

Forms of inorganic phosphorus in carbonatic soils in the Pantanal of Mato Grosso do Sul, Brazil

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Abstract. The western region of the southern Pantanal is characterised by soils with high phosphorus (P) contents, derived from materials from the surrounding lithostratigraphic units, accumulated by fluvial transport. However, studies on forms and availability of P in these soils are scarce. The objective of this study was to evaluate the different forms of inorganic P and their relationship with some attributes of carbonatic soils in the Pantanal of Mato Grosso do Sul, Brazil. Hedley's sequential fractionation scheme was used to evaluate the forms of P in soil profiles: Kastanozems (profiles P1 and P3) and Gleysol (profile P2). Total P contents were similar in the three profiles, 3782–5637 mg kg⁻¹, with mean values of 22% for organic P and 46% for inorganic P (P.i). The P.i results indicated that in the profiles there was a predominance of inorganic forms of P in the following order: P-NaOH 0.5 mol L⁻¹ > P-NaHCO₃ > P-NaOH 0.1 mol L⁻¹ > P-HCl, that is, adsorbed to microaggregates, labile, adsorbed to oxides and precipitated with calcium (Ca), respectively. The highest values of total organic P were verified in the surface horizons, with high correlation with total P contents. Residual P contents were high in all profiles, representing 29.0–33.3% of the total P, being correlated with CaCO₃ contents. The studied profiles had high contents of labile P, with the highest values in the fraction P.i-NaHCO₃, possibly associated with the processes of reduction of iron during the periods of floods, making the adsorbed P available. The contents of P.i-NaOH 0.1 mol L⁻¹, a moderately labile fraction adsorbed to oxides, showed few differences compared to the non-labile fractions associated with Ca (P.i-HCl). Possibly, organic matter was bound to Ca, inhibiting the formation of precipitates of Ca with P and making P available for the more labile fractions.

Keywords: Secondary carbonate, Flood cycle, Phosphorus fractionation, Organic matter, Iron reduction.

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Introduction

The Pantanal, with an area of ~150 988 km² (IBGE 2019), is the largest humid complex in the world (Por 2012) and has 11 distinct sub-regions. The sub-regions called the Pantanal Formation and Apa River Complex, located in the south-west of the state of Mato Grosso do Sul, with an area of 35 229 km² (Silva and Abdon 1998), are formed by limestone sediments, mainly from *Serra da Bodoquena*, which represents one of the most extensive continuous karst areas in Brazil, with an extension of around 7000 km² (Oliveira 2009; Pereira *et al.* 2013).

The Pantanal Formation is the most outstanding, and its main characteristic is the accumulation of sediments from other lithostratigraphic units, transported through the river systems and accumulated along the plain (Assine and Silva 2009). This dynamic possibly contributes to the enrichment of phosphorus (P) in water resources and consequently in the soil, but scientific reports regarding the dynamics of P in this formation are still incipient.

Among the lithostratigraphic units, the Corumbá Group and the Apa River Complex are the main sources of phosphatic sediments and soluble P for the Pantanal Formation, consisting

of rocks with traces of phosphorites (Boggiani *et al.* 1993; Adorno *et al.* 2017). Boggiani *et al.* (1993) and Piacentini *et al.* (2007), in their studies with phosphatic rocks in *Serra da Bodoquena* in the state of Mato Grosso do Sul, highlight that the pelitic-carbonatic sediments of the Corumbá Group have significant occurrences of phosphatic rocks, with P contents ranging from 6% to 40%, possibly associated with marine upwelling that occurred during the Cambrian period. Thus, the dynamics of P in the soils of the Pantanal Formation depend directly on the weathering of surrounding geological materials, their transport and deposition along the water basins.

In general, the P dynamics of the Pantanal humid region are very unique and possibly very dependent on seasonality, favouring the process of reduction of elements such as iron (Fe) and manganese in these environments, and the release in the most labile forms of P precipitated with these elements (Couto and Oliveira 2011). Due to containing carbonate material, it is expected that the free P in the soil solution will bind to Ca ions, and this bond is highly stable, resulting from the alkalinity condition of the medium, giving rise to insoluble calcium phosphates (Chacón *et al.* 2005).

In alkaline soils, free P in the solution is adsorbed mainly by calcium (Ca) ions forming Ca–P minerals (Ma *et al.* 2019). Initially, dicalcium phosphate dihydrate is formed, which is later transformed into octacalcium phosphate (OCP) and hydroxyapatite (HAP), which are more crystalline, less soluble phases and precursors of apatite formation (Wang and Nancollas 2008; Weyers *et al.* 2017). In general, there are low contents of P bound to Ca in tropical soils, due to intense weathering processes, but in carbonated soils the formation of Ca–P precipitate results in the immobilisation of this element in the long-term, which is the non-labile form (Ma *et al.* 2019).

The formation of Ca and P precipitates can be inhibited by low and high molecular-weight organic compounds, which compete with P for the adsorption sites or complexation of metal ions, making them unavailable to form Ca–P precipitates (Weyers *et al.* 2017). Organic compounds also delay the process of precipitation of low-crystallinity forms, amorphous calcium phosphates to more crystalline forms such as HAP (Lei *et al.* 2018). In this way, organic compounds can contribute to the increase of P available in the soil, in labile forms (Ma *et al.* 2019).

Transport and deposition of P-rich sediments by river systems, together with the climatic conditions that determine the flood and drought periods in the Pantanal plain, may be conditioning peculiar processes in P dynamics, forming or inhibiting the precipitation of metal ions with P, thus determining the forms of availability of this element in the Pantanal soils.

