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Phosphorus Forms and Their Spatial Distribution in Permanent Pasture on Karst Landscapes

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Abstract. Grazing pasture implies recycling of nutrients from manure and accumulation of soil nutrients, especially P which raise an environmental concern. We applied a modified Hedley P fractionation procedure to soils from three sinkholes grazed by cattle as well as a wooded ungrazed sinkhole to describe soil P distribution as a function of soil depth and sinkhole topography. Absolute concentrations of all P fractions were highest in the upper 0-5 cm soil depth for all sinkholes and decreased with depth. Changes in inorganic P (Pi) and organic P (Po) differed with depth leading to increased Pi:Po ratio with depth for the NaOH extracts, but decreased for the $NaHCO_3$ extracts. The most pronounced difference between the grazed and ungrazed sinkholes was for resin extractable Pi, which is an indicator of biologically available P and is therefore of environmental concern. Higher levels of resin extractable P, especially near the bottom center of the grazed sinkholes, indicates a high potential for surface P transport. Erosion and surface runoff may have occurred resulting all soil P fractions to move from the outer rim toward the bottom center of the sinkholes.

Introduction

The impact of agricultural activity on water quality in karst landscapes is an important consideration for resource management in the Appalachian Region of the eastern U.S. Karst areas constitute about 18% of the land area (Davies, 1984), and an estimated one-third of the Region's farms, cattle, and agricultural enterprises (Boyer and Pasquarell, 1995). In general, the deposition of manure by grazing cattle can elevate P contents in the soil to levels that enrich surface and subsurface water with P (Sharpley and Moyer, 2000; Sims *et al.*, 1998). The severity of this process is expected to be more pronounced in pastures on karst landscapes as, along with thin soil, limestone contains many joints and fractures creating a direct connection between the surface and ground water (Gerhart, 1986), allowing rapid contamination of ground water. Elevated levels of organic matter especially in surface soil layers as well as in deeper soil layers (Hayness and Williams, 1993) may influence the forms, mobility and transport of P to surface and ground water.

To investigate the effects of manure on soil P, the identification and quantification of individual P compounds in the soil is needed. This is difficult to achieve as the chemistry of soil P is complex. However, this type of data would improve the understanding of P mobility and transformations in the soil matrix. As an alternative, sequential fractionation can facilitate separating soil P into classes of compounds that are often defined functionally depending on the extractants by which they are removed (Yaobing *et al.*, 1999). Data from a sequential fractionation of soil P forms in soils of pasture on karst landscapes is lacking and would be useful for the interpretation of the effect of grazing on P build up and distribution among fractions. It would also be useful to evaluate soil P dynamics in permanents pastures of karst landscapes and the potential for transport into ground water by surface and subsurface runoff (Sharpley and Moyer, 2000).

As summarized in a review article by Potter *et al.* (1993), the Hedley P fractionation method (Hedley *et al.*, 1982) was used in a number of studies

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to evaluate various aspects of P cycling including long term changes, P transformations during pedogenesis, effects of management on P transformations and others. For example, O'Halloran (1993) used the procedure to evaluate the impact of tillage and P fertilization on P dynamics in two soils in Canada and in an earlier study (O'Halloran *et al.*, 1987) evaluated the impacts of 14 years of tillage systems on P forms in a loam soil in western Nebraska. Tiessen *et al.* (1983) used the procedure to evaluate the effects of long-term cultivation (60-90 years) on soil P dynamics. Cross and Schlesinger (1995) concluded that the Hedley fraction procedure (Hedley *et al.*, 1982) could be used as an index to study the importance of biological process on soil P forms across soil weathering gradients. To date no one has used the Hedley P fractionation procedure (Hedley *et al.*, 1982) to evaluate the impacts of animal agriculture on soil P forms or the spatial distribution of soil P forms in karst landscapes.

