better performance of maize under intensive cultivation (8 disc harrow passes) could be attributed to improved rooting ability that contributed to greater acquisition of nutrients. The improved amounts of biologically and moderately available P obtained from 8 disc harrow passes could have contributed to the good performance of maize. Previous research showed that maize is a very shallow rooted crop compared to native and introduced pasture species. Since the mobility of P in soil is low, high levels of biologically available P can benefit shallow-rooted crops. In contrast to the maize system, the green manure cropping system had higher yields with 2 disc harrow passes per year that resulted in greater nutrient (N, P, Ca and Mg) acquisition.

Table 25. Changes in bulk density (g cm $^{-3}$) and volumetric moisture content (%) at different soil depths as influenced by the intensity of disc harrowing and agropastoral systems. LSD values are at the 0.05 probability level. NS = not significant.

Soil depth (cm)	Number of disc harrow passes per year	Native savanna	Agropastoral system			LSD $_{0.05}$
			Pasture	Green manure	Maize	
	Bulk density (g cm^{-3})					
0-5	0	1.26				
	2		1.16	1.28	1.37	NS
	4		1.17	1.20	1.39	0.19
	8		1.13	1.47	1.31	0.12
	LSD $_{0.05}$		NS	0.23	NS	
5-10	0	1.48				
	2		1.40	1.34	1.39	NS
	4		1.37	1.37	1.41	NS
	8		1.40	1.58	1.44	0.16
	LSD $_{0.05}$		NS	0.16	NS	
10-20	0	1.42				
	2		1.46	1.46	1.43	NS
	4		1.38	1.43	1.40	NS
	8		1.42	1.48	1.35	0.11
	LSD $_{0.05}$		NS	NS	NS	
	Volumetric moisture content (%)					
0-5	0	35.6				
	2		31.9	32.2	32.8	NS
	4		31.7	32.0	32.0	NS
	8		30.5	35.8	30.5	NS
	LSD $_{0.05}$		NS	NS	NS	
5-10	0	39.5				
	2		37.5	32.0	33.3	3.0
	4		40.3	35.0	32.7	5.9
	8		37.7	38.2	35.2	1.5
	LSD $_{0.05}$		NS	2.5	1.3	
10-20	0	37.5				
	2		38.3	34.3	34.5	1.7
	4		37.1	36.9	33.2	2.4
	8		37.4	35.3	33.9	2.7
	LSD $_{0.05}$		NS	2.1	NS	

Table 26. Soil chemical characteristics at different soil depth layers as influenced by the intensity of disc harrowing and agropastoral systems

Soil depth (cm)	Disc harrow passes per year	Soil parameter						
		pH	C	P	K	Ca	Mg	Al
			(%)	(mg kg^{-1})	(cmol kg^{-1} soil)			
				Native savanna				
0-5	0	4.8	1.1	3.2	0.1	0.2	0.2	2.6
5-10	0	5.0	1.3	4.6	0.1	0.4	0.3	2.0
10-20	0	4.9	1.2	1.2	0.04	0.1	0.2	2.2
20-40	0	4.4	1.1	1.3	0.02	0.2	0.2	1.8
				Pasture				
0-5	2	4.9	2.6a	12.0ab	0.15a	0.72	0.46	1.7
	4	4.9	2.0b	15.8a	0.13ab	0.72	0.42	1.7
	8	4.7	2.0b	9.5c	0.10c	0.53	0.36	1.9
5-10	2	4.7ab	2.1	6.0	0.10a	0.48	0.21	1.9
	4	4.8a	1.9	4.6	0.08b	0.55	0.20	2.0
	8	4.6b	1.9	4.3	0.08b	0.57	0.20	2.0
10-20	2	4.6	1.7	1.8	0.06a	0.25	0.13	2.1
	4	4.6	1.5	1.3	0.06a	0.18	0.13	2.1
	8	4.6	1.4	1.3	0.04b	0.25	0.15	2.2
20-40	2	4.7	1.2	1.1	0.06a	0.15	0.12	1.6
	4	4.6	1.1	1.4	0.04b	0.12	0.11	1.7
	8	4.6	1.1	1.0	0.04b	0.13	0.12	1.6
				Green manure				
0-5	2	5.0	2.5a	36.7b	0.16	1.22	0.50	1.3
	4	5.0	1.8b	38.4b	0.15	1.00	0.48	1.4
	8	4.8	1.9b	56.8a	0.12	0.98	0.37	1.7
5-10	2	4.7b	2.0a	6.6b	0.13	0.44	0.30	2.1
	4	4.9a	1.6b	9.3ab	0.09	0.65	0.30	1.9
	8	4.7b	2.0a	10.7a	0.10	0.67	0.31	2.0
10-20	2	4.6b	1.7	2.1a	0.12a	0.23	0.14	2.1
	4	4.8a	1.5	2.1a	0.07b	0.26	0.17	1.9
	8	4.6b	1.5	1.4b	0.07b	0.19	0.15	1.9
20-40	2	4.7	1.3	1.5	0.07a	0.16	0.12	1.8
	4	4.7	0.9	1.0	0.04b	0.16	0.14	1.7
	8	4.6	1.3	1.1	0.05ab	0.15	0.13	1.7
				Maize				
0-5	2	4.8	2.1	20.5	0.26a	0.96	0.45ab	1.1ab
	4	5.1	1.9	21.0	0.22b	1.29	0.56a	0.9b
	8	4.9	1.8	27.0	0.22b	1.01	0.42b	1.5a
5-10	2	4.8	1.9	18.5b	0.15a	0.64	0.30	1.7
	4	4.9	2.0	17.1b	0.11b	0.87	0.37	1.5
	8	4.9	1.8	24.2a	0.15a	0.79	0.35	1.8
10-20	2	4.7	1.8	7.0	0.13a	0.41a	0.22b	2.0ab
	4	4.7	1.3	4.6	0.08b	0.30b	0.20b	1.7b
	8	4.7	1.5	6.4	0.11a	0.43a	0.26a	2.3a
20-40	2	4.6	1.6	2.2ab	0.09a	0.26	0.14	2.1
	4	4.6	1.4	1.3b	0.06b	0.21	0.16	1.8
	8	4.6	1.5	3.0a	0.08a	0.24	0.16	2.1

Means in a given column followed by common letters are not significantly different ($P < 0.05$) using Duncan's Multiple Range Test.

Table 27. Leaf, stem and total shoot biomass production and nutrient uptake by grass alone pasture, green manure and maize systems as influenced by the intensity of disc harrowing.

Cropping system	Disk harrow passes per year	Leaf biomass (kg ha^{-1})	Stem biomass (kg ha^{-1})	Total shoot biomass (kg ha^{-1})	Nutrient uptake				
					N	P	K	Ca	Mg
					(kg ha^{-1})				
Pasture	2 passes	726	1030	1756	19 a	4.7 a	34 ab	3.6	6.0 a
	4 passes	506	1107	1613	17 a	4.8 a	43 a	2.4	4.8 a
	8 passes	415	1079	1494	12 b	2.5 b	28 b	2.0	3.7 b
Green manure	2 passes	5076	1257	6333 b	185 b	18 ab	81	90 b	21 b
	4 passes	6154	1679	7833 a	227 a	20 a	88	135 a	35 a
	8 passes	4923	1091	6014 b	192 b	14 b	70	82 b	22 b
Maize	2 passes	4472 b	1855 b	6327 c	40 b	10 b	65 b	12 b	9 b
	4 passes	5417 b	2049 b	7466 b	54 b	8 b	94 b	18 ab	12 ab
	8 passes	8803 a	3316 a	12119 a	99 a	16 a	141 a	23 a	14 a

Means followed by different letters within a column and within a cropping system are significantly different ($P < 0.05$) using Duncan's Multiple Range Test.

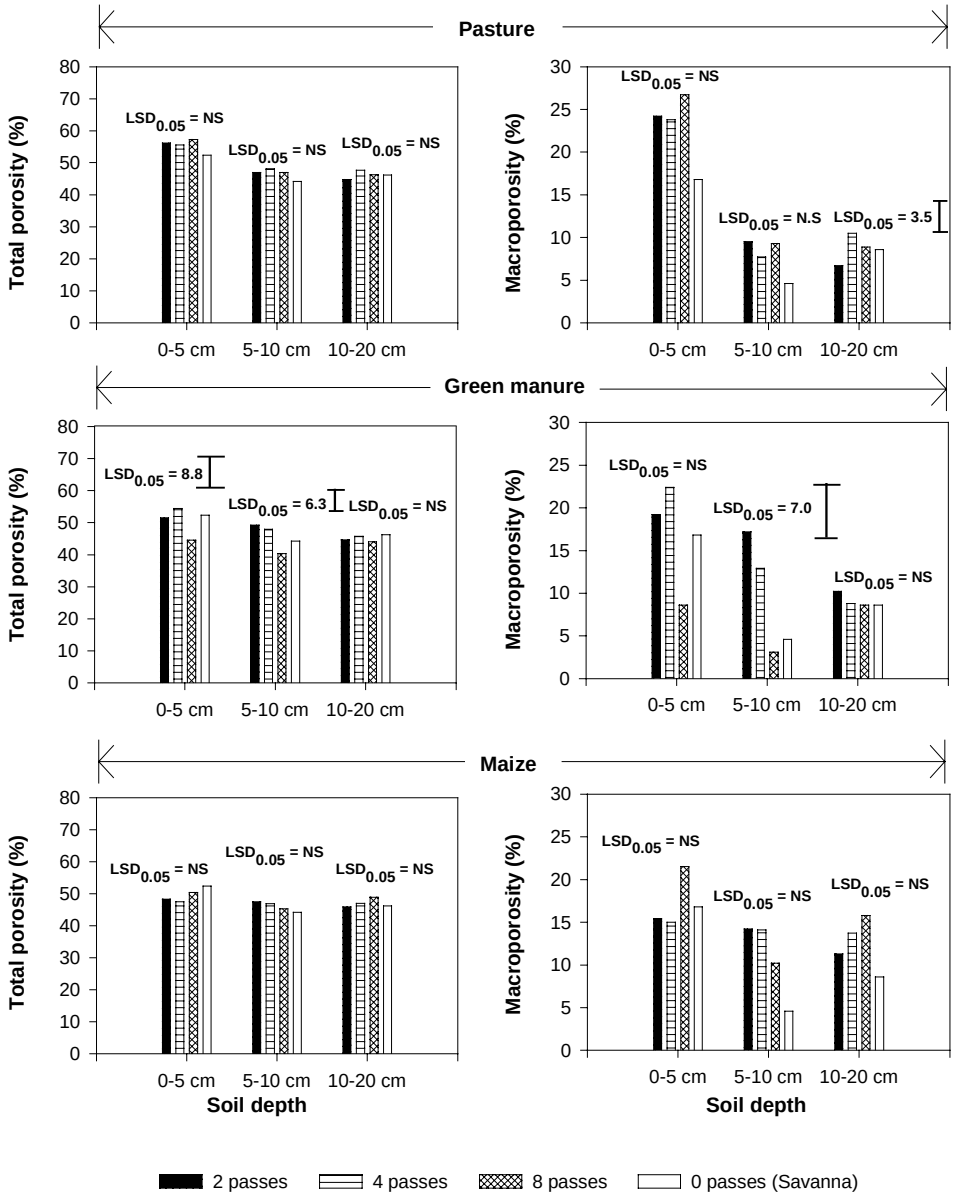

Figure 17. Changes in total porosity and macroporosity at different soil depth layers as affected by the intensity of disc harrowing and agropastoral systems. LSD values at 0.05 probability level. NS = not significant. The 0 number of passes represent the native savanna system.

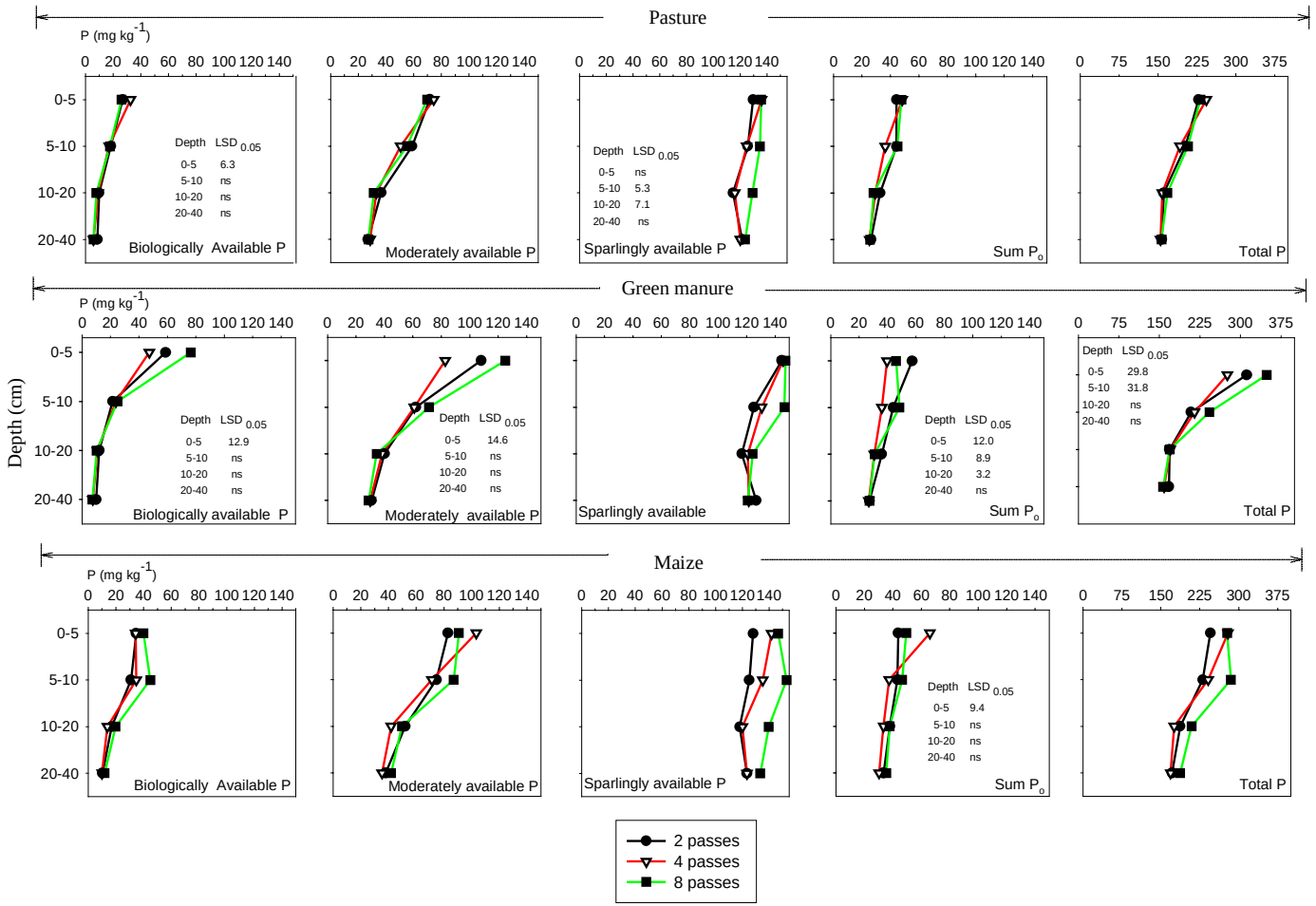

Figure 18. Soil profile distribution of the P fractions as affected by intensity of disc harrowing under the grass alone pasure, green manure and maize cropping systems.

Impact:
The results of this study showed that disc harrowing could reduce bulk density and improve total porosity and macroporosity, volumetric moisture content, and soil P availability in the topsoil layer of P-fixing Oxisols. However the impact of intensive disc harrowing (4 or 8 passes per year) on soil physical and chemical properties was dependent on the agropastoral system used. The maize and green manure cropping systems were better than the grass alone pasture system at separating the effect of increased number of disc harrow passes on soil physical and chemical characteristics.

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Activity 2.2 Develop strategies for nutrient acquisition and replenishment via efficient nutrient cycling and integrated nutrient management

2.2.1 Impact of contrasting agricultural systems on P cycling as determined by sequential P extraction of ^{33}P-labelled oxisol

Highlight:
- Showed that organic P is involved in short term P dynamics in soils with low or no P fertilization.

Purpose:
To evaluate the impact of contrasting agricultural systems on P cycling as determined by Sequential phosphorus extraction of ^{33}P-labeled oxisol.

Rationale:
Knowledge of the P dynamics in the soil/plant system and especially of the short- and long-term fate of P fertilizer in relation to different management practices is essential for the sustainable management of tropical agroecosystems. Chemical sequential extraction procedures have been and still are widely used to divide extractable soil P into different inorganic and organic fractions. The underlying assumption in these approaches is that readily available soil P is removed first with mild extractants, while less available or plant-unavailable P can only be extracted with stronger acids and alkali.

In the fractionation procedure, the P fractions (in order of extraction) are interpreted as follows. Resin-P_i represents inorganic P (P_i) either from the soil solution or weakly adsorbed on (oxy)hydroxides or carbonates. Sodium bicarbonate 0.5 *M* at pH 8.5 also extracts weakly adsorbed P_i and easily hydrolysable organic P (P_o)-compounds like ribonucleic acids and glycerophosphate. Sodium hydroxide 0.5 *M* extracts P_i associated with amorphous and crystalline Al and Fe (oxy)hydroxides and clay minerals and P_o associated with organic compounds (fulvic and humic acids). Hydrochloric acid 1 *M* extracts P_i associated with apatite or octacalcium P. Hot concentrated HCl extracts P_i and P_o from more stable pools. Organic P extracted at this step may also come from particulate organic matter. Residual P, i.e. P that remains after extracting the soil with the already cited extractants, most likely contains very recalcitrant P_i and P_o forms.

Several studies related these different P fractions in tropical soils to plant growth. By testing the influence of land-use and the fate of applied fertilizers, these studies resulted in contrasting assignments of P fractions to pools of different availability. The results obtained in these studies suggest that, in tropical soils, the amounts of P in the different pools measured by sequential P extraction procedures and the fluxes of P between pools are controlled both by physico-chemical factors such as sorption and desorption and by biological reactions such as immobilization and mineralization. However, the importance of these processes for different land-use systems, such as monocropping, pasture or intercropping, remains largely unknown.

The objective of this study was to assess the effect of different land-use systems (native savanna, rice monocropping, rice green manure rotation, grass legume pasture) on some physico chemical and biological reactions involved in P cycling in a Colombian Oxisol. Surface soil sampled in the different cropping systems was labeled with carrier-free radioactive P (^{33}P). After various incubation times, P was sequentially extracted and ^{31}P and ^{33}P were measured in each fraction.

Materials and Methods:

Soils: The soils included in the study were sampled during the rainy season in September 1997 from a field experiment located at CORPOICA-CIAT (Corporacion Colombiana de Investigacion Agropecuario; Centro Internacional de Agricultura Tropical) research station, Carimagua, Meta, Colombia (4°30'N, 71°19'W). Mean annual temperature is 27° C, average rainfall 2200 mm. The soils are well drained Oxisols (tropeptic haplustox, isohyperthermic).

The surface soil layer (0-20 cm) was sampled in the long-term "Culticore" field experiment, which was established in 1993 with the objective to test the effect of different farming systems on plant productivity and soil fertility. The experiment had a split-plot design with four replicates with treatment sub-plots of 0.36 ha size. The soil samples used for this study were taken at random in two replicates of each treatment and the replicates were mixed for the laboratory analysis. For our study, the following treatments were included:

SAV (Native savanna): native grassland annually burned in February, not grazed; no fertilizer application.