The present study aimed to evaluate the different forms of inorganic P and their relationships with some attributes of carbonatic soils in the Pantanal of Mato Grosso do Sul.

Material and methods

Study area description and soil sampling

The study area is located in the municipality of Corumbá, western region of the state of Mato Grosso do Sul, within

19°21'12"–19°28'47"S and 57°01'59"–57°33'53"W (Fig. 1). The municipality has a predominantly gently undulating relief, with topographic gradient that decreases from east to west, accompanying the tributaries of the Paraguay River. The region has a great diversity of vegetation, formed by a mosaic of different shapes, determined directly by the topography, such as the Cerrado, Seasonal Forest and Chaco, in addition to planted pasture in some parts (Assine and Silva 2009).

The climatic conditions, together with the relief of the region, can be considered one of the main factors of formation of the Pantanal soils. Combined, these factors contribute to the dynamics of flooding and drought, thus resulting in the often-imperfect drainage (Coringa *et al.* 2012). The climate of the region is type Aw, humid tropical according to Köppen's classification, with precipitation between 800 and 1200 mm year⁻¹ and monthly average air temperature fluctuating between 22°C (July) and 32°C (December) with a very high evapotranspiration exceeding rainfall in the dry months (Soares and Oliveira 2009).

The soil profiles are located along the BR-262 highway, the road to Forte Coimbra and the road of Passo da Lontra, in a plain area with gently undulating relief and variable elevation between 95 and 140 m of altitude, being described in the field and sampled to collect disturbed and undisturbed samples for subsequent analysis (Santos *et al.* 2015). The profiles were classified according to the Brazilian Soil Classification System (SiBCS) (Santos *et al.* 2018): *Chernossolo Háplico Órtico vertissólico* (profile P1) in native pasture, *Gleissolo Háplico Ta Eutrófico típico* (profile P2) in secondary remnants of native vegetation and *Chernossolo Háplico Carbonático típico* (profile P3) in mountain range area under acuri (*Attalea phalerata*) forest. According to World Reference Base for Soil Resources (IUSS Working Group WRB 2015), profiles P1 and P3 are equivalent to Kastanozems and profile P2 is equivalent to Gleysol.

Chemical and physical analyses

Physical and chemical analyses were performed in air-dried fine earth (ADFE) samples, according to Teixeira *et al.* (2017). Particle-size analysis was performed in the ADFE by the pipette method, using a solution of 1 mol L⁻¹ sodium hydroxide (NaOH) as chemical dispersant and mechanical homogenisation in Wagner shaker for 16 h. Particle density was determined by the volumetric flask method.

The pH was determined in H₂O and 1 mol L⁻¹ KCl (soil: solution ratio of 1:2.5); exchangeable cations of Ca²⁺ and magnesium (Mg²⁺) were extracted with 1 mol L⁻¹ KCl and determined by complexometry; exchangeable sodium (Na⁺) and potassium (K⁺) were extracted by Mehlich-1 solution and determined by flame photometry; H⁺Al was extracted with 0.5 mol L⁻¹ Ca acetate solution buffered at pH 7.0 and determined by titration; and the total organic carbon (TOC) content, by oxidation with potassium dichromate in sulfuric medium. The contents of calcium carbonate (CaCO₃) equivalent were quantified by acid attack with 0.5 mol L⁻¹ hydrochloric acid (HCl) and titration with standard NaOH solution. In the surface and subsurface diagnostic horizons, the contents of SiO₂, Al₂O₃ and Fe₂O₃ oxides were determined after acid and alkaline digestion (sulfuric attack method)