Soil P fractionation procedures allow soil P to be separated and characterized by sequential extraction of soil with different extractants, each extractant removing specific forms of soil P. In the Hedley procedure (Hedley *et al.*, 1982), the initial extract is a measure of anion exchange resin extractable P (resin-P) which is considered by some researchers to be equivalent to bioavailable P in the (Wright and Coleman, 1999; Schoenau *et al.*, 1989; Wagar *et al.*, 1986; Stevenson, 1986; Tate, 1984, 1985; Headley *et al.*, 1982). Resin extractable-P along with the second extract in the sequence, bicarbonate ($NaHCO_3$) extractable-P, are collectively referred to as labile soil P. The bicarbonate extract is thought to extract P on surfaces of more crystalline P compounds, sesquioxides or carbonates (Mattingly, 1975). Hydroxide (NaOH) extractable Pi is extracted next and is considered to be less available for plant uptake, since this form of P is associated with amorphous and some crystalline Al and Fe oxides (Williams, 1980). Acid extractable (HCl) Pi is mostly Ca (apatite) bound P (Williams, 1980). Several of the extracts are analyzed for inorganic (Pi) and organic-P (Po) forms. Bicarbonate extractable Po is considered easily mineralized and contributes to biologically available P (Bowman and Cole, 1978). More stable forms of Po involved in long-term transformations of P in soil are extracted with NaOH (Tiessen *et al.*, 1984). The residual P forms from the Hedley fractionation procedure (Hedley *et al.*, 1982) also account for a resistant mixture of occluded Pi bound with sesquioxides, Ca-Pi included in other minerals, and non-extractable Po (Tiessen *et al.*, 1984), which is usually extracted by complete digestion of the soil by H_2SO_4/H_2O_2 (Hedley *et al.*, 1982).

Our objectives were to quantify the distribution of soil P at various depths among different fractions as influenced by grazing on karst landscapes. Soils were sampled along transects crossing the sinkholes to define changes in forms of soil P with topographic position and soil depth. Special emphasis was placed on the influence of manure deposited by grazing animals on the potential for different soil P fractions to influence water quality, because bioavailable P form can stimulate eutrophication.

Materials and Methods

The study site (Fig. 1) includes three sinkholes [Little sink, Big sink, and Burns (0.5 km east of the sites shown on the map) sink] in a pasture grazed intermittently by cattle from April through October each year and a non-grazed Wooded sinkhole used as a control. The soil is a Caneyville series (fine-loamy, mixed, active, mesic, Typic Eutrudept) in sinkholes floors and a Frederick series (clayey, mixed, active, mesic, Typic Paleudult) on the side slope. The dominant forage species in the grazed sinkholes were tall fescue (*Festuca arundinacea* Schreb.) and red clover (*Trifolium pratense* L.). Soil test results show pH above 5.5 so lime is not spread most years. However, the site did receive 4.94 Mg ha^{-1} in the fall of 1994 and in the fall of 2000. Mineral fertilizers N, P, and K are applied each spring at rates 34, 100, and 56 kg ha^{-1}, respectively. Vegetation in the ungrazed Wooded site consisted of trees, shrubs and forbs. The dominant species were shagbark hickory (*Carya ovata* Mill. K. Koch), multiflora rose (*Rosa multiflora* Thunb.), and poison ivy (*Rhus radicans* L.). Sinkhole diameters ranged between 102-120 m in width and between 5-29 m in depth. Average slopes, as measured from the sinkhole rims, were 13, 17, 8, and 33% along an east-west transect for Little, Big, Burns, and the Wooded sinkholes, respectively.

Soil sampling

Soil samples were collected at 6 to 9 m intervals along a single transect (east-west) across each of the four sinkholes near the sinkhole rims and more closely spaced (3 m) near the sinkhole drain. Eighteen locations and four soil depths (0-2.5 cm; 2.5-5.0 cm; 5-10 cm; 10-20 cm) were sampled. The samples were air-dried and ground to pass 2-mm sieve and then 100 mesh sieve prior to analysis.

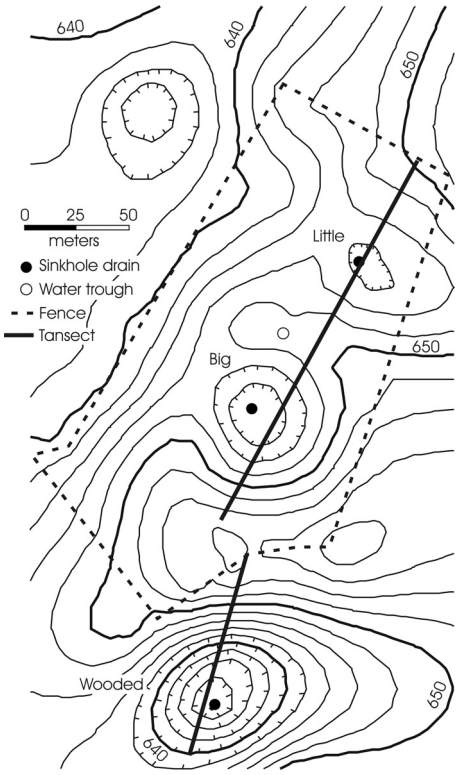

Fig. 1. Contour map of the study area (Burns sinkhole, located 0.5 km east, is not shown). Contour interval = 2 m.