GL (Grass-legume pasture): rice in 1993, with undersown pasture, since then grass-legume pasture with *Brachiaria humidicola* CIAT 679, *Centrosema acutifolium* CIAT 5277, *Stylosanthes capitata* CIAT 10280, and *Arachis pintoi* CIAT 17434. The pasture was partly resown for renovation in June 1996 with legumes (the same *Arachis pintoi*, additionally *Centrosema acutifolium* cv Vichada CIAT 5277 and *Stylosanthes guianensis* CIAT 11833). Grazing intensity was on average 2.7 steers ha^{-1} during 15 d followed by a 15 d ley regrowth phase.

CR (Continuous rice): rice (*Oryza sativa* cv Oryzica Sabana 6, cv Oryzica Sabana 10 since 1996) grown in monoculture; one crop per year followed by a weedy fallow incorporated with early land preparation at the beginning of the rainy season before sowing rice.

RGM (Rice green manure rotation): Rice followed by cowpea (*Vigna unguiculata*, var. ICA Menegua) in the same year. The legume was incorporated at the maximum standing biomass level in the late rainy season before sowing rice in the following rainy season.

At the beginning of the experiment all treatments except SAV were limed using 500 kg dolomitic lime ha^{-1}. Fertilization of rice was 80 kg N ha year^{-1} (urea, divided among three applications), 60 kg P ha year^{-1} (triple superphosphate), 99 kg K as KCl, 15 kg Mg and 20 kg S (as $MgSO_4$) and 10 kg Zn ha^{-1} at establishment and according to plant needs afterwards. With cowpea additionally 20 kg N and 40 kg P ha year^{-1} and 60 kg K, 10 kg Mg, 13 kg S and 10 kg Zn ha^{-1} at establishment and in adequate rates afterwards were applied. The introduced pasture (GL) received additional fertilization only in 1996 (per ha: 20 kg P, 20 kg Ca (lime), 10 kg Mg (lime), 10 kg S (elemental) and 50 kg K (KCl)). Phosphorus input-output balances were estimated by subtracting the P removed from the system by grain and/or with animal live weight gains from the P applied in mineral fertilizers. Phosphorus exports in grain were calculated by multiplying weighed rice grain yields with measured P contents in grains. P exported in the animals was assumed to be 8 g per kg of live weight gain. Live weight gains in GL were on average 68 kg ha^{-1} yr^{-1}. Cultivated soils were tilled to a maximum of 15 cm depth. Topsoil samples (0-20 cm) were air-dried and sieved at 2 mm before they were used for chemical analysis in the analytical service laboratory of CIAT or shipped to Switzerland where they were stored in air-dried condition until use for the fractionation experiment in 2000.

Soil preparation: Before starting the sequential P fractionation, the soils were preincubated in a climate chamber (24°C and 65 % relative atmospheric humidity, no light) for two weeks in portions of 100 g at 50% of their water holding capacity (300 g water kg^{-1} soil dry weight). Soil water content was controlled and adjusted every other day by weighing.

Soil characterization: Bray-II P was extracted using dilute acid fluoride (0.03 *M* NH_4F, 0.1 *M* HCl) at 1:7 soil solution ratio using 2 g soil and 40 sec shaking time. Total soil P (P_{tot}) was determined on samples of 0.25 mg soil with addition of 5 mL concentrated H_2SO_4 and heating samples to 360° on a digestion block with subsequent stepwise (0.5 mL) additions of H_2O_2 until the solution was clear. Microbial P, C and N (P_{Chl}, C_{Chl} and N_{Chl}) were determined on the same moist, preincubated samples as for the sequential P fractionation by extraction, of chloroforme fumigated and unfumigated samples, with Bray I (0.03 *M* NH_4F, 0.025 *M* HCl) (P_{Chl}) or K_2SO_4 (C_{Chl} and N_{Chl}). No k-factors were used to calculate P_{mic}, C_{mic} or N_{mic} from measured P_{Chl}, C_{Chl} and N_{Chl} since there are no proper estimates for these acid tropical soils. P_{Chl} was corrected for sorption of released P. Dithionite-citrate-bicarbonate extractable and oxalate extractable Fe and Al (Fe_d, Fe_{ox}, Al_d, Al_{ox}) were determined. The mineralogy of the soils was determined on total soil samples, pretreated with H_2O_2 to remove organic C, using X-ray diffraction analysis (XRD) (Table 28). The samples were ground under acetone in a tungsten carbide vessel of a vibratory disk mill (Retsch RS1) for 10 minutes. Longer grinding times were not applied due to the detrimental effect that further grinding can have on the crystallinity of minerals, especially Fe (hydr) oxides. For the Cu Kα, the Bragg-Brentano geometry was chosen as an XRD routine setup. The measurement were carried out on a Scintag XDS 2000 equipped with a solid-state detector.

Sequential P fractionation of labeled soils: The preincubated soils were labeled in portions of 15 g with 120 MBq ^{33}P kg^{-1} which were added with 10 µl deionized water per g soil. The mass of P introduced with the ^{33}P label can be neglected (<2.5 x 10^{-3} µg P g^{-1} soil, Amersham product specification, July 2000). Therefore, the term 'P concentration' always refers to ^{31}P and specific activities (SA) are calculated as:

$$SA\ (Bq\ \mu g^{-1}\ P) = {}^{33}P/{}^{31}P \qquad [Eq.\ 1]$$

Soil P was fractionated sequentially with three replicates per soil with HCO_3-saturated resin strips (BDH # 55164, 9 x 62 mm), followed by 0.5 *M* $NaHCO_3$ (referred to as Bic-P), 0.1 *M* NaOH, (these first three steps each with an extracting time of 16 h) and concentrated hot HCl at 80° C for 10 minutes. The step using diluted cold HCl was omitted, as Ca-phosphates are only present at very low levels or are absent in highly weathered acidic soils. Residual P was extracted as described previously for determination of P_{tot}.

The amount of soil extracted was doubled from 0.5 to 1 g using the original volumes of extractants (2 resin strips in 30 mL H_2O, 30 mL $NaHCO_3$, 30 mL NaOH, 15 mL concentrated HCl, 5 mL conc. H_2SO_4) in order to get higher ^{33}P-concentrations in the extracts. This was preferred to the alternative of higher label application as the radiation might affect microbes. After each extraction, the samples were centrifuged at 25000 µg for 10 minutes before filtering the solutions of the Bic- and the NaOH-extraction through 0.45 µm pore size millipore filters (Sartorius, cellulose acetate), and the hot HCl and residual P extract through a Whatman filter Nr. 40.

Phosphorus concentration in all extracts was measured colorimetrically after neutralization. This method was used directly, after neutralization of the extracts, for the P recovered from the resin strip and for P_i determination in the HCl extract. Organic matter was first precipitated by acidification in the Bic- and the NaOH-extracts prior to P_i determination. Total P (P_t) in the Bic-, the NaOH- and the HCl-extracts was measured after digestion of P_o with potassium persulfate. Organic P was calculated as the difference between total P and P_i in the Bic-, NaOH- and hot HCl extracts.

To partition soluble $^{33}P_i$ and $^{33}P_o$ in the Bic-, the NaOH- and the hot HCl-extracts into separate solutions before counting, 5 mL of the extracts were shaken with acidified ammonium molybdate dissolved in isobutanol. With this method, P_i is extracted into the isobutanol while P_o remains in the aqueous phase. The complete recovery of P_i in the isobutanol phase was verified with the addition of a standard amount of ^{33}P in 0.5 *M* HCO_3, 0.1 *M* NaOH and in 2.3 *M* HCl; recovery rates of added ^{33}P in the isobutanol phase were between 97 % and 103 %, which was not significantly different from 100%. Counts in the aqueous phase were 1.1 % (HCO_3), 0.3 % (NaOH) and 0.1 % (HCl) of the original solutions showing that hardly any P_i goes into this phase. Determination of total P in the aqueous phase is not possible because the presence of the molybdate interferes with the analysis.

The radioactivity in each phase was determined with a liquid scintillation analyzer (Packard 2500 TR) using Packard Ultima Gold scintillation liquid in the ratio (extract to liquid) 1:5. The values were corrected for radioactive decay back to the day of soil labeling. All extracts were tested for possible quenching effects by adding defined ^{33}P spikes. Quenching in the acid resin eluate could be prevented by dilution of 250 µl eluate with 750 µl deionized water for counting. The quench effect in the hot concentrated HCl extract could be avoided by counting in the solutions separated with acidified isobutanol because the separated phases were not affected by quenching. All other extracts were not affected by quenching.

The recovery of the label as sum of all fractions, including residual P, was never complete. Therefore, subsamples of the soil residue after final acid digestion were dried and weighed into scintillation vials. These subsamples were then counted after addition of 1 mL water and 5 mL of scintillation cocktail.

As fractionation data were compared to parameters measured with an isotopic exchange batch experiment described below, the influence of the experimental conditions of the isotopic exchange procedure on the ^{33}P and ^{31}P distribution in the different P fractions was checked. Six 1 g samples of each soil were shaken for 16 h on an overhead shaker with 9.9 mL deionized water. The samples were then stirred on a magnetic stirrer plate and labeled with the same amount as above of ^{33}P per g soil introduced in 100 µl deionized water per 1 g sample. The samples were stirred for 100 minutes to simulate the conditions of an isotopic exchange experiment and afterward left without further stirring at room temperature. The sequential soil P fractionation was started 4 hours after labeling by adding two resin strips to each sample. Whether the soil was labeled in a 1:10 shaken soil suspension or at 50 % of water holding capacity did not significantly influence either the ^{31}P or the ^{33}P concentrations in the different fractions 4 hours after labeling (data not shown).

Isotopic exchange kinetics: The procedure of isotopic exchange kinetics was used to assess the exchangeability of P_i in the soils sampled in the different land-use systems. Suspensions of 10 g of soil and 99 mL deionized water were shaken for 16 h on an overhead shaker to reach a steady state equilibrium for P_i. Then, at t = 0, 1 mL of carrier free $H_3{}^{33}PO_4$ tracer solution containing 1.2 MBq was added to each continuously stirred soil water suspension. Three subsamples were taken from each sample after 1, 10 and 100 minutes, immediately filtered through a 0.2 µm pore size micropore filter, and the radioactivity in solution was measured by liquid scintillation as described previously. To determine the ^{31}P concentration in the soil solution (C_p, mg P L^{-1}) 10 mL of the solution were filtered through a 0.025 µm filter (Schleicher & Schuell, NC 03) at the end of the experiment. The smaller filter pore size was used to exclude any influence of suspended soil colloids on C_p determination. The P concentration in the filtrate was measured in a 1 cm cell using the Malachite green method with a Shimadzu UV-1601 spectrophotometer. As the concentrations in the solutions of SAV and GL were close to the detection limit, they were additionally measured in samples concentrated by evaporation (5:1). This procedure resulted in C_p values that were not significantly different from the non-concentrated solutions.

Assuming that at any given exchange time the specific activity (SA) of inorganic phosphate in the solution is equal to the SA of the total quantity of phosphate which has been isotopically exchanged, it is possible to calculate the amount of isotopically exchanged P (E_t, mg P kg^{-1} soil). The amount of P exchangeable within one minute (E_1), indicating the immediately available P, is expressed as:

$E_1 = R \times 10 \times C_p / r_1$ [Eq. 2]

where R is the introduced radioactivity and r_1 is the radioactivity remaining in solution after 1 minute of isotopic exchange. The factor 10 results from the soil solution ratio of 1:10.

Statistical analysis: The effects of land-use systems and incubation time after labeling on P fraction size were tested by two-way ANOVA and Tukey's multiple range over all treatments and times of fractionation. A separate one-way ANOVA was used to test the difference on label recovery and fraction size between samples labeled in soil water ratio 1:10 and samples labeled in incubated moist state 4 hours after labeling. Percentage recovery data were log-transformed to meet the requirements of analysis of variance. Time and soil treatment influences for each repetition in time of sequential fractionation were tested by ANOVA.

Results and Discussion:

The mineralogy (Table 28) and the Fe and Al (oxy)hydroxides contents (Table 29) of the surface soil from the four treatments was normal for this type of soil and were almost identical among the different land-use systems (SAV, GL, CR, RGM). This implies that any difference seen in the P dynamics among land-use systems was mainly due to the land-use system and not to differences in the soil mineralogy.

Total soil P and P balance induced by the different treatments: The amounts of total P directly extracted from the soil samples (P_{tot}) were not significantly different from the sum of P (P_{sum}) extracted in the different fractions of the sequential extraction for SAV and CR while the direct extraction led to significantly higher values ($P<0.05$) for GL and RGM (Table 30). To evaluate whether differences in total P content in soils were related to P fertilization, the increase in P_{tot} content (calculated as the difference between total P extracted from fertilized GL, CR or RGM) and P_{tot} extracted from non fertilized SAV was compared to the estimated P balance of these treatments (significant correlation, $r^2=0.87$; $P<0.001$). The increases in P_{tot} were of the same order of magnitude as the calculated P balance. Given the imprecision of the methods used to determine total P contents and of the estimations made to calculate the P balance, these results suggest that most of the P added with fertilizers and not taken up by plants remained in the surface layers of the studied soils. Soil tillage may have mixed P in the 0-10 cm soil layer with soil in the 10-20 cm layer, resulting in incomplete recovery of P in the 0-10 cm sampling depth.

Isotopic exchange characteristics: The effect of the four land-use systems on P_i exchangeability in the surface layer of the studied soil is presented in Table 4. The ratio r_1/R, which is inversely correlated to the P sorbing capacity of soils, was below 0.05 for all treatments suggesting that these soils have a high P sorbing capacity. Furthermore, the r_1/R-values of the four treatments were positively correlated with the directly extracted total soil P ($r^2=0.76$ $P<0.001$). This suggests that the different land-use systems have resulted, through their different P fertilization and cropping, in different sorption rates of P_i on soil minerals. Since in Oxisols P sorption is governed by the Al and Fe (oxy)hydroxides, these treatments probably induced different degree of P_i saturation on the soil metallic (oxy)hydroxides such as gibbsite, which has been identified in these samples (Table 28).

The P_i concentration in the soil solution (C_p) was close to the detection limit in SAV, GL and CR treatments (Table 31). Although significantly different between all treatments, C_p was significantly increased only in the RGM treatment ($P<0.001$). In SAV, GL and CR, C_p was much lower than the critical concentration needed to sustain optimal growth for a large range of crops. The P_i concentration in the soil

solution was not correlated with the total soil P content. The clear C_p increase in RGM was therefore not only due to an increase in total P but also to other mechanisms. The strong increase in soil biological activity observed in land-use systems including legumes might partly explain this higher C_p value. Variation in the amount of P_i isotopically exchangeable in one minute (E_1) followed the same trend as the variation in C_p.

P concentrations in different fractions of the sequential extraction: The positive P balances of the fertilized GL, CR and RGM treatments resulted in significantly higher P concentrations ($P<0.001$) compared to the savanna soil in all fractions except the organic fractions and residual P (Table 32). Our results show that resin-P_i, Bic-P_i and NaOH-P_i increased with P fertilizer input, with the NaOH-P_i fraction being the main sink for the applied P. This P sink function of the NaOH-P_i fraction can be explained by the adsorption of P_i through ligand exchange with hydroxyl groups located on the surface of Fe and Al (oxy)hydroxides and by the desorption of P_i from the surface of (oxy)hydroxides in the presence of 0.5 M NaOH.

During the continuous 2-week incubation of the soil samples, the resin and the Bic-P_i fractions increased significantly ($P<0.05$) between the first and second fractionation date for all soils (between 4 and 14 mg kg^{-1} for the sum of resin and Bic-P_i). There was no significant and corresponding decrease in any fraction although total extractable P_o tended to decline (between 8 and 18 mg kg^{-1}) for all soils (Table 32). The absence of significant compensating movements of P out of P_o fractions may be due to the high variability of the results, especially for the organic fractions where coefficients of variation for Bic-P_o were between 13 and 70 % and for NaOH-P_o between 7 and 45 %. Since P_o is determined by the difference between P_t and P_i there are multiple sources of error.

Increases in resin and Bic-P_i between 4 hours and 1 week of incubation suggest that mineralization of P_o led to the release of labile P_i from P_o fractions. As the first fractionation was started 4 hours after labeling, the disturbance by mixing the soil with the label and the momentarily increased humidity might additionally have stimulated the microbial activity despite preincubation. A temporary stimulation of the microbial activity by the thorough mixing when labeling soil was indicated in microbial turnover studies conducted on soils from the same field experiment. This assumption seems likely, as there were little changes in fraction sizes between the second and the third fractionation indicating a stabilization of the system.

Distribution of ^{33}P among P fractions and dynamics over time: The fraction of ^{33}P recovered in the resin-P_i fraction 4 hours after labeling varied between 22 % in SAV and 60 % in RGM (Figure 19). The ^{33}P recovery in this fraction was positively correlated to the content of total P of the soils ($r^2=0.87$; $P<0.001$, 4 h after labeling). The corresponding decrease of ^{33}P in the resin fraction in RGM and CR corresponded with an increase in label recovery in Bic- and NaOH-P_i, while in SAV and GL the decline in resin ^{33}P was accompanied by an increase in ^{33}P in NaOH-P_o (GL also NaOH-P_i), HCl-P_i and residual-P. For SAV and GL, the label recovered in the resin-P_i, and Bic-P_i did not change much between the 1st and the 2nd week and the amount of ^{33}P in NaOH-P_i was stable over the entire incubation time. This shows that in SAV and GL the label was rapidly exchanged between these fractions and that equilibrium with the (labeled) soil solution was reached. In contrast, ^{33}P in the Bic-P_i and the NaOH-P_i of CR and RGM was still increasing after one week while the resin-$^{33}P_i$ continued to decrease, showing that the exchange between these fractions was incomplete.

Only small amounts of the label were found in organic fractions after 4 hours, but there were already significant differences in NaOH-^{33}Po ($P<0.001$) in the order:
SAV (4%) ≈ GL (2%) > CR (0.4 %) ≈ RGM (0.1 %).
This might be due to differences in microbial activity. Actually, the microbial biomass in incubated soils, indicated by measured P_{Chl}, C_{Chl} and N_{Chl} values, was significantly different between the soils (Table 33),

despite the fact that the samples had been stored in air-dried condition for more than three years before being used in this study. The assumption that recovery of the label in organic fractions was actually due to active processes and not to any analytical artifact is supported by the observed increases of NaOH-$^{33}P_o$ and HCl-$^{33}P_o$ for all soils over time. The total recovery of 20 % (SAV) or 14% (GL), respectively, of the label in organic fractions two weeks after labeling shows that these compartments have to be taken into account to understand the fate of P in these very low-P soils.

The proportion of label in the hot HCl and residual P fractions increased significantly with incubation time in all soils. This contradicts the prevailing opinion of recalcitrance of the P in these fractions. While the total P content in the residual fraction varied significantly with time (Table 32), this was not the case for hot HCl extractable P_i, while hot HCl extractable Po tended to decrease. This suggests that the movement of the label to these fractions was not due to net P-movement but to exchange processes.

Total ^{33}P label recovery: At all fractionation dates, in total between 67 % and 94 % of the applied ^{33}P label could be recovered in the sum of all fractions (Figure 19). This sum was generally in the order SAV<GL<CR<RGM. These incomplete recoveries can be explained by the fact that the method used to assess total P or residual P was not efficient enough to extract all P. Comparative studies have shown that total P can only be reliably extracted by alkali fusion, which could not be used in this work. The analysis of soil residues after the acid extraction of residual P (Table 34) indicated indeed that significant amounts of the label remained unextracted, these being higher for SAV and GL than CR and RGM. Although counting of ^{33}P bound to solid phases is generally possible, problems of phase, impurity, self absorption of scintillations by the soil particles or color quenching effects are difficult to correct, as these influences might be highly variable between samples. However, the recovery of standard additions of ^{33}P to our soil residues was complete and the correlation of the measured radioactivity in the different soil treatment residues with the sample weight was linear (data not shown), thus confirming the qualitative information obtained from the counting of the soil residues.