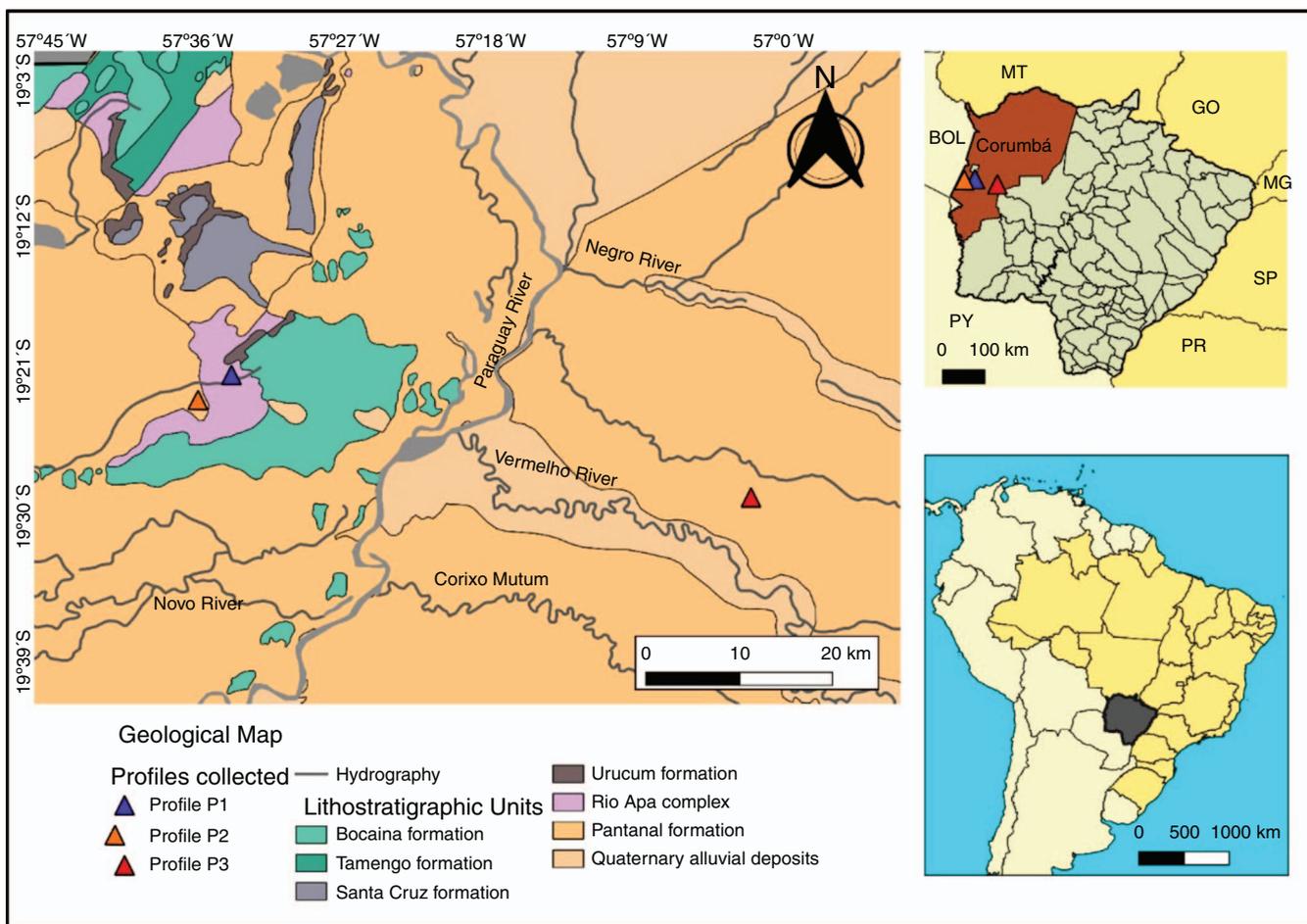


Fig. 1. Location of the collected soil profiles of calcareous sediments in the west (Corumbá City) of Mato Grosso do Sul, Brazil.

(Camargo *et al.* 1986). The extracts were subjected to reading in atomic absorption spectrophotometer to determine the oxides. The contents of pedogenic Fe and Al oxides represented as Fe_o and Fe_d , and Al_o and Al_d , respectively, were determined by atomic absorption spectrophotometry, after extraction with sodium dithionite-citrate-bicarbonate (DCB) solution, according to Mehra and Jackson (1960), and with 0.2 mol L^{-1} ammonium acid oxalate solution at pH 3, in the absence of light, according to Schwertmann (1964), respectively.

P fractionation

The available P content was determined using the Mehlich-1 extractant, used to quantify the assimilated inorganic P (labile), and the sequential fractionation of inorganic P was performed according to Hedley *et al.* (1982) with modifications proposed by Condron *et al.* (1985). In this method, the fractions of P present in the soil are extracted in decreasing order of instability according to the decreasing availability to plants. The extraction was performed using 0.5 g of ADFE from each of the horizons of the three profiles, in triplicate. The first fraction to be determined was the labile inorganic P (P.i-labile) via anion-exchange resin strips (AER), recovered with HCl at 0.5 mol L^{-1} (P.i-AER); after the

extraction of AER, sodium bicarbonate at 0.5 mol L^{-1} was added in the same samples (P.i- $NaHCO_3$), also extracting the P.i-labile. Subsequently, extraction with NaOH at 0.1 mol L^{-1} (P.i-NaOH I) was performed to obtain the inorganic P chemically adsorbed by Fe and Al components. After the extraction of P.i-NaOH I, the inorganic P bound to Ca obtained by HCl at 1.0 mol L^{-1} (P.i-HCl) was determined and, finally, NaOH at 0.5 mol L^{-1} was added (P.i-NaOH II), extracting the inorganic P retained in the microaggregates. Until this stage of extraction, each protocol was subjected to shaking for 16 h on an orbital shaking table and soon after centrifuged for 15 min, collecting the supernatant and centrifuging again with KCl at 0.1 mol L^{-1} to remove P extracted from the previous fractions, being placed together with the supernatant.

From the results obtained with the extractions, residual P (P-residual) was determined by the method of Brookes *et al.* (1982). Total P (P-total) was obtained independently according to Olsen and Sommers (1982). Due to some extraction problems, the method proposed by Hedley *et al.* (1982) had modifications in the extraction of organic forms of P, presenting only the result of total organic P (P.o-total) (Garcia-Montiel *et al.* 2000; Hou *et al.* 2018). The estimated

value of P.o-total was obtained by subtracting the results of total inorganic P plus residual P from the results of total P: (P-total) – (P.i-total + P-residual).

Inorganic P contents of the alkaline extracts of sodium bicarbonate and NaOH were quantified by the method of Dick and Tabatabai (1977), determined in a visible UV spectrophotometer with absorbance wavelength of 700 nm. The P of the acid extracts: AER, HCl, residual P and total P were quantified according to Murphy and Riley (1962) by colourimetry in a visible UV spectrophotometer with wavelength of 882 nm.

Statistical analyses

Correlations between soil attributes and P fractions were evaluated through Pearson's correlation coefficient using the statistical program R (R Core Team 2019), adopting $P < 0.05$ and $P < 0.01$ as significance criteria.