Soil analysis

Prior to soil P fractionation, soil samples were analyzed for total N, organic C, soil pH, and EC. Total N and C were analyzed using a Carlo Erba analyzer (EA1108 CHNSO, Milan, Italy). Soil pH and EC were determined in a 1:1 soil to water ratio (Kuo, 1996).

Soil P fractionation

Soil phosphorus was fractionated using a slight modification of the Hedley procedure (Hedley *et al.*, 1982) (Fig. 2). Subsamples (0.5 g) of air-dried soil were weighed into 50 mL centrifuge tubes and extracted sequentially by shaking for 16 hrs on a reciprocating shaker at a rate of 200 stroke min^{-1}. Initially, the soil was extracted with 30-mL deionized water plus an anion exchange resin bag (1.5 g resin, IRA-402 transformed from original Cl^- form to the HCO_3^- form). After shaking, the anion exchange resin bag was removed and the tubes were centrifuged at 5,000 rpm for 15 min. The anion exchange resin bags were extracted using 30-mL of 0.5 M HCl for 2 hrs, and the resulting extracts were analyzed for PO_4-P. In the standard Hedley procedure (Hedley *et al.*, 1982), the water supernatant from the anion exchange resin extraction is discarded. However, in our investigations the water supernatant was filtered through a 0.45 µm filter, checked for inorganic P by the molybdate-ascorbic method followed by analysis for total P by ICP representing organic-P (Po), as this fraction was not retained by the anion exchange resin.

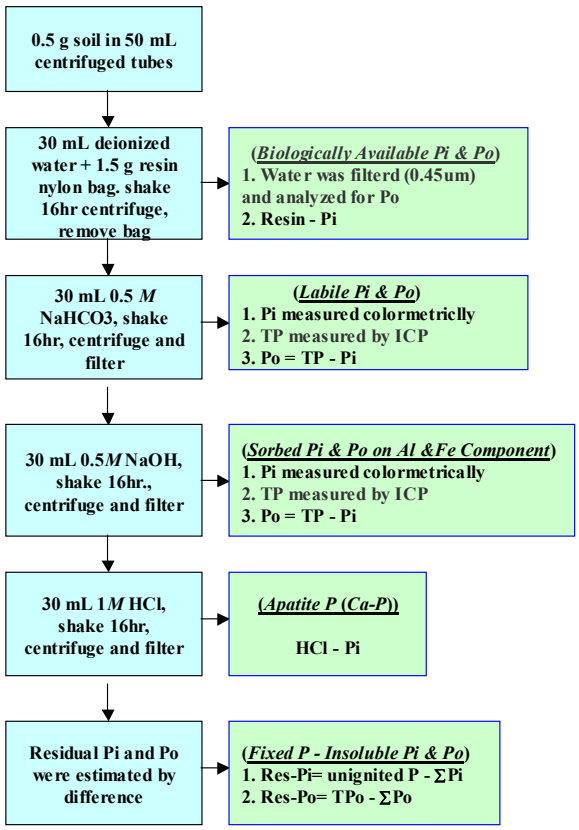

Fig. 2. Sequential P fractionation scheme, modified from the original Hedley P-fractional procedure (Hedley *et al.*, 1982).

Following the resin extraction procedure, soil remaining in the tubes was extracted sequentially with 0.5 *M* $NaHCO_3$, 0.5 *M* NaOH, and 1 *M* HCL, respectively. Each extraction was carried out by adding 30 mL of the respective extracting solutions and shaking for 16 hrs, followed by centrifugation for 15 min at 5,000 rpm and collecting the supernatant. Supernatants were filtered through Whatman No. 1 filter paper and analyzed for inorganic-P (Pi) and total P. Phosphorus extracted in this manner represent labile P ($NaHCO_3$), sorbed P on Al and Fe components (NaOH), and apatite P (HCl), respectively (Hedley *et al.*, 1982). Total P (Pi + Po) in $NaHCO_3$ and NaOH extractants were measured by ICP which enabled us to analyze a large number of samples to evaluate spatial differences in P forms across the sinkhole topography. The validity of ICP

for measurement of total P in various extractants was checked by comparing an average of 25 values obtained by ICP and acid- persulfate digestion (EPA, 1971). Linear regressions between total-P values obtained by ICP and the digestion method are in very good agreement and correlations were highly significant (r^2 = 0.9889***, 0.9874***, 0.9482***, 0.9981***, and 0.9981*** for water, $NaHCO_3$, NaOH, HCl, and H_2SO_4 extracts, respectively).