Altogether the results suggest that the transfer of ^{33}P among the different fractions determined by the sequential extraction was strongly dependent on the degree of saturation of soil Al and Fe (oxy)hydroxides with P_i, and therefore on the bonding energy of P_i to the soil minerals. It is indeed known that a high P_i saturation of metal oxide surfaces causes a more negative charge on the surface and prevents the specific sorption of further P_i ions. In the P poor soils (SAV and GL), most P_i would be sorbed with such a high energy that their exchangeability would be very limited. A specific sorption of ^{33}P to the surface of Al and Fe (oxy)hydroxides of these soils, although unlikely, cannot be excluded. In contrast, in the P rich soils (CR and RGM), the annual P additions might have resulted in the build up of relatively larger quantities of P_i exchangeable with ^{33}P.

Specific activities in the fractions determined by the sequential extraction: The highest specific activities (SA) observed in this incubation experiment were obtained in the resin extract after 4 hours of incubation (Table 35). This is consistent with the assumption that the amount of P desorbed from the soil by a resin is in very rapid exchange with P_i in the soil solution, as suggested by other studies. The subsequent decrease in the SA of resin-P_i reflected the process of isotopic exchange between ^{33}P and stable P_i located on the soil's solid phase. The order of the SAs in the P_i fractions after 4 hours of incubation followed the extraction sequence (resin-P_i>Bic-P_i>NaOH-P_i>HCl-P_i>residual P), showing that the strongest reactants extracted either large quantities of slowly exchangeable P or a large quantity of P in which only a small part was rapidly exchangeable. After 2 weeks the SAs of resin-P_i, Bic-P_i and NaOH-P_i became closer, suggesting that equilibrium with respect to P transfer between these fractions was being approached. The SAs of resin-P_i, Bic-P_t and NaOH-P_i were not significantly different in SAV and GL while the SA of resin-P_i was still significantly higher than the SA of Bic-P_i and NaOH-P_i in CR and RGM. These observations show that it is not possible to discuss the exchangeability of a certain P fraction without relation to a defined time of exchange.

Although the SAs of the NaOH-P_o and HCl-P_o fraction were relatively low they showed that, depending on land-use, these fractions were connected through active processes with the soil solution, most probably through microbial activity. This indicates that the determination of plant available P with short-term isotopic exchange experiments might lead to errors since the dynamics of organic P forms could be excluded.

Table 28. Mineralogy of the studied Colombian Oxisol under different agricultural systems, determined with X-ray diffraction.

Treatment †	Quartz	Kaolinite	Anatase	Rutile	Gibbsite	Vermiculite
	% of total soil weight					
SAV	72	21	3	2	2	<1
GL	67	23	4	2	4	<1
CR	65	25	4	2	3	<1
RGM	67	24	4	2	3	<1

† SAV: Native savanna, GL: Grass-legume pasture, CR: Rice monoculture, RGM: Rice-green manure rotation.

Table 29. Selected chemical and physical properties of the surface soil (0-20 cm) of studied Colombian Oxisol under different agricultural systems. Values are the average of four analytical replicates, except Fe- and Al-contents (three replicates#).

Treatment †	Total C	Total N	pH in water	Al-Saturation	$Fe_{d‡}$	$Fe_{ox§}$	$Al_{d‡}$	$Al_{ox§}$	Clay	Bulk density
	g kg^{-1}			%	———	g kg^{-1}	———		%	Mg m^{-3}
SAV	27	1.64	4.8b	86.8b	26.7	3.6	7.8	2.0	35.0a	1.27
GL	29	1.55	4.9b	71.7a	26.4	3.6	7.7	2.0	39.3b	1.27
CR	26	1.45	4.3a	75.4a	26.2	3.7	7.6	2.0	39.9b	1.21
RGM	26	1.49	4.3a	76.3a	26.9	3.5	7.8	2.0	39.0b	1.24

† see Table 28.

‡ Extraction with dithionite.

§ Extraction with oxalate.

Means followed by the same letter are not significantly different (P=0.05) by Tukey's multiple range test. The absence of letter in a column shows that no significant differences were observed between the treatments.

Table 30. P status and calculated P balances of the studied Oxisol under different land-use systems. Total P as sum of the sequential P fractionation (P_{sum}) or extracted directly with H_2O_2 and H_2SO_4 (P_{tot}).

Treatment†	Bray II P‡	P_{sum} ‡	Δ P_{sum}§	P_{tot} ‡	Δ P_{tot}§	P-Balance¶
	mg kg^{-1}					
SAV	0.9a	165aA	0	172aA	0	0
GL	2.0b	190bA	25	213bB	41	28
CR	17.2c	290cA	125	293cA	121	92
RGM	35.5d	335dA	170	376dB	205	153
F-test (soil)	***	***		***		

† see Table 28.
‡ P concentrations followed by the same lower case letter (within columns) or upper case letter (comparison of P_{sum} and P_{tot} within rows) are not significantly different (P=0.05) according to Tukey's test.
§ Δ P calculated as the difference between P_{sum} or P_{tot} of fertilized treatments – SAV.
¶ Calculated by subtracting the P removed by grain and/or animals from the P applied with mineral fertilizer.

Table 31. Parameters of isotopic exchange †

Treatment‡	r_1/R§	c_p ¶ (mg l^{-1})	E_1 # (mg kg^{-1})
SAV	0.02a	0.0015a	0.7a
GL	0.03a	0.002b	0.6a
CR	0.04a	0.003c	0.8a
RGM	0.055b	0.015d	2.7b
F-test	***	***	***

† Values are the average of three replications.
‡ see Table 28.
§ ratio of radioactivity remaining in soil solution to radioactivity added at time 0 after 1 minute of isotopic exchange.
¶ P concentration in the soil solution measured at soil:water ratio 1:10.
# Quantity of P exchangeable within 1 minute.

Table 32. Distribution of P in various fractions of the modified Hedley fractionation in different agricultural systems with and without P application on an Oxisol, at three times of incubation after mixing the soils for label application.

Treatment ‡	Incubation Time	resin P_i		Bicarbonate P_i		Bicarbonate P_o	NaOH P_i		NaOH P_o	Hot HCL P_i		Hot HCL P_o		Residual P_t		Total P		Total P_o
		mg kg-1																
SAV	4 hours	0.9	g†	1.4	g	12.4	22	de	46	37	b	6.1	ab	44	ab	172	ef	65
GL	4 hours	2.0	ef	2.8	fg	11.8	27	de	56	34	b	8.6	a	43	b	185	ef	76
CR	4 hours	4.8	d	9.7	def	15.0	102	b	48	56	a	9.1	a	49	ab	298	cd	72
RGM	4 hours	10.0	b	21.4	bc	6.7	100	bc	62	65	a	5.2	abc	47	ab	321	abc	74
SAV	1 week	2.0	ef	4.3	fg	5.7	20	e	42	36	b	4.1	bc	42	b	157	f	52
GL	1 week	2.4	e	6.4	efg	10.0	33	d	47	38	b	3.3	bc	43	ab	184	ef	61
CR	1 week	8.0	c	14.3	cde	14.3	89	c	47	53	a	2.5	bc	50	ab	279	d	64
RGM	1 week	16.4	a	29.8	a	12.8	119	a	40	63	a	3.3	bc	54	ab	338	ab	56
SAV	2 weeks	2.0	ef	4.1	fg	6.3	20	e	42	36	b	4.1	bc	48	ab	164	f	52
GL	2 weeks	4.2	d	6.4	efg	10.3	33	d	49	38	b	2.9	bc	62	a	207	e	62
CR	2 weeks	7.5	c	16.6	cd	11.0	90	bc	56	58	a	1.2	c	61	ab	305	bcd	68
RGM	2 weeks	15.8	a	27.5	ab	15.9	118	a	45	63	a	4.3	bc	62	a	354	a	65
Treatment		***		***		n.s.	***		n.s.	***		**		n.s.		***		n.s.
Time		***		***		n.s.	n.s.		n.s.	n.s.		***		***		*		n.s.

*,**,*** Significant at the 0.05, 0.01, and 0.001 probability level, respectively

† values within a column followed by the same letter do not differ significantly (P=0.05) according to Tukey's test.

‡ see Table 28.

Table 33. Size of the soil microbial biomass nutrient pool in different agricultural systems after 20 days of incubation of the formerly air-dried soils. Values are the averages of three replicates†.

Treatment ‡	C_{Chl}	N_{Chl}	P_{Chl}
	mg kg^{-1}		
SAV	88.7a	13.7a	1.6a
GL	80.8a	13.5a	1.2ab
CR	72.9a	8.5b	0.7b
RGM	48.2b	6.1b	0.5b
F-Test	**	***	***

,* Significant at the 0.01, and 0.001 probability levels, respectively.
† Means followed by the same letter are not significantly different (*P*=0.05) by Tukey's multiple range test.
‡ see Table 28.

Table 34. Radioactivity measured in soil solid residues by scintillation counting after extraction of residual P by sequential P fractionation starting 1 week after soil labeling.

Soil treatment	Bq g^{-1} soil (decay corrected)†		% of initial label
SAV	2251	(111)	4.4%
GL	1843	(357)	3.6%
CR	427	(215)	0.8%
RGM	348	(140)	0.7%

†Average of three replications, standard error in brackets.decay corrected to the day of soil labeling.

Table 35. Specific activities ($^{33}P/^{31}P$) in isotopic exchange soil solution and in extracts of the Hedley sequential fractionation in the labeled Oxisols derived from different agricultural systems at different times after labeling. †

Time	treatment	resin P_i		Bic-P_i		NaOH-Pi		NaOH-Po		HCl-Pi		HCl-Po		residual P	
		kBq mg P^{-1}													
4 hours	SAV	32.9	aA	5.9	AC	1.8	aD	119 x 10^{-3}	aE	180 x 10^{-3}	aE	8 x10^{-3}	F	3 x10^{-3}	aF
	GL	24.5	bA	3.3	BB	1.6	aC	44 x 10^{-3}	bE	138 x 10^{-3}	bD	3 x10^{-3}	F	3 x10^{-3}	aF
	CR	13.8	cB	1.3	CC	0.4	bD	11 x 10^{-3}	bF	54 x 10^{-3}	cE	0		1 x 10^{-3}	bF
	RGM	7.9	dA	0.6	CB	0.3	bC	3 x 10^{-3}	bE	33 x 10^{-3}	dD	0		1 x 10^{-3}	bF
	F-test ¶:	***		***		***		***		***		n.s.		***	
1 week	SAV	5.1	abA	2.7	AA	1.9	aB	480 x 10^{-3}	aC	430 x 10^{-3}	aD	280 x 10^{-3}	E	157 x 10^{-3}	aF
	GL	6.4	aA	2.2	BB	1.3	bD	293 x 10^{-3}	bE	436 x 10^{-3}	aE	497 x 10^{-3}	DE	140 x 10^{-3}	aF
	CR	5.3	abA	1.1	CC	0.5	cD	64 x 10^{-3}	cE	138 x 10^{-3}	bE	271 x 10^{-3}	DE	26 x 10^{-3}	bE
	RGM	3.1	bcA	0.6	CC	0.4	cD	35 x 10^{-3}	cE	76 x 10^{-3}	bE	159 x 10^{-3}	DE	18 x 10^{-3}	bE
	F-test:	*		***		***		***		***		n.s.		***	
2 weeks	SAV	2.1	ABC	1.6	AB	2.1	aAB	587 x 10^{-3}	aC	290 x 10^{-3}	aD	566 x 10^{-3}	C	154 x 10^{-3}	aE
	GL	2.1	B	1.4	AC	1.6	aBC	357 x 10^{-3}	bD	249 x 10^{-3}	bD	741 x 10^{-3}	D	135 x 10^{-3}	aE
	CR	2.6	A	1.1	abB	0.7	bB	70 x 10^{-3}	cD	99 x 10^{-3}	cCD	22 x 10^{-3}	D	43 x 10^{-3}	bD
	RGM	1.9	A	0.8	bBC	0.5	bC	48 x 10^{-3}	cDE	75 x 10^{-3}	cD	56 x 10^{-3}	DE	26 x 10^{-3}	bE
	F-test‡:	n.s.		*		***		***		***		n.s.		***	

*,**,*** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

† All values are the average of three replicates. Decay corrected to the day of soil labeling.

‡ ANOVA was calculated separate for each time, means followed by different lower case letters within one column at one time are significantly different (*P*=0.05) by Tukey's test
The same is valid for means within one row followed by different upper case letters.

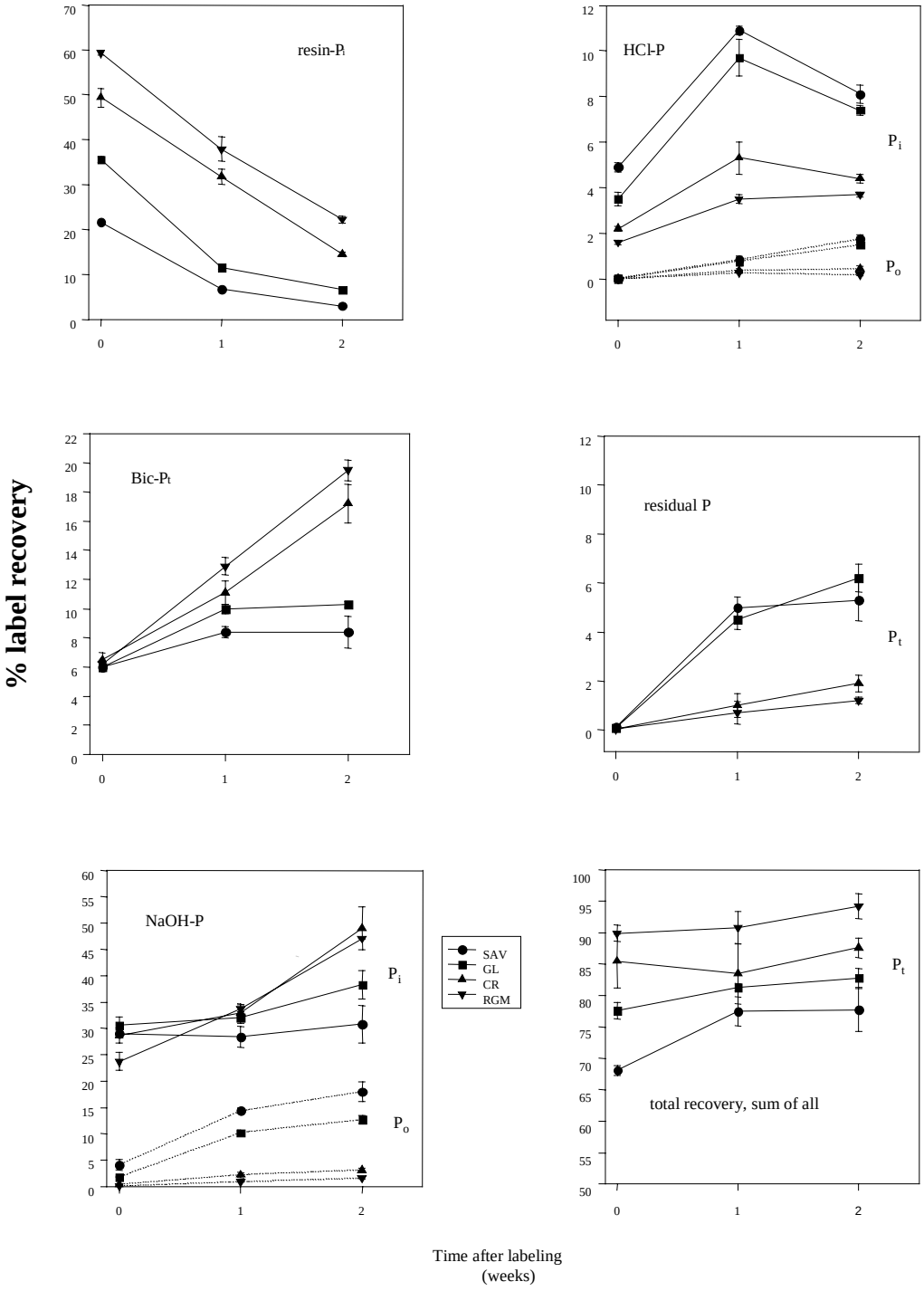

Figure 19. Percentage of label recovery in the different fractions of the sequential P extraction and in the sum of all fractions at 4 hours, 1 and 2 weeks after labeling soil (Means of three replicates ± SD).

Impact:
The effect of contrasting land-use systems on the P fractions extracted by the sequential fractionation procedure was assessed in an Oxisol during a 2-week incubation on soils labeled with carrier free ^{33}P. The results show that in the studied Oxisol, the quantities of ^{31}P and ^{33}P recovered in the different fractions were strongly dependent on the total P content of the soil, which was determined by the amount of P added by fertilizers and by plant P uptake. The importance of organic P pool for natural and low P input systems was pointed out from this detailed study. The extensive native savanna systems and introduced grass alone pasture systems in tropical Latin America on low P acid soils function with almost either no or very little external inorganic P inputs. This study indicates that an efficient cycling through organic P may be an important mechanism for the survival and adaptation of native and introduced tropical grasses in acid low P soils.

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2.2.2 Phosphorous fractions and dynamics in surface earthworm casts under native and improved grasslands in a Colombian savanna Oxisol

Highlight:
- Showed that earthworm surface casts represent a significant source of easily available P for plant roots.

Purpose:
To assess the effect of a native earthworm species on phosphorous availability in an Oxisol from the Colombian Llanos.

Rationale:
At present there exists increasing evidence of improvement of soil fertility through the effects of soil macroinvertebrate activities due to their role in soil organic matter transformations and nutrient dynamics at different spatial and temporal scales, what probably improves nutrient uptake by plants. Within the numerous animals that inhabit the soil, a few invertebrates have been defined as the "soil ecosystem engineers". By definition, ecosystem engineers are "those organisms that directly or indirectly modulate the availability of resources to other species by causing physical state changes in biotic or abiotic materials". This means that they are capable to regulate the trophic (organic matter) and spatial (habitat availability) resources in the soil through the production of physical bio-structures (e.g. casts, galleries and nests). Soil invertebrates are major determinants of soil processes, especially in tropical ecosystems.

Earthworms through their burrowing activities, mixing soil with litter and egesting casts inside the soil or at the soil surface, they affect the physical properties of soils, nutrient cycling and plant dynamics. To assess the contribution of these organisms in soil processes and ecosystem function, a previous step is the description of the phenomena that occur in the casts. Yet the impact on nutrient cycling has not been investigated in detail in tropical anecic earthworms, even though they significantly cast at the soil. For example, some studies have revealed higher contents of available phosphorous (P) (that can be uptaken by plants) in earthworm casts than in the control soil. Effects of earthworms on P are especially interesting since part of the pool, which is normally adsorbed onto the soil solid phase, may be desorbed after gut transit. These organisms have a notorious impact on mineralisation of P[δ], and are able to increase its

[δ] Phosphorous mineralization is an enzymatic process and a group of phosphatases are involved in the catalysis and release of phosphate from organic P compounds to the soil solution.

availability for plants in their casts. This process has been largely documented even for tropical and temperate species, and is the result of their high efficient digestive system while they excrete intestinal and cutaneous mucus that leaves nutrients in excess. In consequence, earthworms have an important role in improving nutrient availability and cycling in natural and agricultural ecosystems.

About 75% of soils in Neotropical savannas are strongly weathered, acidic and infertile soils belonging to the order of Oxisols. Low total and available P contents and a high P fixation capacity due to high contents of Fe and Al oxides characterize them. Most crops recover less than 20% of total fertilizer P applied to soils in these agroecosystems. The remainder is gradually rendered less available to succeeding crops by processes that slowly move P into more stable inorganic and organic pools in the soil. One strategy to increase the productivity and sustainability of production in such agroecosystems is to increase P recovery from these less accessible forms using crop and forage cultivars that are more efficient in acquiring P from theses pools and cycling it into pools more available to crops. The role of soil macroinvertebrates in P cycling must also be considered for their potential to release and make soil P available for plant uptake.