Results

Soil attributes

In all profiles, the clay contents were lower than 280 g kg^{-1} , and the highest contents were verified in the subsurface horizons of profiles P1 and P2; whereas, in P3, the clay content decreased in subsurface (Table 1). In the horizons of profiles P1 and P2, there was a hypocarbonatic character with CaCO_3 equivalent content below 150 g kg^{-1} ; and in profile P3 the CaCO_3 equivalent content exceeded 150 g kg^{-1} , identifying the carbonatic character in this profile (Santos *et al.* 2018).

All values of pH in KCl were lower than the values of pH in water, which resulted in negative ΔpH in all horizons

(Table 1). The TOC contents were high in the surface horizons, decreasing in subsurface, with range of $6.11\text{--}20.60 \text{ g kg}^{-1}$ in profile P1, $7.79\text{--}28.19 \text{ g kg}^{-1}$ in profile P2 and $17.34\text{--}27.81 \text{ g kg}^{-1}$ in profile P3.

The range of SiO_2 content was $14\text{--}140 \text{ g kg}^{-1}$, Al_2O_3 was $4\text{--}92 \text{ g kg}^{-1}$ and Fe_2O_3 was $2\text{--}25 \text{ g kg}^{-1}$ (Table 2). In general, the studied soils had low contents of high and low-crystallinity oxide forms extracted with DCB (Fe_d and Al_d) and low contents of low-crystallinity oxides extracted with ammonium acid oxalate (Fe_o and Al_o). The Fe_d contents ranged from 0.6 g kg^{-1} in profile P3 to 2.1 g kg^{-1} in profile P2, while Fe_o contents ranged between 0.3 and 0.8 g kg^{-1} in profiles P3 and P1, respectively. For Al oxides, Al_d was higher in the horizons analysed in profiles P1 and P2, between 2.2 and 0.9 g kg^{-1} , respectively, whereas profile P3 differed from the others, with higher contents of Al in the form with lower crystallinity values of $0.2\text{--}0.4 \text{ g kg}^{-1}$ of Al_o and 0.2 g kg^{-1} of Al_d .

The Fe_o/Fe_d ratio was equal to 0.43 , 0.36 and 0.66 in profiles P1–P3, respectively, with higher values in the surface horizons of profiles P1 and P3 (Table 2). The Al_o/Al_d ratio was higher in all profiles, with values between 0.66 in profile P1 and 1.93 in profile P3.

P fractionation

The highest values of P.i-labile extracted by Mehlich-1, AER and NaHCO_3 were in the surface horizons of the profiles; however, in profile P3 there was discontinuity between the fractions, with the lowest P.i-AER value in the surface compared with the subsurface horizons (Table 3 and Fig. 2).

There was a great difference in the values of P-labile in the three extractants: for P.i- NaHCO_3 there were P-labile contents

Table 1. Chemical attributes in carbonate soils of Pantanal, Mato Grosso do Sul, Brazil

S, sum of exchangeable bases ($\text{S} = \text{Ca}^{2+} + \text{Mg}^{2+} + \text{K}^+ + \text{Na}^+$); T, cation exchange capacity ($t = \text{S} + \text{H}^+\text{Al}$); V, base saturation ($\text{V} = 100 \times \text{S}/\text{T}$); TOC, total organic carbon

Horizon	Depth (cm)	pH KCl	Ca^{2+}	Mg^{2+}	K^+	Na^+	H^+Al	S	T	V %	TOC	CaCO_3	Clay
			cmol _c kg ⁻¹							g kg ⁻¹			
Profile P1													
Ap1	0–10	7.37	20.33	7.19	2.77	0.024	1.78	30.31	32.09	94.5	20.60	89.49	222.40
A2	10–29	7.25	22.47	4.95	2.22	0.012	1.73	29.64	31.37	94.5	18.70	57.18	238.84
Biv1	29–50	6.80	19.53	5.22	1.88	0.024	2.32	26.62	28.94	92	11.47	60.16	238.38
Biv2	50–72	6.53	20.45	6.49	1.22	0.049	2.42	28.17	30.59	92.1	14.36	60.60	273.87
Biv3	72–97	4.92	19.33	7.40	1.45	0.036	3.34	28.18	31.51	89.4	20.73	60.33	238.11
2BC	97–111	5.76	18.64	7.40	1.22	0.061	2.77	27.26	30.03	90.8	11.73	58.87	220.62
2CBk	111–137+	7.40	24.10	5.39	0.89	0.025	1.52	30.39	31.90	95.3	6.11	114.10	242.62
Profile P2													
A1	0–8	7.24	15.73	9.20	3.36	0.025	2.52	28.29	30.81	91.8	28.19	117.06	168.16
A2	8–13	7.58	13.98	6.42	0.66	0.024	1.87	21.06	22.93	91.9	11.48	123.88	168.16
BA	13–25	7.14	7.63	3.75	0.76	0.024	1.79	12.14	13.92	87.2	10.57	60.54	108.66
2Bg1	25–43	6.78	13.51	3.86	0.66	0.024	2.47	18.03	20.50	87.9	9.29	56.83	181.46
2Bg2	43–64	7.33	14.76	6.78	0.77	0.048	1.85	22.31	24.16	92.4	3.33	59.42	197.10
2Bgz3	64–92	7.73	16.46	7.10	0.44	0.375	0.71	24.00	24.71	97.1	4.05	63.67	215.53
2Bgz4	92–108+	7.70	18.41	7.74	0.67	0.561	1.53	26.83	28.36	94.6	8.26	62.67	215.74
Profile P3													
Ak1	0–15	7.84	13.27	0.74	7.90	0.025	1.04	21.91	22.95	95.5	27.81	242.63	127.48
Ak2	15–45	7.91	10.78	1.59	6.79	0.025	0.52	19.15	19.67	97.3	26.07	242.38	120.48
Ak3	45–65	8.15	9.03	1.37	9.83	0.026	0.45	20.22	20.67	97.8	21.76	242.75	88.94
ABk	65–86+	8.06	9.03	1.65	12.49	0.026	0.06	21.34	21.41	99.7	5.38	245.50	58.20