Total organic phosphorus

Subsamples (0.5 g) of air-dried soil were analyzed for total organic P (TPo) by the ignition method (Kuo, 1996). Ignited (550°C for 1 hr) and unignited soil samples were extracted for 16 hrs with 30 mL of 1 *M* H_2SO_4. In this procedure, total organic P (TPo) is determined by subtracting P extracted from the unignited samples from P extracted from the corresponding ignited samples.

Determination of phosphorus

Inorganic P (Pi) in the $NaHCO_3$ and NaOH extracts was analyzed using the ammonium molybdate-ascorbic acid method (Murphy and Riley, 1962). Total P in solutions and total P in the aqueous supernatant of the resin extraction (Fig. 2, Step 1) are measured by ICP (Jobin Yvon Model JY46P ICP, Longjumeau, France) according to the settings recommended by the manufacturer that permits P determination for heavily salted solutions. Total phosphorus (TP) in the 0.5 *M* HCl, and 1 *M* H_2SO_4 extracts was also measured by ICP. Organic P (Po) in each extractant was calculated as the difference between TP obtained by ICP and molybdate reactive P (Pi). Residual Pi and Po were estimated as the difference between unignited P, TPo and the sum of Pi and Po fractions, respectively (Forssard *et al.*, 1989; Sharpley *et al.*, 1995).

Determination of dissolved organic carbon

Dissolved organic C (DOC) was determined in the water supernatants generated from resin exchangeable P (Fig. 2, Step 1) using a modification of the Sims and Haby organic matter method (Sims and Haby, 1971). Duplicate 2 mL subsamples of soil water extracts were placed in 20 mL glass tubes, along with 2 mL of 0.167 *M* $K_2Cr_2O_7$ and 2 mL of concentrated H_2SO_4. Tubes contents were mixed by swirling and allowed to stand for 20 min in a hot water bath (70°C). After oxidation, the volume of the mixture in each tube was adjusted to 10 mL with distilled water. Absorbance was measured spectrophotometrically at 600 nm. Standards containing 0-1000 µg of organic carbon (as sucrose) were treated exactly the same way as the samples.

Statistical analysis

The effect of grazing on P forms for various soil depth increments was analyzed by analysis of variance (ANOVA) using SAS procedures (SAS, 1999). Mean separation was also determined for variables in each soil depth increments using the least significant difference (LSD = 0.05).

Results

Total soil C and N, while decreasing gradually with increasing soil depth regardless of grazing management (P = 0.0001), were higher in Burns and Wooded than Little and Big sinkholes (Table 1). The decrease in total soil C with depth was greater than total N leading the C:N ratios to decrease with depth in all sinkholes, the decrease being the least in Burns sinkhole. Soil pH remained almost unchanged with depth in grazed sinkholes and decreased significantly with depth (P = 0.0001) in the ungrazed Wooded sink (from 6.51 in the surface 0-2.5 cm layer to 5.69 at the 10-20 cm soil depth). Soil pH values were lower at all soil depths in Burns compared to Little and Big sinkholes (Table 1). Grazing did not influence EC values, which decreased with soil depth in all sinks. All ions extracted with 1*M* ammonium acetate (S, Ca, Mg, K, and Na) decreased with soil depth. With the exception of Na, the concentrations of all ions were significantly higher in Wooded compared to grazed sinkholes (Little, Big, and Burns).

Fractionation of soil phosphorus

Average values for P distribution among the various forms in the pastured sinkholes versus the ungrazed Wooded sinkhole are presented in Table 2. For the simplicity of the discussion, spatial distribution of P fractions, total Po, and TC will be presented from the Burns sinkhole for the surface soil layer (0-2.5 cm), which is the most important soil depth influencing soil and water quality (Figs. 3-9).

Table 1. Characteristics of various soil depth increments (cm) for Little, Big, Burns, and Wooded sinkholes