The potential of the Colombian savannas, an isohyperthermic ecosystem dominated by Oxisols, for both crop and livestock production systems is limited by the lack of available P for primary production. The conversion of native savanna into intensive pastures generally leads to a huge increase in earthworm biomass. *Martiodrilus carimaguensis* (Glossoscolecidae) is a large anecic native savanna earthworm from the Colombian "Llanos" which has been shown to affect soil processes and potential to take advantage from its activities in tropical agroecosystems.

Casts of large anecic *M. carimaguensis* are enriched in labile organic P, which suggests that this species improves the supply of P in soil under pastures by creating an available organic P pool. There is some evidence from soil P fractionation analyses which indicated that a high level of P cycling in intensive pastures contributed to their sustainability with very low P inputs. Based on these observations, we hypothesized that *M. carimaguensis*, through greatly increased population and their effect on soil P dynamics, is a major contributor to the sustainable productivity of intensive pastures on Colombian savannas Oxisols. The objective of this study was to determine the temporal dynamics of P fractions in casts and quantify P availability and enhanced P cycling in an Oxisol of the Colombian Llanos. The study involved both laboratory and field experiments to assess the impact of this species of high casting surface activity in nutrient cycling. Since earthworms can accelerate the mineralisation of organic matter by reducing the size of residues to particles more available to microbial populations one laboratory experiment aimed at the effect of incorporating finely ground vegetative material on P mineralization.

Materials and Methods:

Study site: This study was conducted during May-September 1994 at the CIAT-CORPOICA experimental station at Carimagua (4°30'N; 71°19'W; 150 m above sea level) located 320 km east of Villavicencio in the Eastern Plains of Colombia. The site is representative of the well-drained isohyperthermic savanna ecosystem. Mean annual rainfall and temperature are 2240 mm and 26.5 °C respectively. Rainfall distribution is characterized by a 4-month sharp dry season from December to March. Native vegetation varies with topography accordingly: open herbaceous savannas in the uplands ("altos"), and gallery forests or flooding savannas in the low-lying areas ("bajos"). Samplings were done in a well-drained silt-clay Oxisol (typic haplustox, fine kaolinitic, isohyperthermic). They are characterised by favourable physical properties, e.g. porosity and water retention, but high Al saturation (>80%) and low chemical fertility (Table 36).

Table 36. Soil physico-chemical properties in the native savanna and intensive pasture.

Treatment	pH(H_2O)	Total C	Total N	Bray-II P	Exchangeable cations				
					Al	Ca	Mg	K	H
		- - - - - - - mg g^{-1} - - - mg k^{-1}			- - - - - - - - - - - - - - - $cmol^{+}$ kg^{-1} - - - - - - - - - -				
Savanna	4.80	23.5	1.45	1.3	2.42	0.26	0.11	0.08	0.27
Pasture	4.96	24.9	1.67	2.2	1.90	0.89	0.23	0.09	0.26

Background ecology of M. carimaguensis: Martiodrilus carimaguensis is a large dorsally pigmented earthworm 9.3 mm in diameter, 194 mm in length and weighing 9.2 g (in 4% formaline) on average. Its morphology and life history traits are like most anecic species but its feeding regime seems to be close to endogeic type and it only eats litter in an opportunistic way and exhibits a feeding regime mostly based on small casts of other earthworm species. The species is native to the well-drained savannas and is quite common in both the natural and managed ecosystems of Carimagua. The establishment of intensive pastures results in a spectacular increase of density compared to the native savanna, and the strategy to support the drought stress during the dry season is quite surprising. Juveniles enter into a physiologically-induced diapause deep in the soil by the middle of the rainy season, whereas adults present a similar behaviour with a lag of several months, i.e. at the end of the rainy season or just after their reproductive period, i.e. when they lay large cocoons (16% of adult weight) from September onwards.

Casts of M. carimaguensis: Martiodrilus carimaguensis remains active in the same burrow during at least the first week after cast deposition begins on the soil surface. The size of these towerlike casts ranges from 3 to 6 cm in diametre, 2 to 10 cm in height and 25 g dry weight on average, and large casts can reach up to 15 cm height and 400 g dry weight. At the beginning of the deposition casts are a fresh pasty structure, and during the following week, a combination of dry and fresh material at the bottom and the top of the cast, respectively, can be observed. Whether the earthworm leaves its semi-permanent burrow or it goes deep into the soil profile to begin diapause the cast dries completely, and remains at the soil surface for more than one year after having been excreted. Their half-life range from 2 to 11 months in a trampled and protected pasture, respectively, and 5 months in ungrazed natural savanna. The disappearance of casts from the soil surface is attributed to several environmental and anthropogenic factors, i.e. rainfall impacts, fire, cattle trampling and the activity of burying small invertebrates.

Experimental design: Three experiments were performed during the rainy season of 1994: one field study and two laboratory/incubation studies using soils collected from the same plots where the field work was conducted.

Field experiment: The field experiment was carried out from May to August 1994 in adjacent paddocks of open herbaceous native savanna dominated by *Andropogon bicornis* L. and *Trachypogon vestitus* Anders. and a 18 year-old pasture of *Brachiaria decumbens* cv Basilisk and *Pueraria phaseoloides* (Roxb.) Benth. CIAT 9900 (Kudzu). In each paddock, three areas (repetitions) of 4x4 m and 1 m^2, respectively, were located at random and cleaned of all existing surface earthworm casts. In the *B. decumbens*-Kudzu pasture, plots were protected from cattle trampling with wire exclusion cages. On the following day, plots were surveyed and freshly deposited casts of *M. carimaguensis* were identified, displaced slightly to the side of the earthworm gallery and tagged with a plastic peg to identify the time of in situ ageing at which it would be sampled (the day of displacement being “day 0”). Samples were taken at six different ages, i.e. 1, 4, 8, 16, 32 and 64 days. For a given date, casts from each area were picked up and placed in an ice chest for transport to the laboratory. Approximately 70 g were collected for each sample, of which

approximately 40 g were dried under forced draft in an oven at 40 °C and 30 g were stored in a refrigerator at 4 °C prior to analysis as described below. Control soil samples were taken in each paddock by splitting it into four areas where five 0-15 cm soil cores were taken per area and mixed. Samples were further prepared for analyses by crushing and sieving through a 2-mm mesh. Samples for microbial P determinations were maintained fresh under refrigeration as mentioned above.

Laboratory experiment: Twelve pots or experimental units were prepared using 6 kg of soil (air-dried and sieved to 2 mm) collected from the top 15 cm of the two paddocks where the field experiment was conducted. Two different laboratory experiments were conducted. In the first (LE1), no additional treatments other than the paddock field treatments were applied. In the second (LE2), two additional treatments were applied in factorial combination with the two field treatments, one without and the other with added green vegetative material collected from the same field treatments. The vegetative material was oven-dried and finely ground and mixed with the corresponding soil at a rate of 20 g kg^{-1} soil, in order to test the effect of plant material addition on P dynamics. The twelve experimental units corresponded to the systems studied, savanna and introduced pasture, two treatments (with and without organic amendments) and three replications. In each pot the soil was adjusted to pF 2 moisture content (25 g g^{-1}) 5 days prior to earthworm introduction. A total of 160 adults of *M. carimaguensis* were collected in the field, and twelve individuals were placed in each pot for a 6-day period of conditioning to void field-ingested material. Afterwards, all earthworms were transferred directly to pots containing 2-kg of sieved and similarly preconditioned soil. After 1 day, these second pots were examined and earthworms were moved to another set of similar pots. This procedure was repeated six times to complete the temporal lag, i.e. 1, 4, 8, 16, 32, 64 days. Surface cast material in the pots was also sorted from the soil and placed in a Petri dish containing moistened filter papers to maintain the humidity of the samples, which were incubated at ambient temperature (24 ± 3 ºC) in the laboratory for periods of 1, 4, 8, 16, 32 and 64 days. After incubation, casts were broken up, mixed and separated into two other samples (one air dried, and the other moist) as described for the field experiment above. Control (non-ingested) soil was sampled from pots when earthworms were introduced and prepared in the same manner as the incubated casts.

Determination of soil P fractions in casts and controls: Phosphorous in soil and earthworm casts were fractionated according to a modified method of Hedley et al. using successively the following increasingly aggressive extractants: H_2O with anion exchange resin in HCO_3^- form, 0.5 M $NaHCO_3$, 0.1 M NaOH, 1 M HCl, and hot concentrated HCl. Inorganic P in extracts was determined by the molybdate-ascorbic acid method. Total P in the H_2O, $NaHCO_3$ and NaOH was measured after digestion with $K_2S_2O_8$. Total soil P was determined by perchloric acid digestion. Controls and one day-old casts were fractionated according to the full method while a reduced method using the first three extractants was applied to the remaining samples.

Inorganic P removed by anion exchange resin comes either from solution or is desorbed from the Al and Fe oxyhydroxide colloid surfaces in the soil. Sodium bicarbonate (0.5 M at pH 8.5) also extracts weakly adsorbed Pi. Together these two Pi fractions constitute a highly available Pi pool in soil. More slowly available Pi (also called "secondary Pi") is extracted by 0.5 M NaOH and is associated with amorphous and crystalline Fe and Al oxyhydroxides. Highly labile soluble organic P compounds are found in the water phase of the resin-H_2O extractant. The weakly alkaline $NaHCO_3$ extractant also removes easily hydrolyzable organic P (Po) compounds such as ribonucleic acids and glycerophosphate while the more strongly alkaline NaOH solution extracts less labile Po associated with fulvic and humic acids. Dilute HCl (0.1 M) extracts Pi from apatite or octocalcium phosphate, neither of which are likely to be present in Oxisols unless they have been fertilized with phosphate rocks. Hot concentrated HCl extracts more stable pools of Pi and Po, some of which may be associated with particulate organic matter. The P remaining in the sample (residual Pt) contains very recalcitrant Pi and Po forms that likely participate in P cycling processes only at long term.

Phosphorus in the microbial biomass and acid soil phosphatase activity were estimated on moist samples using procedures described by several authors. This procedure was used since air drying of cast and soil samples may cause loss of inorganic P from microbial biomass and give erroneous results.

Total carbon and nitrogen were analyzed on previously 2 mm sieved subsamples. A LECO CR-12 furnace with CO_2 infrared detection was used to determine total C, and the standard Kjeldahl digestion to measure total N contents. A titration method was used to extract exchangeable Al and H using 1 *M* KCl. Cations were extracted with 1 *M* NH4-acetate and determined by atomic absorption spectrometry using standard methods.

Statistical analysis: Data were log transformed prior to analysis to reduce the asymmetry of the frequency distribution. A two-way analysis of variance (ANOVA) was performed with system and cast age as the fixed main effects. The organic and inorganic P fractions extracted with H_2O/resin, bicarbonate and NaOH solutions, and for total P, total C, Bray-II P, phosphatase activity, microbial P and pH were analysed. Comparisons of means were performed by the Tukey HSD test. The software Statistica 5.1 for Windows was used in all statistical analyses.

Results and Discussion:

Total P, microbial P, phosphatase activity and other chemical properties: Total P content was significantly (MANOVA, $F = 20.25$; $p<0.001$) higher in 1-day old earthworm casts than in control soil for both laboratory incubated and field aged samples (Table 37). Total P calculated from the sum of P fractions did not differ significantly from that determined directly by perchloric acid digestion for any particular treatment (not shown). In the laboratory experiments, casts contained 10-20 mg-P kg^{-1} soil or 5-10% more total P than the soil from which they were produced. Casts produced in the field had approximately 100 mg-P kg^{-1} soil or 50% more P in native savanna, and >200 mg-P kg^{-1} soil or >100% more P in introduced pasture than the bulk soil (Table 37).

Phosphatase activity in soils and casts ranged from 120 to 313 mg-nitrophenol kg^{-1} $hour^{-1}$ in laboratory experiment samples, and from 249 to 312 mg-nitrophenol kg^{-1} $hour^{-1}$ in the *in-situ* (field collected) pasture and savanna samples, respectively. Except in *in-situ* samples from the native savanna phosphatase activity was significantly lower in casts than in the control soil (Table 37), probably due to the manipulation of soil or because enzymes were partly degraded during gut transit. In field samples, phosphatase activity was significantly higher in earthworm casts than in soil in the native savanna system but significantly lower in the intensive pasture.

Bray-II P and microbial biomass P as well as total C concentrations were significantly higher in 1-day-old casts than in the corresponding control soil for field samples while no significant differences were observed for the laboratory samples (Table 37). The pH was increased significantly in earthworm casts compared to the control soil in both native savanna and intensive pasture systems and for both field and laboratory produced samples.

P dynamics: The immediate effects of casting on the concentration of P in soil P pools in the field and laboratory are shown in Tables 38 and 39, respectively. Under field conditions, almost without exception P fractions were larger in casts than in the bulk soil (corresponding to the increase in total P content). Increases ranged from 0% (HClhc-Po) to 875% (Resin-Pi) in the egested savanna soil and 46% (Residue Pt) to 814% (Resin-Pi) in the egested pasture soil, and were relatively greater in the labile Pi fractions (resin-Pi and $NaHCO_3$-Pi). In both savanna and pasture derived casts, about 60% of the increased P content was found in Pi fractions, 30% in Po fractions and 10% in the residual Pt fraction. Most of the total P increase in cast over soil was found in secondary (NaOH) Pi and Po pools in both savanna and pasture derived casts, with significant amounts entering stable P pools as well, especially in the pasture casts.

Table 37. Chemical properties (mean ± standard deviation) of soil and one day-old casts of *M. carimaguensis* in the laboratory (LE1 experiment) and field experiment.

Experiment	pH (H_2O)	Total C (%)	Bray-II P (mg-P kg^{-1})	Phosphatase activity (mg kg^{-1} h^{-1})[§]	Microbial P (mg-P kg^{-1})	Total P[‡] ($HClO_4$ dig.) (mg kg^{-1})
Laboratory Experiment						
Native savanna						
Soil	4.6 ± 0.01	2.6± 0.8	2.6 ± 0.3	215 ± 23	4.1 ± 0.1	208 ± 2
Casts	5.2 ± 0.04	2.5 ± 0.01	2.9 ± 0.2	120 ± 18	4.1 ± 0.8	225 ± 7
B. decumbens – Kudzu pasture						
Soil	4.6 ± 0.01	2.9 ± 0.05	4.2 ± 0.3	313 ± 29	6.0 ± 0.9	248 ± 1
Casts	5.2 ± 0.1	3.0 ± 0.06	4.1± 1.1	242 ± 12	5.4 ± 0.4	272 ± 1
Field Experiment						
Native savanna						
Soil	5.1 ± 0.1	2.1 ± 0.1	1.0 ± 0.2	254 ± 15	2.5 ± 0.3	179 ± 5
Casts	5.4 ± 0.1	4.1 ± 0.1	6.3 ± 0.6	312 ± 7	4.0 ± 0.6	267 ± 15
B. decumbens – Kudzu pasture						
Soil	5.2 ± 0.1	2.1 ± 0.1	2.5 ± 0.6	299 ± 14	4.1 ± 1.3	194 ± 5
Casts	5.8 ± 0.1	5.2 ± 0.3	11.0 ± 2.3	249 ± 12	10.9 ± 1.5	396 ± 69

[§] mg ρ-nitrophenol kg^{-1} h^{-1}

[‡] from laboratory experiment 2 (LE2)

Under laboratory conditions where the increase in total P content due to casting (11-17%) was much smaller than in the field, increases in the sizes of P fractions ranged from 0% to 344% (Table 39). In the savanna soil casts, most of the added P was found in the secondary P pools (20% NaOH-Pi and 64% NaOH-Po) whereas substantial amounts were also found in the stable P and residual Pt pools in the pasture soil casts. The addition of green material residues had no significant effect on either Pi or Po fractions in the laboratory experiment, neither in the soil nor in the casts.

The dynamics of labile pools of P in ageing casts is shown in Figures 20 and 21 for laboratory and field incubation experiments, respectively. Pi extracted by resin was increased strongly in fresh casts but then slowly declined to the levels in soil during the following 64 days of incubation. In contrast, organic P (Po) extracted by bicarbonate and hydroxide increased during 1 to 8 days after casting, rather than during transit of the earthworm gut, and then remained relatively constant. Afterwards these Po-fractions remained relatively constant or declined slightly during the remaining 56 days of incubation. Inorganic P in bicarbonate and hydroxide was not affected significantly by casting and did not change significantly with time of incubation.

Similar patterns in P dynamics in Pi and Po fractions were observed during *in situ* ageing of *M. carimaguensis* casts produced in the field although of much lower magnitude, especially for Po fractions. The most marked changes occurred during transit of the earthworm gut whereas, after the initial increase, Pi and Po in all fractions remained relatively constant during the 64 days of field incubation.

Table 38. Phosphorus fractions in soil and fresh (1-day-old) casts of *M.. carimaguensis* collected in the field from the native savanna and the intensive pasture[1].

		H_2O-Po	Resin-Pi	$NaHCO_3$		NaOH		1*M* HCl-Pi	HCl hc		Residue-Pt	Total Pi	Total Po	Total P
				Pi	Po	Pi	Po		Pi	Po				
		mg P kg^{-1} soil												
Savanna	Soil	0.5 b	0.8 b	1.6 c	8.6 b	22 bc	42 ac	0.3 b	38 c	22 ac	59 b	63	73 b	195 c
	Cast	1.4 a	7.8 a	9.9 b	17.0 a	52 ac	68 a	0.9 b	52 b	21 ac	68 b	123	108 a	299 b
	%increase	180	875	519	98	137	64	200	36	-5	15	95	47	53
	%P added[2]	1	7	8	8	29	25	1	13	-1	9	58	33	100
B. decumbens + Kudzu	Soil	0.8 b	1.4 b	2.8 c	9.1 b	26 b	43 bc	0.8 b	45 bc	10 bc	60 b	76	62 b	199 c
	Cast	2.0 a	12.8 a	19.0 a	18.8 a	82 a	82 a	5.6 a	83 a	32 a	88 a	202	136 a	425 a
	%increase	150	814	579	107	213	92	600	83	234	46	165	117	114
	%P added	1	5	7	4	25	17	2	17	10	12	56	32	100

[1] values within a column followed by te same letter do not differ significantly ($p<0.05$) according to Tukey's HSD test.
2 percentage of total P increase in cast over soil found in respective fraction,

Table 39. Phosphorus fractions in soil and fresh (1-day-old) casts of *M carimaguensis* produced in the laboratory (LE1) from soil collected in the native savanna and the intensive pasture

		H_2O-Po	Resin-Pi	$NaHCO_3$		NaOH		1*M* HCl-Pi	HCl hc		Residue-Pt	Total Pi	Total Po	Total P
				Pi	Po	Pi	Po		Pi	Po				
		mg P kg^{-1} soil												
Savanna	Soil	1.6 ab	0.9 d	4.1 b	1.8	25 d	32 c	0.3	41 b	16	43	71	51 bc	165 b
	Cast	1.9 b	4.0 b	4.3 b	2.4	30 c	50 b	0.4	42 b	17	42	81	71 ac	193 bc
	%increase	19	344	5	33	23	57	33	2	6	-3	14	39	17
	%P added[2]	1	11	1	2	20	64	0	2	3	-5	35	70	100
B. decumbens + Kudzu	Soil	1.7 ab	2.8 c	6.5 a	1.0	34 b	54 a	0.2	51 a	22	46	94	78 a	218 ac
	Cast	2.5 a	6.3 a	6.5 a	1.4	39 a	57 a	0.7	55 a	20	55	108	80 a	243 a
	%increase	47	125	0	40	14	5	250	9	-12	20	14	2	11
	%P added	3	15	0	2	20	11	2	20	-11	38	57	5	100

[1] values within a column followed by the same letter (or no letter) do not differ significantly ($p<0.05$) according to Tukey's HSD test.
2 percentage of total P increase in cast over soil found in respective fraction.