of 472.80–844.49 mg kg⁻¹, which are higher than those obtained by Mehlich-1 and P.i-AER, whose contents were 2.75–232.61 and 21.17–117.04 mg kg⁻¹, respectively. The P.i-NaHCO₃ was the largest fraction of P.i-labile extracted by sequential fractionation, standing out with significant participation in comparison to P-total (with 16.8%, 16.3% and 10.9% in profiles P1–P3, respectively); while P.i-AER had the lowest percentage of P.i-labile in comparison to P-total (correspondingly 1.4%, 1.5% and 0.7%).

The P.i-NaOH I, a moderately labile fraction adsorbed to oxides and kaolinite, showed little differentiation in the contents

Table 2. Iron and aluminium contents extracted with dithionite-citrate-bicarbonate (DCB) and ammonium oxalate and their relationships in carbonate soils of Pantanal, Mato Grosso do Sul, Brazil
Fe_d, iron oxide extracted by DCB; Fe_o, iron oxide extracted by oxalate; Al_d, aluminium oxide extracted by DCB; Al_o, aluminium oxide extracted by oxalate; Fe_o/Fe_d and Al_o/Al_d, estimation of crystallinity

Horizon	SiO ₂	Al ₂ O ₃	Fe ₂ O ₃	Fe _d	Fe _o	Al _d	Al _o	Fe _o /Fe _d	Al _o /Al _d
g kg ⁻¹									
Profile P1									
Akp1	133	92	25	1.8	0.8	2.4	2.2	0.4	0.9
Biv1	140	84	22	1.9	0.8	2.6	2.2	0.4	0.8
2BC	11	8	2	1.8	0.4	1.6	1.1	0.2	0.7
Profile P2									
A1	14	4	2	2.1	0.4	1.3	0.9	0.2	0.7
2Bgn1	89	55	18	1.6	0.6	1.1	0.9	0.4	0.8
2Bgz1	136	70	21	1.4	0.5	0.7	1.3	0.3	1.8
Profile P3									
Ak1	119	9	5	0.7	0.4	0.2	0.4	0.7	1.9
ABk	111	9	4	0.6	0.3	0.2	0.2	0.5	1.4

Table 3. Concentrations of phosphorus fractions in carbonate soils of Pantanal, Mato Grosso do Sul, Brazil

M-1, Mehlich-1; P.i-AER, anion-exchange resin; P.i-NaHCO₃, 0.5 mol L⁻¹ sodium bicarbonate; P.i-NaOH I, 0.1 mol L⁻¹ sodium hydroxide; P.i-HCl, 1.0 mol L⁻¹ hydrochloric acid; P.i-NaOH II, 0.5 mol L⁻¹ sodium hydroxide; P.i-total, total inorganic phosphorus; P.o-total, total organic phosphorus

Horizon	M-1	P.i-AER	P.i-NaHCO ₃	P.i-NaOH I	P.i-HCl	P.i-NaOH II	P.i-total	P.o-total	P-residual	P-total
mg kg ⁻¹										
Profile P1										
Akp1	19.35	117.04	844.48	211.71	199.16	879.29	2251.69	1009.39	1144.63	4405.71
A2	10.09	82.09	527.08	204.04	104.94	720.00	1638.15	1042.54	1416.98	4097.67
Biv1	3.32	82.80	778.41	214.66	132.74	861.00	2069.61	566.00	1394.44	4030.05
Biv2	3.20	29.45	825.02	194.31	248.75	844.48	2142.00	886.18	941.77	3969.95
Biv3	2.80	21.17	659.23	159.50	133.34	874.57	1847.82	472.24	1492.11	3812.17
2BC	2.75	26.83	472.80	155.37	81.50	855.69	1592.20	868.74	1321.19	3782.12
2CBk	5.33	35.96	619.12	158.17	68.13	883.42	1764.80	628.52	1606.69	4000.00
Profile P2										
A1	232.61	98.86	626.20	194.45	161.44	938.70	2019.65	602.81	1632.98	4255.45
A2	69.43	27.89	697.58	180.74	180.98	819.71	1906.89	636.68	1771.98	4315.55
BAn	31.83	88.60	859.82	249.17	202.62	883.42	2283.63	628.09	1065.74	3977.46
2Bgn1	16.40	104.80	829.74	213.78	229.66	1167.79	2545.76	270.17	1093.91	3909.84
2Bgn2	21.21	32.99	498.76	173.22	175.57	892.27	1772.80	1347.41	1090.16	4210.37
2Bgn3	30.88	34.68	617.35	185.16	95.93	788.44	1721.56	808.87	1717.51	4247.93
2Bgz1	12.76	39.28	582.54	171.45	207.57	890.50	1891.34	796.86	1311.80	4000.00
Profile P3										
Ak1	52.90	29.59	541.24	210.83	298.03	950.68	2030.37	2167.98	1439.52	5637.87
Ak2	59.79	33.27	490.50	212.01	170.16	1088.14	1994.08	1869.94	1015.03	4879.04
Ak3	38.34	34.54	593.16	194.16	261.37	925.31	2008.54	1355.85	1394.44	4758.83
ABk	55.34	47.14	522.95	190.47	243.49	857.46	1861.51	653.89	1987.98	4503.38