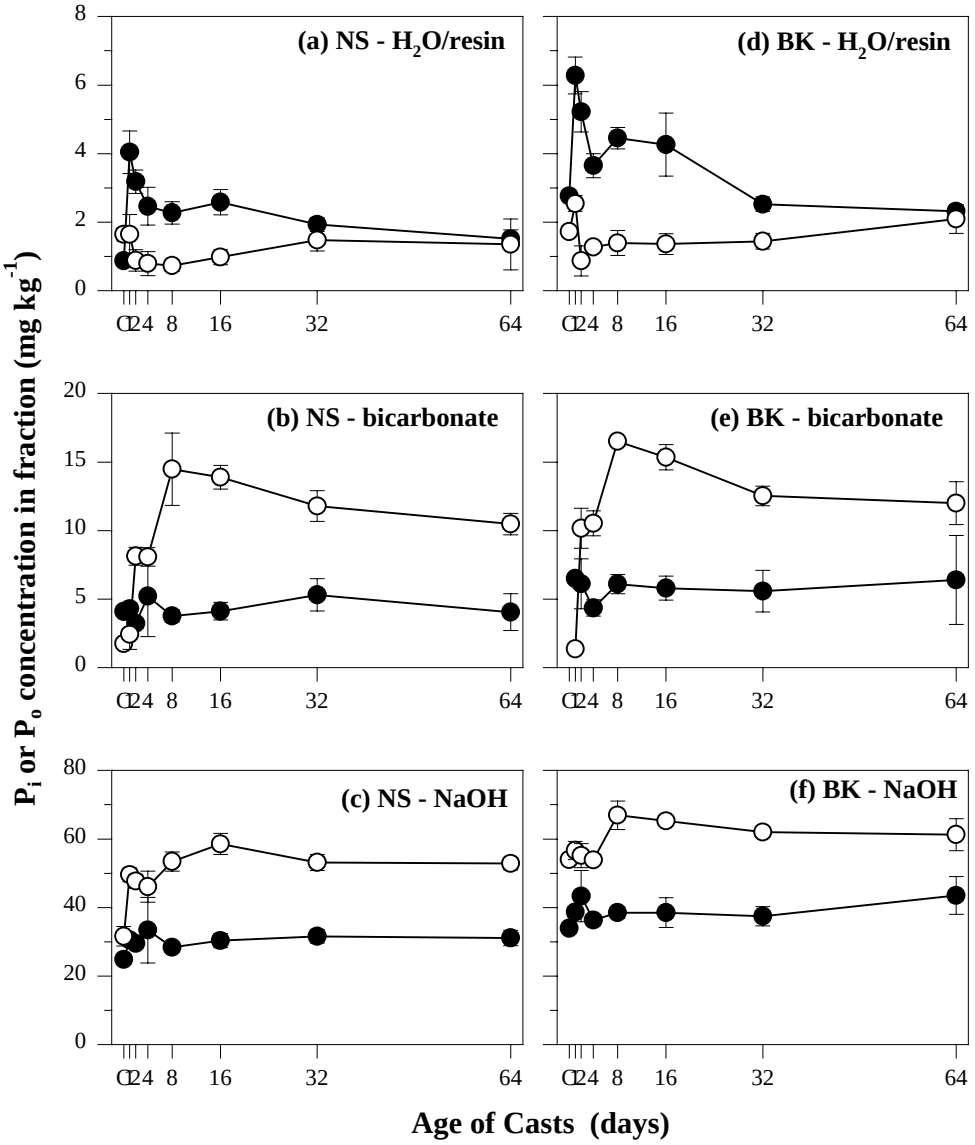

Figure 20. Dynamics of Pi and Po fractions in casts of *M. carimaguensis* produced and incubated in the laboratory (LE1 experiment). *C* Control soil (non-ingested) Bars indicate standard deviation. NS Native savanna, BK *B. decumbens* + Kudzu pasture.

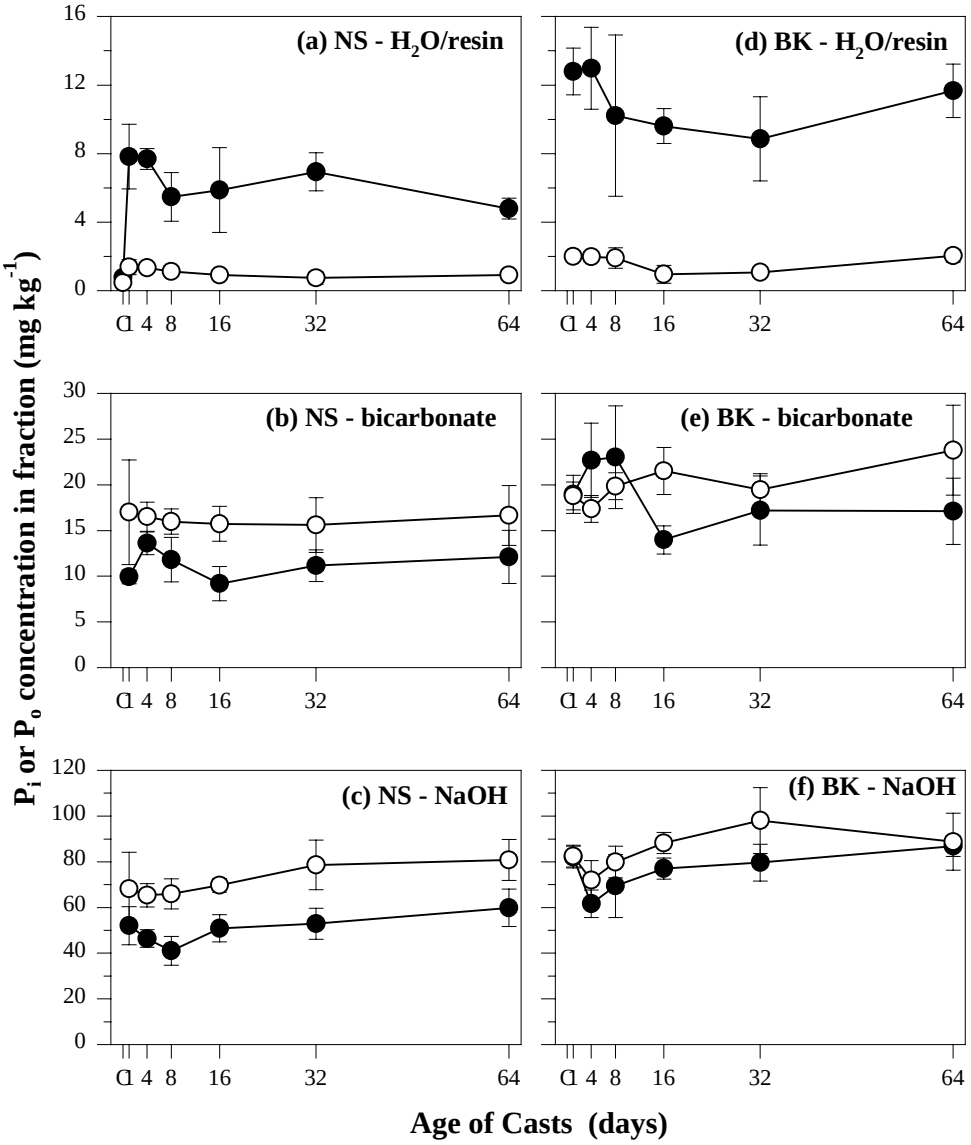

Figure 21. Dynamics of Pi and Po fractions in *in-situ* ageing casts of *M. carimaguensis*. *C* Control soil (non-ingested). Bars indicate standard deviation. NS Native savanna, BK *B. decumbens* + Kudzu pasture.

The use of both field and laboratory experiments in this study helps to better understand the actual dynamics of nutrients in soil and in biogenic structures produced by soil macroinvertebrates. Although the total P contents of control soils in the field and laboratory experiments were similar, ingestion by *M. carimaguensis* increased total P by 53% to 114% under field conditions but by <20% under laboratory conditions. Under field conditions, *M. carimaguensis* apparently incorporated P from sources such as litter, undecomposed plant debris and roots and other holorganic casts that were not present in the soil

under laboratory conditions. Together with mixing of soil and litter, both coprophagy and necro-rhizophagy seem to be the dominant feature of the *M. carimaguensis* diet.

Phosphorus incorporated into soil from "non-soil" sources may enter into all P fractions (organic and inorganic) but the fractionation data indicate that a greater proportion entered into labile Pi fractions, particularly under field conditions (Tables 38 and 39). It is not possible to conclude that *M. carimaguensis* promoted the mineralization of organic P since the proportion of labile P in the substrate may have been greater than that of stable P forms. However, the fact that under laboratory conditions, where organic litter substrate was not provided (LE1) and where the increase in total P due to casting was quite small, relatively large increases especially in the secondary (NaOH-Pi and -Po) pool sizes accompanied by decreases in stable fractions suggests that ingestion of the soil by the earthworm did promote movement of P from stable to more labile P forms. Moreover, the relatively large increases observed in resin-Pi in the laboratory experiment, and in all labile fractions (H_2O-Po, resin-Pi, bic-Pi and bic-Po) in the field experiment (all small pools), suggests some mineralization of less available P from large more stable pools where relative changes would be difficult to detect. This would agree with the interpretation that *M. carimaguensis* likely acts as an endogeic (soil consumer) rather than an anecic (soil + litter consumer) species in terms of feeding regimes.

Although beginning with almost identical total soil P contents, casts produced *in situ* in the *B. decumbens* and *P. phaseoloides* pasture had more than twice the total P content and correspondingly higher P content in all fractions than casts produced from savanna soil (Table 38). This was probably due to higher biomass production, both aboveground and roots, of legume and deep-rooting grasses in the pasture resulting in greater litter fall and root turnover as well as greater dung deposition from greater stocking of cattle. Our study confirmed the results obtained by several authors who reported higher P contents in earthworm casts than in the surrounding soil from grassland ecosystems. Phosphorus contents were at least 30% higher in casts of several earthworm species than in the soil. In our study, water extractable P was 300% higher in casts than in the adjacent control soil under field conditions. Stabilization of P in casts of *M. carimaguensis* occurred between 16 and 64 days after cast deposition, whilst this lag was of 4 days in casts of the endogeic *P. corethrurus*.

In permanent pastures from a New Zealand watershed it was estimated that the total amount of organic-P was 14 kg ha^{-1} yr^{-1} and 11 kg ha^{-1} yr^{-1} accumulated in 30 tons ha^{-1} yr^{-1} of surface earthworm casts. Estimated surface cast production by *M. carimaguensis* at the study site was 1.2 and 13.2 t ha^{-1} yr^{-1} in the savanna and in the pasture, respectively, based on a density of 0.2 and 2.2 fresh casts m^{-2} and considering the active period of the species, at least 4 months. The average dry weight of casts ranges from 25 to 35 g.

Thus, 0.36 kg ha^{-1} yr^{-1} and 5.61 kg ha^{-1} yr^{-1} of total P may be accumulated in fresh casts of *M. carimaguensis*, respectively, in the savanna and the pasture (0.13 and 1.8 kg ha^{-1} yr^{-1} of total Po). This represents a fair contribution to the overall P fluxes in these agroecosystems. For example, the total P uptake in above-ground biomass of a grass-legume pasture (*B. humidicola* plus several legumes), a maize monocrop and the native savanna was found to be 14, 18 and 4 kg P ha^{-1} at the same site. We may say then that, in the case of pasture, total P accumulated in casts is ≈ 40% of the total annual P uptake by grasses, whereas in the case of the savanna it is equivalent to only ≈ 9% of the total P uptake in above-ground biomass. Nonetheless, the global contribution to availability of P for plants may be even greater due to the presence of other earthworm species in the soil and the casts deposited in the soil. Thus, our results confirm that earthworm activity at Carimagua had a significant effect on P availability.

Measurements of phosphatase activity and microbial P on field experiment samples further indicate that *M. carimaguensis* participates in the mineralization of available Po fractions. In the field experiments, phosphatase activity was greater in *M. carimaguensis* casts than in the control soil from the savanna. The contrary observation seen in the laboratory experiment was probably an artifact of the methodology since

the organic residues added to earthworm cultured pots could have already mineralized before ingestion by the earthworms. The high values of phosphatase activity obtained in our study for all treatments show the importance of both biological and biochemical processes in Po mineralization, although these are not always markedly detected, for example, in the case of termite mounds and surrounding soil where no significant differences were observed in the Venezuelan Llanos. A strong enzymatic activity has been reported in fresh earthworm casts from temperate regions as well as in tropical sites.

Microbial activity is enhanced in casts of tropical earthworms due to strong enzymatic activity and available organic C. In this study values of microbial P were similar in both soil and cast samples from the lab experiment, and slightly higher in samples from the pasture. In summary, therefore, the increase in total P content of casts over soils in the field can be explained by organic matter, litter (including roots) and cast selection by *M. carimaguensis* while the comparative increases in labile fractions extracted with water, $NaHCO_3$ and NaOH are due to the reorganization or translocation of P from stable to available pools for plant uptake. Evidence for the latter is found in the increased enzymatic activity (phosphatase and microbial) in casts.

"Ecosystem engineers" and P dynamics in savanna soils: Phosphorous is a limiting nutrient in tropical savannas, especially if aluminum saturation is high since P is immobilized in highly stable Al based compounds. Studies on the role that ecosystem engineers play in P dynamics in Neotropical savannas are very scarce. The activity of soil ecosystem engineers, which include ants, termites and earthworms, influence and control both the amount and distribution of P in savanna soils by construction of nests and galleries. As an example, both the quantity and distribution of P in the savannas of Venezuela depends upon the biological activity of the termite *Nasutitermes ephratae* (Holmgren) (Nasutitermitidae). Termites feeding on plant debris have been shown in other studies to have little or no impact on available P, but soil feeding termites, i.e. humivorous, can increase available P in their nests two or five-fold. This is the result of the alkaline conditions in the anterior hindgut of most humus-eating termites. The availability of mineral P derived from litter or soil can be increased by the activity of earthworms as we have shown in our field study. But P availability also changes with time as casts aged in the field. Similar results have been reported where P retention declined in the biogenic structures produced by the African savanna termite *Cubitermes oculatus*.

There is still a lack of studies dealing with the interactive role that ecosystem engineers play in nutrient cycling both within and among major taxa. For example, casts of *M. carimaguensis* are large resistant structures that persist at the soil surface from two to eleven months on average in intensive pastures (protected or exposed to cattle trampling, respectively), and 5 months in native savannas. Termite species *Armitermes* sp. and *Velocitermes* sp. (Nasutitermitinae) colonize these compact casts and creates channels and deposits faecal pellets at the cast surface. When cast finally splits into smaller aggregates, the nutrients that were preserved from further mineralization processes in these casts are release. Thus these surface casts represent a significant source of direct P easily available for plant uptake, and possibly also explains the increase in root biomass observed under earthworm casts. Further studies should consider the effect on P dynamics of other soil ecosystem engineers' biogenic structures that are also abundant in the savannas of the Colombian Orinoco basin, for example, *Microcerotermes* sp., *Spinitermes* sp. (Termitidae) and *Velocitermes* sp. (Nasutitermitinae) species and the ants *Atta laevigata* and *Acromyrmex landolti*.

Only when the total volume of soil processed by earthworm species including *M. carimaguensis* in the Carimagua savannas is considered can their engineering feat be truly appreciated. Based on an ingestion rate of nearly 20 g dry $soil^{-1}$ day^{-1} by *M. carimaguensis* under laboratory conditions we estimated that the entire first 10 cm of the topsoil passes through the gut of the entire population of earthworms in only 6 years.

Impact:

The importance of *M. carimaguensis* activity in natural and introduced pastures on incorporating P from organic sources into soil P pools, in increasing the labile P pools and improving P cycling was

demonstrated in our short-term studies under laboratory and field conditions. The ecological significance of earthworms in P cycling in the native savanna and the introduced pasture is based on the improved nutritional basis of plant litter from the pasture. There is an enhancement of biotic processes in the pasture, since populations of this species are quite abundant and there is also higher microbial biomass P. The long-term effects of earthworms on P cycling and other nutrients must therefore be tremendous and merit further investigation. The net benefits of earthworms involved in soil quality improvement and soil fertility are still ignored.

Contributors:
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Activity 2.3 Develop appropriate and diverse strategies for controlling soil erosion

- Results of scaling out sugarcane double-purpose live barrier technologies developed in Colombian hillsides to Honduran hillsides will be reported next year. The agronomic information will be complemented with evaluation of the economic contribution of sugarcane live barriers under traditional management to hillside farmers in Honduras and also agroenterprise approaches to make sugarcane live barriers profitable in Cauca, Colombia.

Activity 2.4 Develop strategies to maximize C sequestration in soils and minimize emissions of greenhouse gases

- Results from one PhD thesis in the Colombian savannas in collaboration with Ohio State University and results from collaborative work with Cornell University in the Brazilian Amazon will be reported next year.

Activity 2.5 Characterize soil biodiversity and develop strategies to manage beneficial soil biological processes

2.5.1 The impact of soil organisms on plant diversity and abundance. A case study of a native anecic earthworm species

Highlight:
- Observed direct preference by *M. carimaguensis* for seeds from the soil seed bank and found considerable loss of viability of seeds in earthworm casts.

Purpose:
To describe the main biological and ecological effects of a native earthworm species, i.e. *Martiodrilus carimaguensis* (Oligochaeta, Glossoscolecidae) on permanent soil seed banks in a natural savanna and several introduced pastures from the Eastern Plains of Colombia.

Rationale:
The substitution of the native savanna by introduced grasses from Africa has been a widely accepted practice in the Colombian "Llanos" and in the Brazilian savannas or "Cerrados" during the last 20 years in order to increase the proportion of land used for more intensive food production. Improved pastures based on introduced grasses from Africa, principally *Brachiaria* spp., and *Panicum* spp., with or without forage

legumes, e.g. *Stylosanthes* sp., *Arachis* sp. and *Pueraria* sp., and annual crops with inputs of fertilizers and lime, mainly upland rice and soybeans, are land management options normally found in these areas. In addition, we know little about biological processes involved in these land use changes and how these can alter fundamental ecosystem processes and services. This is especially relevant when it is evident, from studies carried out in the Brazilian "Cerrados", that biodiversity of the neotropical savannas is more threatened than that of the Amazon forest.

The study of diversity and ecological processes linked to ecosystem functioning constitute the basis for an understanding and better management of natural and derived ecosystems. Communities of soil fauna are, in general, sensitive to climatic and edaphic factors that in turn determine the availability of food resources and microclimatic conditions. Besides, human-induced disturbance of natural ecosystems will alter the macroinvertebrate communities in the soil.

Earthworms are the most abundant group of macrofauna in the soil in terms of biomass and they generally participate in the regulation of important soil processes through their functional domains. The effects of earthworms on soil physical properties, nutrient dynamics, organic matter and plant growth have been studied in detail. However more efforts are needed to evaluate the role of the biogenic structures produced by theses organisms in soil functioning and ecological processes.

The substitution of the natural ecosystem (native savanna) by introduced pastures influences the functional structure of the earthworm communities although species richness is the same. *Andropogon gayanus* and *Brachiaria decumbens*, alone or in association with the forage legume *Pueraria phaseoloides*, *Brachiaria humidicola*, alone or in association with *Arachis pintoi* or other legumes are systems that conserved the native macrofauna at Carimagua. These results are exceptional as disturbances to natural systems result generally in a decrease or disappearance of the native species. Normally, pantropical species with a wide range of tolerance to physico-chemical properties (peregrine species) such as *Pontoscolex corethrurus* Müller and *Polypheretima elongata* Perrier become predominant. Of the many life forms that inhabit soils only a small number of macroinvertebrates (earthworms, termites and ants) are capable to plow soil and produce a large variety of organo-mineral structures, e.g. nests, mounds, macropores, galleries and excretions. These organisms are named "ecological or ecosystem engineers" of the soil and their structures have been described as "biogenic structures".

In agreement with the high species richness of the savanna a high functional diversity exists in Carimagua, in terms of different biogenic structures. The soil ecological engineers from Carimagua produce fourteen types of these structures on the soil surface, i.e. 4 termite structures, 8 types of ant nests and 2 types of earthworm casts. It is on one of these biogenic structures and the species that produce them, the anecic *Martiodrilus* sp. (Glossocolecidae, Oligochaeta) where the research presented here has been focused on. These surface casts are formed by a continuous deposition of egested material at the opening of the burrow during several days. The final structure is a towerlike cast with a fresh pasty structure in the top and dry material at its base easily recognisable from other soil surface casts. The term towerlike was prefered although Madge originally created it to describe casts of *Hyperiodrilus africanus*. When the earthworm *Martiodrilus* sp. leaves its semi-permanent U-shaped burrow or when it sinks to start diapause the cast dries completely. Dry casts remain at the soil surface for more than one year after having been egested, and the disappearance of casts is mostly attributed to rainfall drop impacts and to cattle trampling in the grazed pastures.

The functional role of these structures is believed to be of utmost importance and represent sites where certain pedological processes occur. These include the stimulation of microbial activity, the formation of soil structure, the dynamics of soil organic matter or the exchange of water and gases. In this study our objective was to assess some ecological processes at the scale of the biogenic structure and relate them

with the living habits of the species. The effects on permanent soil seed banks in surface casts of *Martiodrilus* sp. were the main objective addressed in this study.

Materials and Methods:

Study site: The study was carried out at the "Centro Nacional de Investigaciones" Carimagua (CIAT-CORPOICA agreement), in the well-drained isohyperthermic savannas of the Eastern Plains of Colombia (4° 37' N and 71° 19' W, 175 metres altitude). Climate is subhumid tropical with a four-month dry period from December to March and an average yearly rainfall and temperature of 2280 mm and 26°C, respectively. Vegetation is characterised by open herbaceous savannas with scattered trees and bushes in the uplands ("altos") and gallery forests and palm trees ("morichales") in the low-lying savannas ("bajos"). Burning is used to get rid of excess vegetation and to stimulate the re-growth of those more nutritional plant material. Soils at the study site are Oxisols (Tropeptic Haplustox Isohyperthermic) in the uplands and Ultisols (Ultic Aeric Plintaquox) in the lowlands (USDA). These soils are characterized by their acidity (pH 4.5, water), a high Al saturation (> 80%) and low concentrations of exchangeable Ca, Mg and K. Phosphorous is a limiting nutrient for plants as it remains fixed by Al in stable compounds, what reduces the productivity of these acid soils.

All the research was done on the same upland edaphic unit (pedon Carimagua) where different plots were located: A native savanna without grazing nor burning where *Andropogon bicornis* and *Gymnopogon* sp. were the predominant plant species, and a grazed pasture, in a two ha plot, that combines an exotic African grass, *B. decumbens* cv. Basilisk, and a tropical herbaceous legume, *P. phaseoloides* CIAT 9900 ("Kudzu"). Cattle stocking rates were 1 animal unit (AU) ha^{-1} in the dry season and 2 AU ha^{-1}in the wet season (1 AU = 250 kg live weight).

Earthworm population sampling: With the objective to assess the biology, ecology and population dynamics of species across time a stratified sampling procedure was performed during 17 months. Physical methods of extraction were applied to collect earthworms. These are often very tedious, hard working and time-consuming. From April 1994 to September 1995 earthworms were hand-sorted from five 1 m^2 soil monoliths monthly. A trench was dug around the monolith to avoid earthworm escaping and ease the separation of 10 cm sucessive layers. Normally, five soil layers were revised, till 50 cm. However, as some species migrate deeper into the soil, e.g. *Martiodrilus* sp., sampling depth varied accordingly. Vertical distribution was not much affected by sampling since a positive correlation between the number of fresh casts in the soil surface and the number of earthworms in the topsoil exists. Soil blocks were carefully hand revised and all earthworms and cocoons collected. Whether any earthworm was in dormant stage, i.e., quiescence or diapause, or not is annotated.

All earthworms were carried to the laboratory where complete specimens were weighed and the maximum preclitelar diameter measured in fragmented worms, since it has been very useful to estimate the weight of the complete earthworm. Biomass is based on formalin preservation, 12% lower than fresh weight on average for *Martiodrilus* sp.

Sampling of biogenic structures: Cast and soil seed banks: Three experimental plots were selected to compare native savanna with different pastoral systems under study at the research site:
(a) A *Paspalum pectinatum* Nees, *Axonopus purpusii* (Mez.) Chase, and *Trachypogon vestitus* Anders. native savanna, protected from grazing for four years and traditionally managed by fire during each dry season.
(b) An 18 year-old associated pasture of *B. decumbens* Stapf. and *P. phaseoloides* Benth. (pasture A), grazed by cattle and maintained with a stocking rate of 1.0 animal unit. ha^{-1} during the dry season and 2.0 animal units. ha^{-1} during the wet season.

(c) A 3 year-old associated pasture of *B. humidicola* (Rendle), *A. pintoi* Krap & Greg, *S. capitata* Vog. and *C. acutifolium* Benth. (pasture B), grazed by cattle with an average stocking rate of 2.0 animals units. ha^{-1}.

Fieldwork was conducted in August 1996 and August 1999, i.e. at the middle of the rainy period. Our idea was to take samples from the permanent soil seed bank, assuming that most of the temporary seed banks had already germinated at the onset of the rainy season. Twenty soil cores (8 cm diametre and 6 cm depth) were taken in each experimental plot and 300 fresh casts were randomly collected. Both soil and casts were air-dried for 15 days and weighed.

Each sample was put on a 2-cm layer of river sand in a plastic seed tray (26x27x6 cm). Soil layers were less than 5 mm in order to allow the germination of the larger part of the seeds as recommended by some authors. Forty trays were used for each plot (20 for each type of sample, i.e. soil and casts) and 10 trays were filled up with sand alone and used as control. Germination trays were located randomly in a greenhouse, kept moistened and exposed to natural light and temperature regimes (approximately 12h/12h darkness/light and 26 °C, respectively). Once a week, the emerging seedlings were identified at the species level and removed. After three months, casts were broken in small fragments (< 5 mm of diameter) before being placed in the trays to continue the evaluation. For each sample, 40 g of dry soil or casts where randomly taken at the beginning of the experiment. Each sample was soaked for a minimum of 30 minutes in a solution of sodium hexametaphosphate (50 g. l^{-1}) and sodium bicarbonate (25 g. l^{-1}). Afterwards, the suspension was poured through a 0.125-mm sieve. Organic debris were washed with a fine spray of water and oven-dried for 48 hours at 70°C. Both damaged and undamaged seeds (i.e. seeds which visually seemed to be intact and resisted gentle pressure) were separated under a stereo-microscope and counted.

Statistics: Simple linear regression analysis (Pearson *r*) was used to study the correlation among variables. Analysis of variance (ANOVA) was employed to test for significant differences between means, and a non-parametric Kruskal Wallis ANOVA was used to compare variables under the assumption of distinct sample size. Asymmetry of data frequency distribution was reduced by Box-Cox transformation with the software Vernorm.

Results and Discussion:

Species ecology: Martiodrilus sp. is an endemic anecic earthworm from Carimagua. It is a large size, dorsally dark-grey pigmented, and surface-casting species. Surface casts of *Martiodrilus* sp. are large and ranged from 3 to 6 cm Ø and from 2 to 10 cm height, with an average dry weight of 25 g (Picture 3). Average monthly fresh cast and total cast numbers were significantly higher ($P < 0.01$, t-test) in the improved pasture than in the native savanna. The number of surface fresh casts declined during the middle of the rainy period due to the fact all the juveniles have already descended to deeper layers to enter diapause. A strong positive correlation between the number of fresh casts and the density of individuals in the first 10 cm was observed in the improved pasture ($r = 0.907$; $P < 0.01$) and it has been proved to be a good estimator of population density.

The highest population density was recorded at the beginning of the rainy season and the new juveniles hatched in October 1994 increase the total number of individuals, despite a reduction in theses numbers due to severe seasonality occurs. Earthworm biomass ranged from 0.24 g m^{-2} (March 1994) to 8.76 g m^{-2} (September 1995), and from 26.5 g m^{-2} (January 1995) to 94.8 g m^{-2} (May 1994), in the native savanna and in the improved pasture, respectively. In the native ecosystem *Martiodrilus* sp. comprised 15.1% of total earthworm biomass, while this value rose to 85.1% in the improved pasture, the highest ever recorded to date for an anecic species. Population density remained stable during the dry season months, therefore it is assumed that mortality occurs during the rainy period and that other factors seem to be responsible for it

Picture 3. Turricule of *Martiodrilus* sp. in the natural savanna from Carimagua (scale: length of picture = 20 cm).

The extremely effective adaptive strategy of *Martiodrilus* sp. is the reason why there is negligible risk of mortality during the dry season. The species has a true diapause, although different patterns were found between adults and juveniles. The latter were only active during the four months following the onset of the rainy period and entered diapause much earlier than adults, which remained active until December. The cease of activity occurred after they sank to deeper soil layers. An aestivating chamber is built, at the end of its semi-permanent burrow, where they coiled themselves up, after emptying their gut contents. The end of the burrow was usually sealed with several septae to avoid loss of tegumental moisture, which is vital to support a minimal rate of respiration (Picture 4). They remained still until the onset of the next rainy season.

Picture 4. Pattern of diapause in *Martiodrilus* sp. with the aestivation chamber at the end of the burrow (dotted line) and the septae (arrows) built with cast material.

A diapause process, physiologically induced, was assumed to be occurring because in controlled conditions there was no response when aestivating earthworms were introduced into soil with water content near field capacity. Aestivating juvenile earthworms were normally found in the 40- to 50-cm layer, whilst both adults and subadults were located in the 50- to 60-cm layer. A significant non-linear correlation was found between diapausing earthworms weight and the depth at which they were found

(Kruskal-Wallis ANOVA, $P = 0.011$). The larger the individual is the deeper it aestivates (Figure 21). The average weight of diapausing earthworms ranged from 1 to 2.5 g (4 g maximum), quite low when compared with fresh adult weight, ca. 11.2 g. This is the result of the earthworms emptying their guts to coil themselves up in their aestivating chambers.

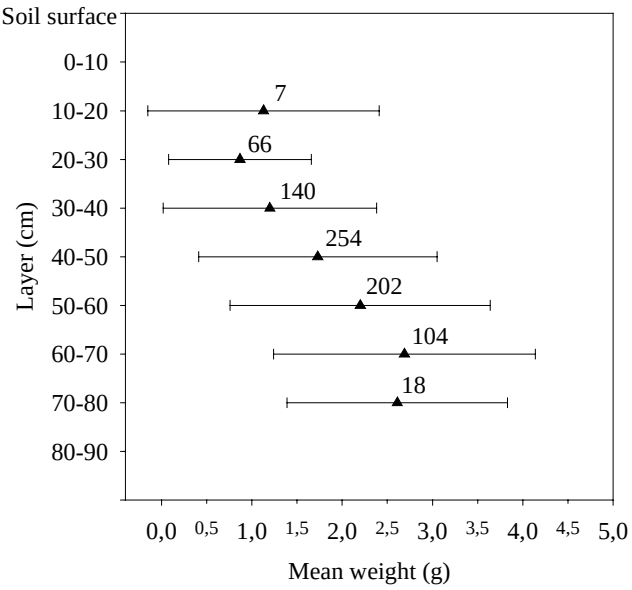

Figure 22. Relationship between the vertical distribution in the soil profile of aestivating individuals of *Martiodrilus* sp. and their weight.

Owing to the life cycle of the species, it is a clear K-strategist with a relatively long life period. New juveniles emerged from cocoons just before the end of the rainy period rapidly sank towards the deeper horizons to inititiate diapause. In the next year they will become active at the onset of the rainy season and be so during the following 3-4 months (July-August), entering again in a new diapause phase (the weight of these juveniles is 4 g maximum) until the next wet season. At the beginning of the following rainy period (second year) these worms will be active until the middle or the end of the humid season, depending on the developmental stage. In the next year (3rd) these worms become adults and two possibilities must be considered here: i) these new adults will not reproduce until the next year (4th), as some inactive adults were found when no cocoon was collected (see below), or ii) in some years of unusual lower rainfall all reproduction will be initiate by most adults. Adults that constitute, in a given period, the population of *Martiodrilus* sp., seemed not to actively participate in the reproduction, as the number of adults was double-fold than cocoons. We actually ignore if these adults participated during the reproductive period, but it seems improbable since no cocoons at the onset of the wet season were found.

Effect of Martiodrilus sp. on plant diversity at the scale of biogenic structures:

- *Cast seed banks*: Martiodrilus sp. seemed to ingest preferentially large quantities of seeds as part of its diet from 58 to 163 seeds 100 g^{-1} of ingested soil. The percentage of germination of seeds is 3 to 40 times lower in earthworm casts than in soil, probably as a consequence of damages suffered by ingested seeds during gut transit.

In all systems seedlings were observed in casts and only 41% of seeds emerged from casts, and the remaining 59% needed cast destruction to germinate (data not presented). In addition, the number of

viable seeds within casts were negligible in comparison to soil seed bank and it only accounted for 0.2-0.6% of the total (Figure 23). However, the total number of seeds collected per 100 g sample was normally higher in casts than in the soil, and the number of viable seeds was greater in the intensive pastures than in the native savanna, and in all systems it was higher in the soil compared with the casts.

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Differences were found in the species composition of soil seed banks between both pastures and the savanna, and it was certainly similar in the two pastures. The species composition of the soil seed banks was dissimilar to the above ground vegetation, where dominant species did not germinate in greenhouse experiment and on the other hand, the most abundant species of soil seedlings were rare in the plant community. The cast seed bank was relatively closer to that of the standing vegetation than soil seed banks in the native savanna, whilst the opposite pattern was found in *B. decumbens* and *P. phaseoloides* pasture.

The number of viable seeds egested in *Martiodrilus* sp. surface casts is up to 8.7 million seeds. ha^{-1}. yr^{-1}, and from 18 to 878 viable seeds. m^{-2}. yr^{-1} can be egested in surface casts, representing from 1% to 13% of the total viable soil seed bank. From 64 to 97% of seed viability was lost in earthworm casts, probably due to damages suffered by seed during gut transit, as suggested by the higher percentage of damaged seeds observed in casts.

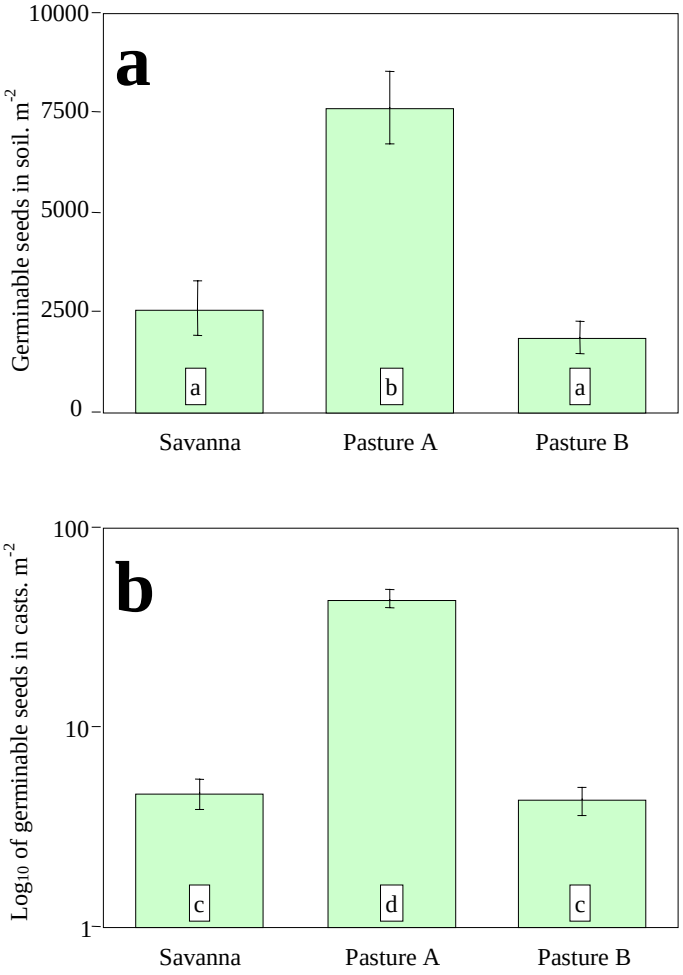

Figure 23. Density of germinable seeds contained in soil (a) and casts (b) in the three systems. Bars indicate standard errors; letters mean differences at $P < 0.05$ (t test).

Casts of *Martiodrilus* sp. are sometimes large and reach up to 15 cm height and 400 g weight. Casts do not dry completely till the earthworm abandons its burrow and the half-life of casts ranges between one and three weeks in native and non-native pastures, respectively. Afterwards, casts are progressively broken until they completely disappear, sometimes by water run-off after a big storm or incorporation into the soil profile.

The results of the present study confirm the complex role played by earthworms in the process of seed bank formation and contribution to the above ground vegetation. In such systems, the number of viable seeds egested each year in the surface casts of *Martiodrilus* sp. is important (up to 8.7 million seeds. ha^{-1}. yr^{-1}), compared with results obtained with *Lumbricus terrestris* L. in temperate pastures (890 thousand seeds. ha^{-1}. yr^{-1} on average). From 18 to 878 viable seeds. m^{-2}. yr^{-1} can be egested in surface casts, representing from 1% to 13% of the total viable soil seed bank.

In our study from 64 to 97% of seed viability is lost in earthworm casts, probably due to damages suffered by seed during gut transit, as suggested by the higher percentage of damaged seeds observed in casts. Earthworm burying activity of seeds may have important consequences for plant community dynamics as seeds of many species survive better buried than when left at the soil surface, and there are few other natural mechanisms that explain how seeds are buried in soil. Moreover, earthworm faeces may protect seeds from early germination processes and participate in the formation of important and persistent seed banks.

In the savanna protected from fire, casts sometimes remain at the soil surface for more than one year, while burning of the savanna suddenly leads to their destruction by depriving them of the protective effect of the vegetation and litter cover. This may lead to the formation of a pool of seeds potentially ready to germinate, that may be indirectly dispersed by fire on the nearby soil surface where they further benefit from suitable conditions to germinate (*i.e.* bare ground, higher light intensity, available nutrients in ashes). Important chemical constraints on seedling emergence and survival (e.g. low nutrient content, high aluminum saturation) may also be alleviated in casts. Therefore, seeds in casts, egested at the soil surface may be more likely to germinate than in the soil seed bank. This can explain why the composition of the cast seed bank was closer to that of the standing savanna vegetation than that of the soil seed bank. Further research should focus on the effects of earthworm activities in above standing vegetation at larger spatial and temporal scales.

Impact:

Martiodrilus sp. is an endemic earthworm from the natural savannas of Carimagua (Colombia) well adapted to introduced pastures, where it increases its abundance significantly and for instance its effects on some soil processes. The adaptive strategies of earthworms are diverse and are the result of living in a complex environment and facing limiting factors such as poor nutrient contents, movement in a compact environment and sometimes a strong seasonality. This species maintains quite abundant populations in the introduced pastures and its adaptive strategy is extremely efficient to maintain an almost constant density through the dry season. Earthworm casts could be considered as an important regeneration niche for the plant community, and earthworm activity could be an indispensable factor of ecosystem sustainability and diversity.