in the three profiles, ranging within 155.37–249.17 mg kg⁻¹, corresponding to 4.6%, 4.7% and 4.1% of P-total for profiles P1–P3, respectively. The results of P.i-NaOH I showed little differentiation compared to those of P.i-HCl, a non-labile fraction corresponding to the inorganic forms of P associated with Ca. For the fractions bound to Ca, there were contents from 68.13 to 298.03 mg kg⁻¹, whose percentages in relation to P-total were 3.4%, 4.3% and 4.9% in profiles P1, P2 and P3, respectively. However, we acknowledge that this assumption of the P.i HCl associated with Ca-linked fractions is an oversimplification (Barrow *et al.* 2021).

The highest contents of inorganic P were in the P.i-NaOH II fractions, fraction of P occluded in microaggregates, with contents higher than 1000 mg kg⁻¹, representing 21%, 22% and 19% in profiles P1–P3, respectively in relation to P-total. The P.i-total is the fraction with the highest means of P extracted from each profile (Table 3), with contents of 1638.15–2545.76 mg kg⁻¹.

The highest contents of total organic P (P.o-total) occurred in the surface horizons, representing 19.5%, 17.6% and 30.6% in relation to P-total for profiles P1–P3, respectively. Regarding the total organic P contents, the highest values were in profile P3, being associated with the highest contents of TOC and CaCO₃ of this profile.

In general, P-residual contents were higher than those found in tropical soils (Table 3 and Fig. 2), representing 33.3% and 29.0% of P-total, in profiles P2 and P3, respectively. The P-total contents were high, ranging within 3782.12–5637.87 mg kg⁻¹, and were higher than those observed in soils formed under tropical climate conditions (Agbenin and Tiessen 1994; Araújo *et al.* 2004).

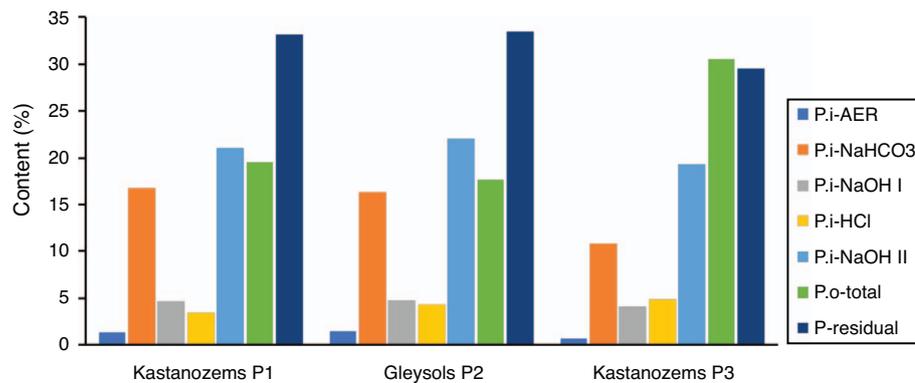


Fig. 2. Average proportions of inorganic P (P.i-AER, P.i-NaHCO₃, P.i-NaOH I, P.i-HCl and P.i-NaOH II), residual P and total organic P (P.o-total) in soils developed in carbonate soils of the Pantanal Sul Mato Grossense.

Correlations between P fractions and soil attributes

The main positive correlations of P-labile were verified for the contents of P.i-NaHCO₃ (Table 4) with P.i-AER ($r = 0.89$, $P < 0.01$), P.i-NaOH I ($r = 0.73$, $P < 0.05$), P.i-total ($r = 0.86$, $P < 0.01$) and Fe_o ($r = 0.87$, $P < 0.01$), and there were correlations between the labile forms of P and the Fe oxides of low crystallinity.

For contents of moderately labile P, the values of P.i-NaOH I showed positive correlations with P.i-NaHCO₃ ($r = 0.73$, $P < 0.05$) and P.i-total ($r = 0.82$, $P < 0.01$).

For contents of non-labile P, the values of P.i-HCl showed positive correlations with P-total ($r = 0.74$, $P < 0.05$), pH in KCl ($r = 0.82$, $P < 0.01$), CaCO₃ ($r = 0.69$, $P < 0.05$), Fe_o/Fe_d ($r = 0.79$, $P < 0.05$) and Al_o/Al_d ($r = 0.73$, $P < 0.05$). This fraction also had negative correlations with Fe_d ($r = -0.76$, $P < 0.05$) and Al_d ($r = -0.68$, $P < 0.05$). However, the values of P.i-NaOH II showed a positive correlation only with P.i-total ($r = 0.77$, $P < 0.05$).

The only positive correlation verified for P-residual was with CaCO₃ contents ($r = 0.71$, $P < 0.05$). For contents of P.o-total, a positive correlation was observed only with P-total contents ($r = 0.88$, $P < 0.01$). The P-total also showed positive correlations with CaCO₃ ($r = 0.83$, $P < 0.01$) and pH in KCl ($r = 0.69$, $P < 0.05$), demonstrating the influence of these two attributes on the P dynamics of Pantanal soils.