Contributors

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2.5.2 Properties of the structures created by ecosystem engineers on the soil surface of a Colombian savanna

***Highlight*:**

- Showed that biogenic physical structures produced by soil macrofauna on the soil surface can be related to their ecological function in the soil.

Purpose:

To assess the diversity, abundance and physical (aggregate size and stability, and bulk density) and chemical (C, N, and P contents, and pH) properties of the biogenic structures and establish a functional classification for the savannas of the Eastern Plains of Colombia

Rationale:

"Ecosystem engineers" or "ecological engineers" are those organisms that can physically structure the environment in which they live by producing "biogenic" structures or artefacts. Through these structures the engineer organisms can modulate the availability or accessibility of one or more resources used by other organisms. Their activities, including the building of biogenic structures, are therefore capable of modifying the abundance and/or community structure of populations of other organisms without being directly involved in any trophic relationship (e.g., predation, parasitism, mutualism, or competition).

Earthworms, termites, and ants form the main groups recognized as "soil engineers", and the spatio-temporal scale of their structures (e.g., casts, galleries, and domes) may serve to evaluate the impact on both the soil and other organisms living in it. The physico-chemical nature, abundance, size, and, morover, dynamics of construction and degradation of these structures are therefore parameters with which to evaluate the type and breadth of indirect effects that these structures have on the environment. So biogenic structures reflect certain functional attributes of the species that produce them (attributes directly linked to the definition of engineers). Their description can be used to establish a functional classification of soil engineers.

This study aimed to develop a classification of engineer organisms based on the nature of the physical structures that they build. Toward this end, we described the "biogenic structures" most commonly found on the soil surface in a well-drained savanna of Colombia. A typology of structures was thus established, based on 12 variables that describe the physical nature of the aggregates produced (bulk density and aggregate size), their structural stability, and their organo-chemical nature (pH, organic C, N, P, Ca, K, Mg, Al, and Al saturation).

Materials and Methods:

Study area: The study was carried out at the Centro Nacional de Investigación Agropecuaria at Carimagua (jointly run by the Corporación Colombiana de Investigación Agropecuaria [CORPOICA] and CIAT). Located at 4°37'N, 71°19'W, the center is situated in the phytogeographic region of well-drained isohyperthermic savannas in the Colombian Eastern Plains. The climate is subhumid tropical, with an average annual temperature of 26 °C, an average annual rainfall of 2300 mm, and a highly marked dry season from November to March. Vegetation is influenced by topography, with herbaceous savanna in the higher zones, and gallery forests and flooding zones in low-lying savanna. The soils are ferrallitic and kaolinitic (Oxisols) in the higher zones, with ferrisols and plinthites (Ultisols) occurring in the low-lying zones. The two types of soil have a characteristically granular structure and very low chemical fertility, with a pH (H_2O) <5, Al saturation >80%, and CEC <5 meq/100 g (CIAT database).

Experimental plots: Observations and sampling were carried out in plots on a savanna with ferrallitic soil. Vegetation consisted of ungrazed native savanna, dominated by *Trachypogon vestitus* Anders. and *Paspalum pectinatum* Nees. The plots were managed traditionally, with an annual burn during the dry

season (February). Sampling was carried out in 1996 at the beginning of the rainy season (May), a period when soil macrofauna are mostly active.

Identification and morphological description: The plots were thoroughly checked (one hour per plot) by three people. All biogenic structures found on the soil surface of the native savanna were described, and the macroinvertebrates responsible for their construction identified as precisely as possible (family, genus, or species). This sampling did not aim toward an exhaustive list of all biogenic structures found in the savanna but, instead, to establish a detailed description of the most representative found in this type of vegetation. The color, size, shape, and general aspect of the structures were described for each species inventoried. The forms were likened to simple geometric forms to more easily evaluate the volume of soil moved through each type of structure on the soil surface. About 500 g of fresh material were collected from each structure found.

Chemical analysis: Contents of organic C and mineral elements were determined for those biogenic structures that were sufficiently abundant to permit the collection of enough material. We sampled the top layer of soil (0 cm–10 cm) with a cylinder 5 cm Ø × 10 cm long. Analyses were carried out, using standard procedures recommended by the Tropical Soil Biology and Fertility (TSBF) Programme. Hydrosoluble carbohydrates are considered to play an important role in stabilizing soil aggregates. The concentration of these carbohydrates were measured in both soil aggregates (0 cm–10 cm) and a representative selection of biogenic structures such as two types of earthworm casts, two types of termite mounds, and two types of ant nests, all chosen for their abundance on the soil surface being studied. The biogenic structures were air-dried, then sieved to 0.25 and 2.0 mm. Later, 2 g of each sample were agitated for 8 h in 20 mL of hot (80°C) water. The resulting solution was centrifuged for 6 min at 13,000 rpm. The supernatant was centrifuged again under the same conditions. Carbohydrate contents were determined, using a standard colorimetric method.

Bulk density: Bulk density was evaluated for all biogenic structures, using samples dried at 75 °C for 48 h. For soft structures (ant nests), apparent density was measured according to the volume occupied by the sample in a metal cylinder (5 cm Ø and 10 cm height). Hard structures (earthworms casts and termite mounds), however, were made impermeable with paraffin paper (Parafilm), then measured, using the displacement-of-water method. For savanna soil, bulk density was estimated by weighing a known volume of soil that had been sampled with a cylinder, 5 cm Ø × 10 cm long, and dried at 75 °C for 48 h.

Soil aggregates' distribution among size classes and their structural stability: Distribution of soil aggregates into size classes was determined for all biogenic structures. A minimum of four samples for each structure described was collected from the savanna. Eight cylinders (8 cm Ø × 10 cm long) of soil were also extracted to compare the aggregates in the structures with those in the topsoil. To evaluate the distribution of aggregates into size classes, we used a method of dry sieving, already used in other studies of Colombian savanna soils. The samples were air-dried for 4 days before being dropped from a height of 2 m onto a hard surface to break up the aggregates. They were then sieved, 20 times, through a sieve column of 0.053, 0.125, 0.250, 0.5, 1.0, 5.0, and 10.0 mm opening. Each fraction was dried for 48 h at 75 °C, then weighed. Aggregate distribution for an entire sample was expressed as the mean weight diameter (MWD), according to the following equations:

$$MWD = \sum_{i=1}^{n} \bar{x}_i w_i \qquad [1]$$

and the fraction of aggregates $(w_i) = \frac{M_{sieve\ i}}{M_{total\ sample}}$ [2]

where,

$\bar{x}_i$ is the average diameter of each fraction of aggregates;
$M_{sieve\ i}$ is the dry weight of particles retained in sieve *i*; and
$M_{total\ sample}$ is the dry weight of the total sample.

The water stability of aggregates (WAS) was evaluated in those samples used for estimating the concentration of hydrosoluble carbohydrates. The method used involved the application of a destructive force of a certain range. Five grams of aggregates (2 cm–5 cm Ø) were air-dried, then humidified by placing them on a humid sand layer at a suction of about 1 cm of water for less than 45 min. The sample was then placed on a sieve with a 1-mm mesh, immersed in water, and, using an automatic apparatus, agitated up and down for 3 min at a rate of 34 oscillations per minute and through a 3-cm range. The soil remaining on the sieve was dried at 105 °C and weighed.

Sand contents were estimated for the total sample and for the soil left in the sieve by dispersing the aggregates in a solution of 5% hexametaphosphate, then sieved (1-mm mesh). The WAS was expressed as the weight of the stable aggregates (corrected according to sand contents) divided by the weight of the sample (corrected according to sand contents), as follows:

$$WAS = \frac{M_{stable\ sample} - M_{sands\ in\ stable\ sample}}{M_{total\ sample} - M_{sands\ in\ total\ sample}} \times 100\% \quad [3]$$

where,

$M_{total\ sample}$ is the dry weight of the initial sample;
$M_{stable\ sample}$ is the dry weight of the particles retained by the sieve after dispersion by water;
$M_{sand\ in\ total\ sample}$ and $M_{sand\ in\ stable\ sample}$ are the dry weights of sand contents in, respectively, the initial sample and the sample dispersed with water.

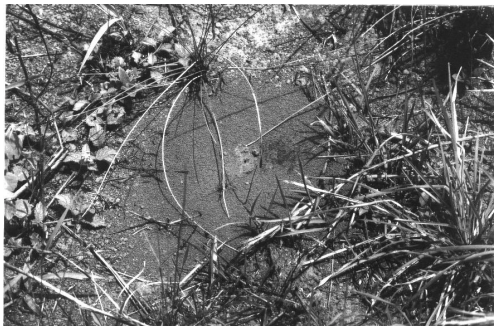
Statistical analysis: Mean comparisons were performed with ANOVAs and with Fisher PLSD tests at the significance level of 0.05. To establish the typology of structures, a principal component analysis (PCA) was carried out. The chemical and physical parameters available for each structure identified (chemical properties, apparent density, and weighted average diameter of aggregates) were included in the analysis. Of the structures built by ants we only used those for which a complete data set was available. The matrix used contained 11 rows (objects = 11 sampled structures) and 12 columns (variables = physical and chemical characteristics). The analysis was conducted with the help of the computer package ADE-4.
Linear regressions were used to evaluate relationships that could exist between carbohydrate contents of the samples and the WAS. The normalcy of the data had been previously verified through the Kolmogorov-Smirnov test, carried out with the statistics program, VerNorm 3.0 of the «R Package». The normalcy of the data was accepted at the 0.05 significance level for all sets of data.

Results and Discussion:

Micromorphological identification and descriptions: Fourteen species of macroinvertebrates were identified as being responsible for the construction of biogenic structures found on the soil surface in native savanna. Of the structures, we collected eight types of epigeic ant nests (Pictures 5 and 6), three types of epigeic termite domes (one was made of a pasteboard-like material and the other two of an organo-mineral mixture) (Pictures 7 and 8), one type of surface slab, built by a termite species (solid,

surface galleries, Picture 9), and two types of earthworm casts (Picture 10). Table 40 lists the 14 species of macroinvertebrates identified, and briefly describes the respective structure built.

Picture 5. Epigeic ant nest and colony entrance for *Trachymyrmex* sp. in the native savanna (scale: length of photo = 40 cm).



Picture 6. Epigeic ant nest and colony entrance of *Camponotus* sp. in the native savanna (scale: length of photo = 50 cm).

Picture 7. Epigeic termite mound of a *Velocitermes* sp. colony in the native savanna (scale: length of photo = 30 cm).

Picture 8. Epigeic termite mound of a *Spinitermes* sp. colony in the native savanna (scale: length of photo = 1 m).

From a micromorphological viewpoint, the biogenic structures could be considered in terms of Bal's definitions, as follows:

- The result of β-accumulation of aggregates coming from deep soil horizons and transported by invertebrates to the soil surface (terrepleins of ant nests).
- «Agrotubules», that is, tubular structures composed of aggregates cemented together in no particular direction (termite surface channels).
- «Modexis», that is, excrements produced by the animals as individualized three-dimensional structures (earthworm casts). The casts of *Martiodrilus carimaguensis* could be classified more precisely as «modexotubules» because they also have a central, tubular hole that correspond to the earthworm's gallery entrance.
- The three-dimensional networks of the «scatotubules», that is, of those tubular structures consisting of invertebrate excrements that are not clearly individualized from each other (epigeic termite domes).

Table 40. Taxonomic position of macroinvertebrates together with a description of the physical structures they produce.

Code	Macroinvertebrates					Structures					
	Order	Family	Subfamily	Species	Feeding regime	Type	Average dimension		Colour	Shape	Aspect
							In soil	Height			
	Ants										
Campo	Hymenoptera	Formicidae	Formicinae	*Camponotus* sp.	Omnivore	Dome	10-20 cm	1-3 cm	orange	paraboloid	Mobile rubbish
Odon	Hymenoptera	Formicidae	Ponerinae	*Odontomachus* sp.	Predator	Dome	20-25 cm	5-10 cm	grey	paraboloid	Mobile rubbish
Crema	Hymenoptera	Formicidae	Myrmicinae	*Crematogaster* sp.	Nectívoro	Dome	15-30 cm	10-15 cm	grey	truncated cone	Mobile rubbish
Pheid1	Hymenoptera	Formicidae	Myrmicinae	*Pheidole* sp.1	Omnivore - predator	Dome	3-5 cm	2-5 cm	orange	truncated cone	Mobile rubbish
Pheid2	Hymenoptera	Formicidae	Myrmicinae	*Pheidole* sp.2	Omnivore - predator	Dome	5-10 cm	5-10 cm	orange	truncated cone	Mobile rubbish
Atta	Hymenoptera	Formicidae	Myrmicinae	*Atta laevigata*	Fungi growers	Dome	50-100 cm	20-50 cm	orange	truncated cone	Mobile rubbish
Acrom	Hymenoptera	Formicidae	Myrmicinae	*Acromyrmex landolti*	Fungi growers	Dome	15-30 cm	10-15 cm	orange	cone	Mobile rubbish
Trachy	Hymenoptera	Formicidae	Myrmicinae	*Trachymyrmex* sp.	Fungi growers	Dome	10-20 cm	10-20 cm	orange	cone	Mobile rubbish
	Termites										
Micro	Isoptera	Termitidae	Termitinae	*Microcerotermes* sp.	Geophagous	Dome	10-20 cm	10-20 cm	black	cylinder	Pasteboard-like material
Spini	Isoptera	Termitidae	Termitinae	*Spinitermes* sp.	Geophagous	Dome	20-30 cm	20-50 cm	black	cone	cemented material
Veloci	Isoptera	Termitidae	Nasutitermitinae	*Velocitermes* sp.	Litter feeder	Dome	5-20 cm	10-25 cm	grey	cone	cemented material
Rupti	Isoptera	Termitidae	Apicotermitinae	*Ruptitermes* sp.	Geophagous	Slab	5-20 cm	0.5-1 mm	orange	slab	cemented rubbish
	Earthworms										
Mca	Oligochaeta	Glossoscolecidae	Glossoscolecinae	*Martiodrilus carimaguensis*	Anecic	Turricule	3-8 cm	1-10 cm	grey	cylinder	compact material
Andio	Oligochaeta	Glossoscolecidae	Glossoscolecinae	*Andiodrilus* sp.	Geophagous	Turricule	5-10 mm	5-10 mm	grey	sphere	Compact material

Chemical properties: Contents of organic C and mineral elements found in earthworm casts and termite mounds were usually present at levels higher than those of the savanna topsoil; for example, +8.6% and +248.3% of organic C in the casts of *Andiodrilus* sp. and domes of *Microcerotermes* sp., respectively (Figure 24). Likewise, pH increased, whereas Al contents dropped significantly. In contrast, contents of organic C in ant nests and termite slabs were much lower than those of the topsoil, that is, –47.6% for the ants *Pheidole* sp., and –69.2% for the ants *Atta laevigata* Smith and *Acromyrmex landolti* Forel. Contents of mineral elements and pH did not change or were lower, compared with the topsoil, and Al saturation dropped slightly.

Picture 9. Slabs of *Ruptitermes* sp. and casts of *Andiodrilus* sp. in the native savanna (scale: length of photo = 30 cm).

Picture 10. Casts of *M. carimaguensis* in the native savanna (scale: length of photo = 20 cm).

The concentrations of hydrosoluble carbohydrates found in ant nests and casts of *Andiodrilus* sp. were equivalent to or less than those found in the savanna topsoil, at –29.7% for *Trachymyrmex* sp. and–70.3% for *A. laevigata* (Figure 25a). In contrast, the soil aggregates forming the casts of *M. carimaguensis* and termite mounds had concentrations that were higher than those of the topsoil, that is, +12.8% for *M. carimaguensis*, +78.4% for *Spinitermes* sp., and +132.4% for *Velocitermes* sp.

Physical properties: Bulk density of biogenic structures varied widely according to the invertebrate species being considered. Earthworms constructed, for example, compact casts with an apparent density of more than 1.3 g/cm^3, that is, 10% to 20% higher than that of the top 10 cm of savanna soil (Table 41). In contrast, termites and ants built nests that were less compact that the surrounding soil, with an apparent density of 0.90 g/cm^3 or less.

The distribution of size classes of the «bioformed» aggregates differed widely. Earthworm casts and termite mounds were composed of large aggregates, that is, more than 50% of aggregates had diameters larger than 5 mm. In contrast, termite slabs and ant nests were composed exclusively of aggregates smaller than 5 mm in diameter (Figure 26). The MWD of biogenic aggregates therefore differs according to the species that produce them (Table 41). Compared with savanna soil aggregates, MWD increased in earthworm casts and termite mounds (e.g., +12.9% in *Andiodrilus* sp. and +51.0% in *M. carimaguensis*) or, in contrast, was reduced in antnests and termite slabs (e.g., –79.5% and –97.0% in *Odontomachus* sp. and in *Pheidole* sp. 1, respectively).

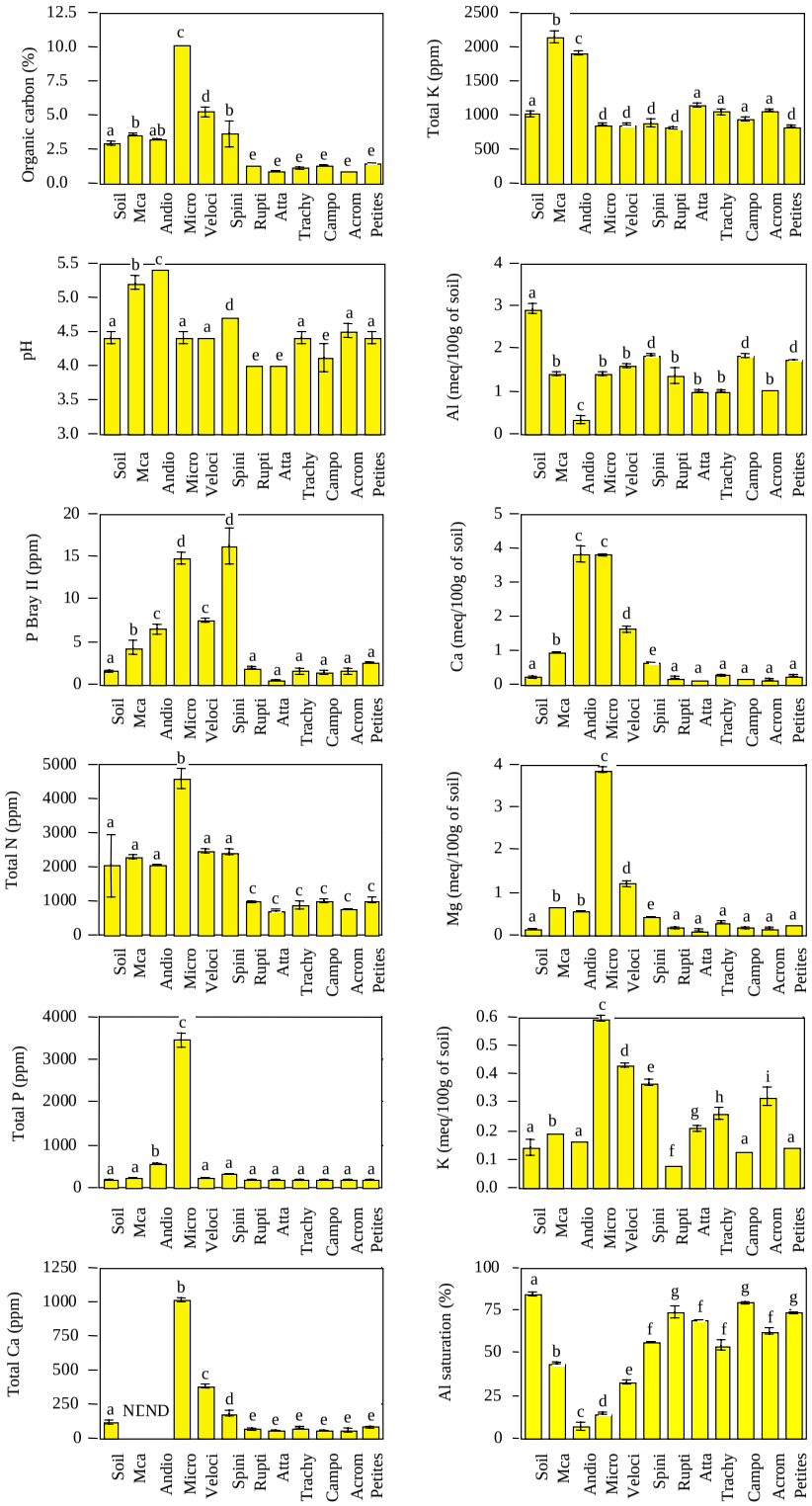


Figure 24. Chemical properties of the biogenic structures and control soil of the native savanna (codes of the structures correspond to those from table 1, except the word «petites» which refers to a combined sample of the structures produced by the smallest ants; different letters mean significant differences at $P<0.05$).

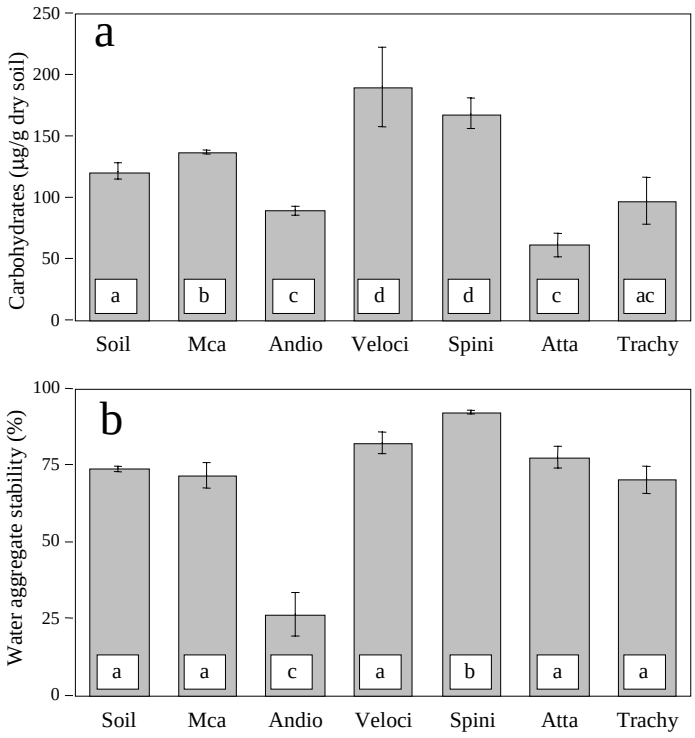


Figure 25. Hydrosoluble carbohydrates contents (a) and water aggregate stability (b) of aggregates (2-5 mm) from the biogenic structures. The results correspond to a representative selection of structures (codes correspond to those from table 1; different letters mean significant differences at P<0.05).

Table 41. Mean weight diameter (MWD) of aggregates and bulk density (BD) of biogenic structures and control soil in the native savanna (standard error within brackets; different letters mean significant differences at P<0.05).

Species	Code	MWD (mm)			BD (g/cm^3)		
Control soil		6.69	(0.78)	a	1.18	(0.07)	a
Earthworms							
Andiodrilus sp.	Andio	7.55	(0.38)	a	1.33	(0.16)	b
M. carimaguensis	Mca	10.10	(1.17)	b	1.39	(0.02)	b
Termites							
Microcerotermes sp.	Micro	-	-		0.60	(0.03)	c
Ruptitermes sp.	Rupti	1.35	(0.32)	c	0.70	(0.03)	c
Spinitermes sp.	Spini	9.05	(0.88)	e	0.91	(0.03)	e
Velocitermes sp.	Veloci	8.50	(0.83)	e	0.73	(0.05)	c
Ants							
A. landolti	Acrom	0.80	(0.04)	cd	0.54	(0.03)	c
A. laevigata	Atta	1.08	(0.05)	c	0.64	(0.02)	c
Camponopus sp.	Campo	0.54	(0.03)	cd	0.80	(0.05)	ce
Crematogaster sp.	Crema	0.32	(0.03)	cd	0.63	(0.09)	d
Odontomachus sp.	Odon	1.37	(0.05)	c	0.46	(0.02)	c
Pheidole sp.1	Pheid1	0.20	(0.02)	d	0.61	(0.09)	c
Pheidole sp.2	Pheid2	0.92	(0.03)	cd	0.71	(0.01)	c
Trachymyrmex sp.	Trachy	0.82	(0.04)	cd	0.71	(0.02)	ce

The WAS for ant nests and termite mounds was about 75% under test conditions. This figure is comparable or higher than that of savanna soil aggregates of equivalent size (–4.8% in *Trachymyrmex* sp. and +25.0% in *Spinitermes* sp.; Figure 25b). Aggregates from casts of *Andiodrilus* sp. were much less stable than those from other biogenic structures and the surrounding soil (–64.2%, compared with the soil). No significant correlation was found between structural stability of aggregates produced by engineer organisms and the concentration of hydrosoluble carbohydrates (Figure 27).

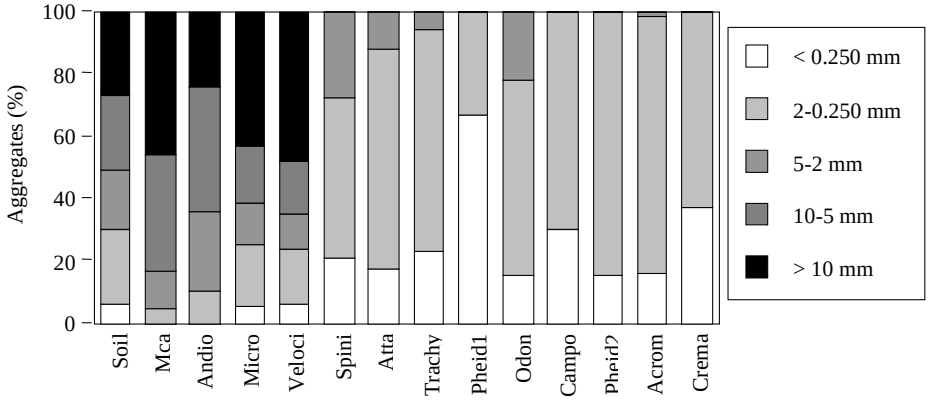


Figure 26. Size class distribution of aggregates from the biogenic structures (codes correspond to those from table 1).

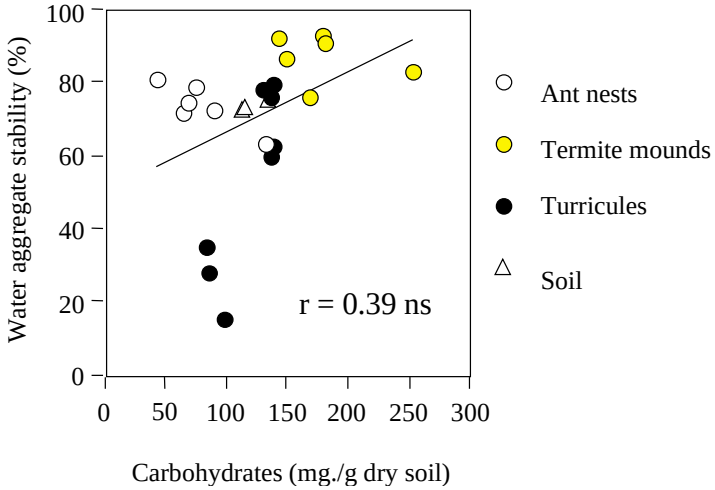


Figure 27. Simple linear regression between water aggregate stability and hydrosoluble carbohydrate concentration (NS = not significant; * = significant at P<0.05; ** = significant at P<0.01; *** = significant at P<0.001).

Principal component analysis: The first two axes of the PCA explained 82.2% of the total inertia (59.6% and 22.6% for the first and second axes, respectively; Figure 28a). The correlation coefficients associated with the variables in axis 1 showed opposition between (1) high percentages of Al saturation and (2) an important MWD and high content of organic C in mineral-element contents of the aggregates (Figure 28b). The representation of objects on axis 1 showed marked opposition between (1) ant nests and termite

slabs and (2) termite mounds and earthworm casts (Figure 28c). Axis 2 was defined by an opposition between (1) Al saturation, and (2) structural density and pH (Figure 28b). On this axis too, termite mounds and ant nests were in opposition with the compact casts of earthworms (Figure 28c).

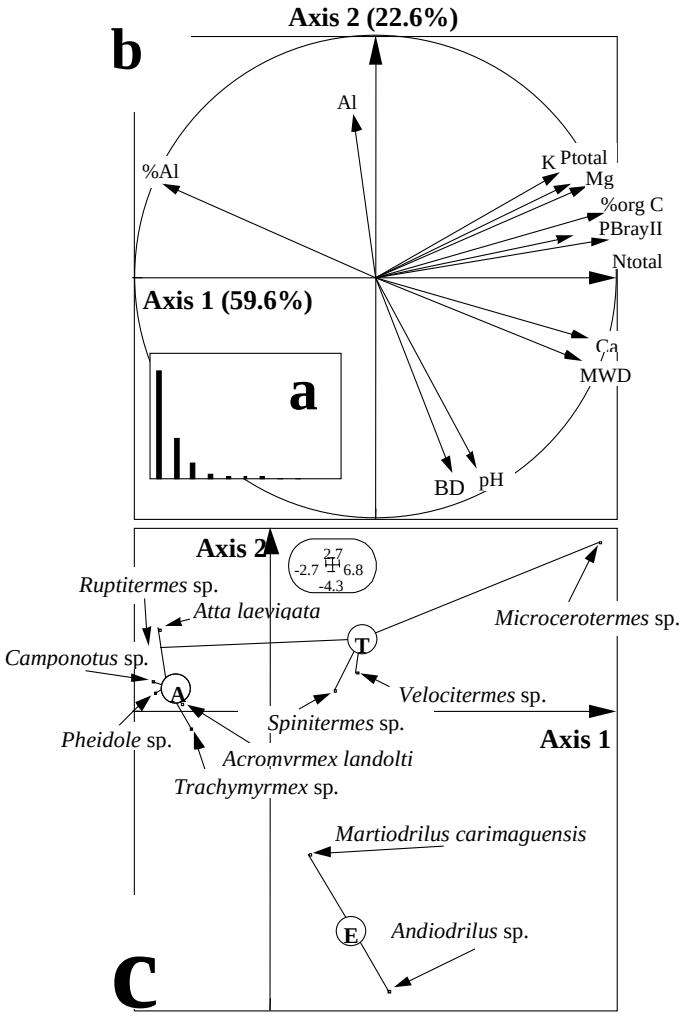


Figure 28. Results from the PCA performed on physico-chemical properties of the biogenic structures collected in the native savanna: (a) Eigenvalues; (b) Correlation circle associated to the first two axes (% org C = organic carbon; PBrayII = Phosphorous (BrayII); N total = total Nitrogen; Ptotal = total phosphorous; Al = exchangeable aluminium; Ca = exchangeable Calcium; Mg = exchangeable magnesium; K = exchangeable potassium; %Al = aluminum saturation; BD = bulk density; MWD = mean weight diameter of aggregates); (c) ordination of the different biogenic structures onto the plane defined by the first two axes (circles correspond to the baricenters within the cloud of points: E = earthworm casts; T= termite mounds and slabs; A = epigeic ant nests).

The strong concentrations of organic C and mineral elements measured in the feces of earthworms from Carimagua are common to numerous oligochaete species that build casts on the soil surface. This result, corroborated by previous findings for feces of *M. carimaguensis*, is explained by the food habitats occupied by the species under study. *Andiodrilus* sp. and *M. carimaguensis* belong, respectively, to the ecological categories “polyhumic” endogeic and “anecic”, where specialization consists of selectively

ingesting a substrate (e.g., organic soil and/or fresh plant material) that is richer in organic matter than the surrounding soil.

Termites that build epigeic domes normally cement soil particles with variable quantities of salivary secretions and excrements rich in organic matter. In certain cases, the walls of the termite mounds are made of a pasteboard-like material that is very rich in organic C (e.g. *Microcerotermes* sp.). The enrichment of fecal organic matter explains the differences in concentrations of both C and mineral elements observed between termite mounds and the soil. The walls or panels of aerial galleries produced by *Ruptitermes* sp cover accesses to food sources, probably playing a role in protection from heat, predators, and competitors. Contrary to what is usually described in the literature the walls analysed in our study were not enriched with organic matter and exchangeable bases.

The epigeic dome built by ants was, in contrast to the structures built by termites and earthworms, in this study, all pale yellow. The soil used was brought up from the deeper horizons of the soil profile, and left to accumulate little by little, on the soil surface. The dome's height is a measure of the excavation for the nest's subterranean spaces. Poor in organic matter, this soil does not undergo significant modifications. Neither is it enriched, as shown by its low contents of C and mineral elements.

The casts of *M. carimaguensis* and *Andiodrilus* sp., compared with the surrounding soil, were compact structures that were composed of large aggregates. The aggregates had a stability that was equivalent to or less than that found in aggregates of the surrounding soil. This finding was surprising because earthworm casts are usually more stable than soil aggregates of comparable size, and a recent study showed that the casts of *M. carimaguensis* to have considerable structural stability. Our result is probably linked to our use of casts that were recently deposited. In fact, earthworm deposits acquire their structural stability as they age, probably under the combined effects of (1) physical processes (hardening under either constant humidity or alternate phases of humidity and dryness), and (2) biological processes (fungi developing on the surface of the aggregates or the production of polysaccharides of microbial origin).

Fecal pellets deposited by termites are composed of organo-mineral aggregates that are smaller than those deposited by earthworms. These are used, together with salivary secretions, to cement soil particles in the construction of walls for the termite mound. During this process, soil porosity diminishes and the reorganized material is much more compact than the original soil. In our study, however, the presence of numerous galleries makes termite mounds cavernous, which explains the low apparent densities observed. Moreover, after a blow, the walls of termite mounds break up into macroaggregates of significant size. One can imagine the same phenomenon occurring naturally, as for example, under the impact of raindrops, thus leading to a slow redistribution of macroaggregates over the soil surface. These aggregates are particularly stable, allowing us to believe that the effects of such processes on soil stability are significant at important spatio-temporal scales. The stability of aggregates produced by termites has been pointed out by other studies, although the processes involved have not been precisely identified.

Although research on the structure of earthworm casts and termite mounds has increased in the last 10 years, the stability of aggregates produced by ant activities has only just begun attracting significant attention. In Carimagua, ant nests are composed of loose aggregates that are brought up to the soil surface from deep horizons of the soil profile and simply piled on top of each other. It is known that the average size of the aggregates is proportional to the size of mandibles of the ants carrying them. Structural stability is comparable with that of similar-sized aggregates in the topsoil. The aggregates are not cemented together with any organic adhesive, thus giving rise to granular structures. Nor are the aggregates joined, and the numerous spaces between them explain the structures' low apparent density.

Hydrosoluble carbohydrates are considered as important components of the stabilization mechanisms of soil aggregates in the Colombian savannas. Among the carbohydrates involved in soil aggregation are

those that represent a specific and important fraction that is derived principally from microbial metabolism and, perhaps, plant tissues. However, our results do not show any correlation between structural stability of the biogenic aggregates and the presence of such components. This result indicates that other mechanisms, and not carbohydrates, are involved in aggregate stability at the soil's surface. For example, certain physical mechanisms, such as hardening of structures under the effects of variable humidity, may play an important role.

The functional significance of structures: By comparing the physico-chemical characteristics of the biogenic structures listed in this study with the surrounding soil, we could establish a classification, comprising three major groups:

- Structures more compact than the soil, enriched with organic C and mineral elements, and composed of large aggregates. This group corresponded, in our study, to the globular casts of the two earthworm species studied.
- Structures less compact than the soil, enriched with organic C and mineral elements, and composed of large aggregates. In our study, these were the epigeic termite domes.
- The least compact structures, built with soil from the deepest layers, granular, containing levels of organic C and mineral elements that are either equivalent or lower than those of the topsoil, and composed of highly stable but small aggregates. These structures were typified by anthills and epigeic termite slabs.

From this typology of biogenic structures we can deduce the impact produced by a given species on the soil. In fact, the nature of the respective biogenic structure could be considered as reflecting certain functional attributes of that species. Those species producing structures typical of groups 1 and 2 (termites and earthworms) accumulate organic C on the soil surface and probably influence dynamics of organic matter and the rate of release of mineral elements assimilable by plants.

Structures belonging to the first two groups are also characterized for their large size and the aggregates constituting them. In contrast, structures from group 3 (termite slabs and ant nests) are much smaller. The production of aggregates with diverse physico-chemical characteristics may result in the efficient regulation of soil structure. This mechanism has been described, for example, for the savannas of Côte d'Ivoire, where the smaller earthworm species break up the casts produced by larger species, thus preventing excessive accumulation on the soil surface. In Carimagua, termites (Nasutitermitinae) carry out a similar regulation—it visibly accelerates the kinetics of degradation of the large casts produced by *M. carimaguensis*. Recent studies carried out on Amazonian pastures showed that the presence of abundant earthworm populations could lead to considerable soil compaction because these populations are not associated with species able to break their casts up into much smaller aggregates.

Biogenic structures on the soil surface probably influence soil structure from the moment that these become disaggregated and the fragments are dispersed over the soil, into which they become progressively incorporated. In our study, the loose aggregates observed on the soil surface probably correspond, in essence, to fragments from biogenic structures that are being incorporated. This hypothesis is supported by the easy observation of the shape of these loose aggregates, most of which are either small casts of *Andiodrilus* sp. or recognizable fragments of *M. carimaguensis* casts (Picture 11) scattered across the soil surface.

Picture 11. Soil surface in the native savanna covered by free aggregates from biogenic structures (scale: length of photo = 40 cm).

Impact:

This study demonstrated the diversity of structures that ecological engineers build on the soil surface in a native savanna. We identified three types of structures on the basis of their physico-chemical properties: (1) compact structures, rich in organic matter (earthworm casts); (2) soft structures, rich in organic matter (termite mounds); and (3) soft granular structures, poor in organic matter (termite slabs and ant nests). The multivariate analysis reflected the wide diversity of structures and indicated both the possibility and complexity of a functional classification of engineer organisms that would simultaneously take into account the different functions reflected by these structures.

This study was limited to describing structures built by engineer organisms on the soil surface. Actually, the large number of structures built under the soil surface has not been taken into account for our typology. Numerous soil invertebrates can build endogeic structures (e.g., nests, galleries, and aggregates) that can influence soil processes in specific ways. For example, at Carimagua, an endogeic species of polyhumic earthworm (Ocnerodrilidae sp.) preferentially ingests the compact casts of *M. carimaguensis* and produces small granular casts below the soil surface. Another species, of medium size, *Glossodrilus* sp., sometimes behaves similarly. These species fall into the category of engineers that build soft granular structures. But because these earthworm casts may be richer in organic matter than the material used in ant nests and termite slabs, a new category must be created to correspond to those structures that are soft, granular, and rich in organic matter.

The definition of functional groups of ecological soil engineers is based on the typology of structures that they produce. A complete description of all biogenic structures built (including aggregates produced both on the soil surface and throughout the soil profile, galleries, and endogeic nests) is needed to precisely describe these organisms' functions. Such a description would also help establish accurately those characteristics of the structures that could be used to describe the functional attributes of a given engineer species. For example, do species building compact structures systematically produce a «compacting» effect on the soil? In contrast, do those that build soft structures have a «decompacting» effect on the soil? Do those that concentrate organic matter in their structures have an effect on the dynamics of organic matter and, if so, what sort of effect? And finally, what effects do these structures have on the living conditions of other soil organisms?

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