Discussion

In the three profiles, high contents of P.i-labile were verified, with P.i-NaHCO₃ being much higher than Mehlich-1 and P.i-AER. Regarding the P extracted by Mehlich-1, the lower values may be associated with the high pH of the soils (greater than 8), and pH of ~6.2 is the maximum recommended limit for this extractant (Yee and Broersma 1987). Mehlich extractants are composed of a mixture of strong acids at low concentrations, with initial pH of 2.5–3, and there may be an increase when the soil pH determined in KCl exceeds 7 or the extractable Ca exceeds 20 cmol_c kg⁻¹ (Table 1), underestimating the results of P extracted from the soil (Yee and Broersma 1987; Zbiral 2000). Another relevant factor that affects the extraction of labile P by Mehlich-1 is the

characteristics and contents of 2:1 clay, where the SO₄²⁻ of the extractant, which acts at the exchange site replacing the adsorbed phosphate ions, is quickly adsorbed at other sites, not yet occupied by P. Despite the underestimation in the results, the Mehlich-1 extractor is still widely used in the analysis and recommendations of P in the soils of Pantanal, generally following the standards of the technical bulletin for Cerrado biome soils elaborated by Lopes and Guilherme (1994).

The dynamics that contribute to the high contents of P.i-labile in the Pantanal soils are initiated and determined by the seasonality of floods. In these periods, the processes of reduction occur with maximum intensity even in profiles with moderate drainage, and the P is made available according to the reduction of Fe changing to freer forms (Jien *et al.* 2016). With the soil saturated by water, depletion of pore gases and consequently the reduction of oxygen content occur. In this period, Fe is used as an electron receptor for the oxidation of organic matter (OM), contributing with the release of P bound to these oxides, and as these metals are reduced the pH increases, since this reaction consumes protons, facilitating the release of P bound to Fe and pH (Shenker *et al.* 2005; Couto and Oliveira 2011; Henderson *et al.* 2012). The positive correlation between P.i-NaHCO₃ and Fe_o contents corroborates this hypothesis, but this extractant may lead to obtaining forms of P bound to oxides, which also explains the positive correlation with the extractant P.i-NaOH I (Jien *et al.* 2016).

In periods of drought, the Fe oxide contents tend to increase, since the oxygen concentration rises, occupying the free pores of the soil. In this period, the free P in the solution binds again to the oxides, changing to moderately labile forms such as P.i-NaOH I (Chacón *et al.* 2006). Low-crystallinity oxides tend to have a larger specific surface, contributing to P adsorption, agreeing with the results found, demonstrating that even in an environment with low concentrations of Fe₂O₃ and Al₂O₃, these may still interfere in the adsorption dynamics (Borggaard *et al.* 1990; Donagemma *et al.* 2008).

The P.i extracted with HCl showed a positive correlation with CaCO₃, P-total and pH in KCl, demonstrating that the

Table 4. Linear correlation coefficient (r) between phosphorus fractions and some attributes present in the three profiles

P.i-AER, anion-exchange resin; P.i-NaHCO₃, 0.5 mol L⁻¹ sodium bicarbonate; P.i-NaOH I, 0.1 mol L⁻¹ sodium hydroxide; P.i-HCl, 1.0 mol L⁻¹ hydrochloric acid; P.i-NaOH II, 0.5 mol L⁻¹ sodium hydroxide; P.i-total, total inorganic phosphorus; P.o-total, total organic phosphorus; COT, carbon organic total; Fe_d, iron oxide extracted by the citrate bicarbonate dithionite (DCB); Fe_o, iron oxide extracted by oxalate; Al_d, aluminium oxide extracted by DCB; Al_o, aluminium oxide extracted by oxalate; * significant at P < 0.05, ** significant at P < 0.01

	P.i-AER	P.i-NaHCO ₃	P.i-NaOH I	P.i-HCl	P.i-NaOH II	P.i-total	P-residual	P.o-total	P	pH KCl	CaCO ₃	COT	Fe _d	Fe _o	Fe _o /Fe _d	Al _d	Al _o
P.i-NaHCO ₃	0.89**																
P.i-NaOH I	0.63	0.73*															
P.i-HCl	-0.07	0.02	0.49														
P.i-NaOH II	0.39	0.43	0.42	0.37													
P.i-total	0.77*	0.82**	0.42	0.42	0.77*												
P-residual	-0.34	-0.58	-0.16	0.16	-0.43	-0.48											
P.o-total	-0.49	-0.38	0.10	0.48	-0.21	-0.21	0.02										
P-total	-0.27	-0.24	0.41	0.74*	-0.04	0.04	0.27	0.88**									
pH KCl	-0.05	-0.07	0.34	0.82**	-0.08	0.15	0.47	0.34	0.63								
CaCO ₃	-0.38	-0.47	0.18	0.68*	-0.16	-0.17	0.71*	0.58	0.83**	0.69*							
COT	0.21	0.03	0.34	0.19	0.04	0.13	-0.05	0.60	0.60	0.16	0.27						
Fe _d	0.58	0.47	-0.04	-0.76*	0.04	0.15	-0.49	-0.51	-0.66	-0.66	-0.84**	0.16					
Fe _o	0.65	0.87**	0.54	-0.21	0.06	0.55	-0.65	-0.22	-0.27	-0.21	-0.59	-0.02	0.53				
Fe _o /Fe _d	-0.21	0.01	0.56	0.79*	0.00	0.22	0.20	0.64	0.82*	0.66	0.71*	0.13	-0.78*	0.02			
Al _d	0.58	0.63	0.21	-0.68*	-0.20	0.18	-0.48	-0.35	-0.49	-0.56	-0.69*	0.04	0.80**	0.83**	-0.38		
Al _o	0.51	0.66	0.21	-0.50	-0.25	0.21	-0.58	-0.25	-0.42	-0.34	-0.70**	-0.02	0.69*	0.91**	-0.24	0.92**	
Al _o /Al _d	-0.59	-0.39	-0.02	0.73*	-0.09	-0.14	0.19	0.63	0.64	0.72	0.55	0.02	-0.78*	-0.33	0.68*	-0.70*	-0.40

solubility of P in these areas decreases with the increase in pH. However, for these P fractions, the contents were close to those extracted with 1 mol L⁻¹ NaOH solution, and the main hypothesis is that Ca is bound to OM, forming organic compounds with low solubilisation and making P available (Perassi and Borgnino 2014). The humic substances present in OM contain carboxylic groups, which can compete with P for adsorption sites, besides inhibiting the formation of more stable precipitates of Ca-P, such as OCP and HAP, precursors of apatite formation (Weyers *et al.* 2017; Lei *et al.* 2018). Thus, OM may be contributing to the high contents of P.i-labile, extracted by NaHCO₃ (Ma *et al.* 2019). The alternation of wetting and drying may also contribute to the values of P bound to Ca²⁺ being low, even if these soils have high CaCO₃ contents in the sediments. The period in which the soils are saturated with water can hinder the process of adsorption of P with Ca²⁺ or even contribute to the dissolution of the most recalcitrant forms of the bond of P with Ca²⁺ (Chacón *et al.* 2005, 2006).

The P.i-NaOH II was the most abundant fraction in the three profiles, characterised by being P occluded in microaggregates, and may be present in strongly or moderately protected form; however, this fraction is difficult for plants to extract (Vázquez *et al.* 2011). The permanent charges of clays with high adsorption power protect the ions inside the aggregates, contributing to the high contents of this fraction of P in the studied soils (Donagemma *et al.* 2008).

The P-residual contents may be associated with the high contents of CaCO₃ related to OM, which promotes chemical protection for P. The OM may react with Ca-P precipitates, forming complex compounds of low molar ratio, co-precipitates of Ca-P-OM, delaying the formation of HAP and apatite (Pereira *et al.* 2013; Lei *et al.* 2019). These co-precipitates of Ca-P-OM hamper the extraction of P by the method proposed for inorganic P associated with Ca, being later extracted by sulfuric digestion and quantified as residual P fraction. The high contents of P-residual may also be related to the high contents of CaCO₃ associated with the forms of inorganic P found in the studied soils, which demonstrates that not all P bound to Ca²⁺ can be extracted by means of acid extractants such as HCl (Araújo *et al.* 2004).

Fractionation limitations and challenges for future research

In recent years, the main P fractionation methods, including Hedley's, have been objects of studies and discussions, being presented by many authors as a methodology of false detection of P (Condrón and Newman 2011; Barrow 2021; Gatiboni and Condrón 2021; Gu and Margenot 2021; Guppy 2021), and considered by Barrow *et al.* (2021) to be 'the soil phosphate fractionation fallacy'. Barrow *et al.* (2021) reacted a sample of goethite and a sample of aluminium oxide with a phosphate solution under conditions chosen to facilitate penetration of phosphate, resulting in detection of discrete compounds even though none were present. However, it is worth noting that works like Condrón and Newman (2011) and Gu and Margenot (2021) do not rule out the soil P fractionation, but recommend the use of existing schemes with more forethought and instead of focusing on discrete P compounds extracted at each stage of

the procedure users should focus on the objective of the procedure, with fractionation being an approximation to reality.

The main objective of the present work is not linked to the recent discussions on the problems of fractionation methods, being a broader discussion that needs new methodological resolutions and epistemological discussions (Gu and Margenot 2021; Guppy 2021). However, in order to stimulate new research in the Pantanal biome, here we open some of the possible problems of the work that must be addressed with the use of more specific techniques in future works allowing a better understanding of the structurally and functionally defined fractions. The main problems according to Condrón and Newman (2011) follow: the high levels of OM, possibly recalcitrant, associated with high levels of CaCO_3 , making it difficult to extract P by the method proposed for the extraction of inorganic P; some inorganic P molecules may be associated with mineral colloids and organic macromolecules that prevent their determination by colourimetry; the studied soils present limitations in drainage and are saturated in the flood periods of the Pantanal, which favours a reducing environment. It is necessary to use adaptations to extract P; in relation to the previous problems, it is possible that the inorganic P was not completely extracted, the extraction of some fractions being incomplete.

Conclusions

The profiles had high contents of labile P, and the highest values were extracted with NaHCO_3 . This fraction had a positive correlation with low-crystallinity Fe oxides (Fe_o) and with P adsorbed to oxides (P.i-NaOH 0.1 mol L^{-1}). The process of Fe reduction during flood periods may have contributed to making adsorbed P available, changing it to more labile forms.

Moderately labile P contents, the fraction adsorbed to oxides and extracted with 0.1 mol L^{-1} NaOH, showed few differentiations compared to the contents of non-labile P associated with Ca (extracted with 1.0 mol L^{-1} HCl). Possibly, OM bound to Ca formed organic compounds with low solubilisation and inhibited or delayed the formation of precipitates of Ca with P, making P available for the most labile fractions.

The non-labile P fraction occluded in microaggregates, extracted with 0.5 mol L^{-1} NaOH, had the highest contents of inorganic P in the studied soils.

Conflict of interest

The authors declare no conflict of interest.

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