Sinkhole	Soil Depth	pH	EC ds/m^{-1}	Total N $g\ kg^{-1}$	Total C $g\ kg^{-1}$	C/N Ratio	1 *M* Ammonium acetate extraction ($mg\ kg^{-1}$)				
							S	Ca	Mg	K	Na
Little	0-2.5	6.22	0.28	4.4	40.4	9.2	29	1413	168	259	14
	2.5-5	6.35	0.15	2.4	20.3	8.4	19	887	83	131	8
	5-10	6.33	0.09	1.3	9.5	7.5	11	602	48	85	7
	10-20	6.21	0.06	0.9	7.0	7.6	11	512	37	63	9
Big	0-2.5	6.12	0.28	5.0	47.8	9.5	26	2001	179	268	7
	2.5-5	6.19	0.22	3.7	32.0	8.6	20	1665	126	193	6
	5-10	6.15	0.13	2.1	16.4	8.0	13	1231	74	124	6
	10-20	6.10	0.10	1.3	9.4	7.1	13	1162	67	102	7
Burns	0-2.5	5.74	0.32	6.0	53.2	8.9	32	1950	260	323	14
	2.5-5	5.52	0.16	4.0	33.4	8.3	21	1435	121	149	11
	5-10	5.59	0.09	2.5	20.3	8.2	14	1104	70	84	11
	10-20	5.63	0.07	1.7	13.8	8.3	14	944	49	57	10
Wooded	0-2.5	6.51	0.28	5.5	52.1	9.4	26	3598	161	296	4
	2.5-5	6.27	0.20	4.1	36.3	8.9	21	2775	115	242	4
	5-10	5.93	0.14	2.7	23.0	8.5	16	1857	81	192	3
	10-20	5.69	0.10	1.8	14.4	8.2	15	1503	60	133	3
Grazing		0.0001	0.0529	0.0001	0.0001	0.0002	0.0001	0.0001	0.0001	0.0001	0.0001
Depth		0.0131	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0013
G x D		0.0002	0.0026	NS	NS	0.0001	0.0013	0.0001	0.0067	0.0349	NS

Table 2. Phosphorus removed in sequential extraction (mg kg^{-1}) for various soil depth increments (cm) for Little, Big, Burns, and Wooded sinkholes. Values in parentheses are percentage of P fraction of total P

Soil	Sinkhole	Inorganic P				Organic P			Total	Residual		Total
Depth		Resin	$NaHCO_3$	NaOH	HCl	H_2O	$NaHCO_3$	NaOH	Po	Pi	Po	P
0-2.5	Little	191b (16)	39b (3)	148b (12)	50b (4)	18b (2)	49b (4)	188c (15)	543bc (45)	247a (20)	287a (24)	1218b
	Big	363a (27)	51a (4)	153ab (11)	73a (3)	19b (2)	28c (2)	191c (14)	582b (43)	125c (9)	345a (26)	1347ab
	Burns	273b (19)	51a (4)	73a (3)	40bc (5)	54a (4)	71a (5)	345a (23)	665a (45)	278a (19)	194b (13)	1473a
	Wooded	67c (8)	9c (1)	40bc (5)	30c (4)	53a (6)	30c (4)	317b (37)	504c (58)	182b (21)	104c (12)	866c
2.5-5	Little	64b (9)	17c (2)	126b (17)	31b (4)	8c (1)	33b (5)	112c (15)	367c (51)	120c (17)	213b (29)	725b
	Big	242a (22)	39a (4)	158a (14)	61a (6)	13c (1)	34b (3)	152b (14)	504a (46)	103c (9)	305a (28)	1107a
	Burns	76b (8)	24b (2)	147a (15)	30b (3)	31b (3)	61a (6)	275a (27)	552a (55)	182a (18)	185b (18)	1012a
	Wooded	46b (8)	7d (1)	81c (4)	30c (4)	39a (5)	30b (4)	280a (37)	447b (59)	150b (20)	99c (13)	761b
5-10	Little	13c (3)	5c (1)	101b (22)	26b (6)	5c (1)	20c (4)	57d (12)	251c (54)	69c (15)	169b (36)	464c
	Big	80a (12)	16a (2)	116a (18)	37a (6)	6c (1)	30ab (4)	92c (14)	344b (52)	69c (10)	216a (33)	664b
	Burns	35b (5)	10b (1)	122a (17)	24b (3)	27b (4)	35a (5)	193b (26)	403a (55)	144a (20)	148b (20)	737a
	Wooded	34b (5)	6c (1)	81c (13)	31ab (5)	33a (5)	29b (5)	221a (35)	358b (57)	122b (19)	75c (12)	632b
10-20	Little	7c (2)	3c (1)	88b (23)	19c (5)	5b (1)	15c (4)	33c (9)	210c (56)	51c (14)	156ab (41)	378c
	Big	20b (5)	5b (1)	79b (18)	29ab (7)	4b (1)	19bc (5)	61b (14)	247bc (58)	49c (11)	163a (38)	429c
	Burns	23b (4)	7a (1)	115a (19)	23bc (4)	24a (4)	23ab (4)	148a (24)	328a (53)	117a (19)	133b (22)	614a
	Wooded	28a (5)	6ab (1)	85b (17)	32a (6)	28a (5)	24a (5)	157a (30)	278b (54)	88b (17)	70c (14)	515b
						$P > F$						
Graze		0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Depth		0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
G x D		0.0001	0.0001	0.0001	0.0001	0.0007	0.0001	0.0012	0.0060	0.0062	0.0001	0.0001

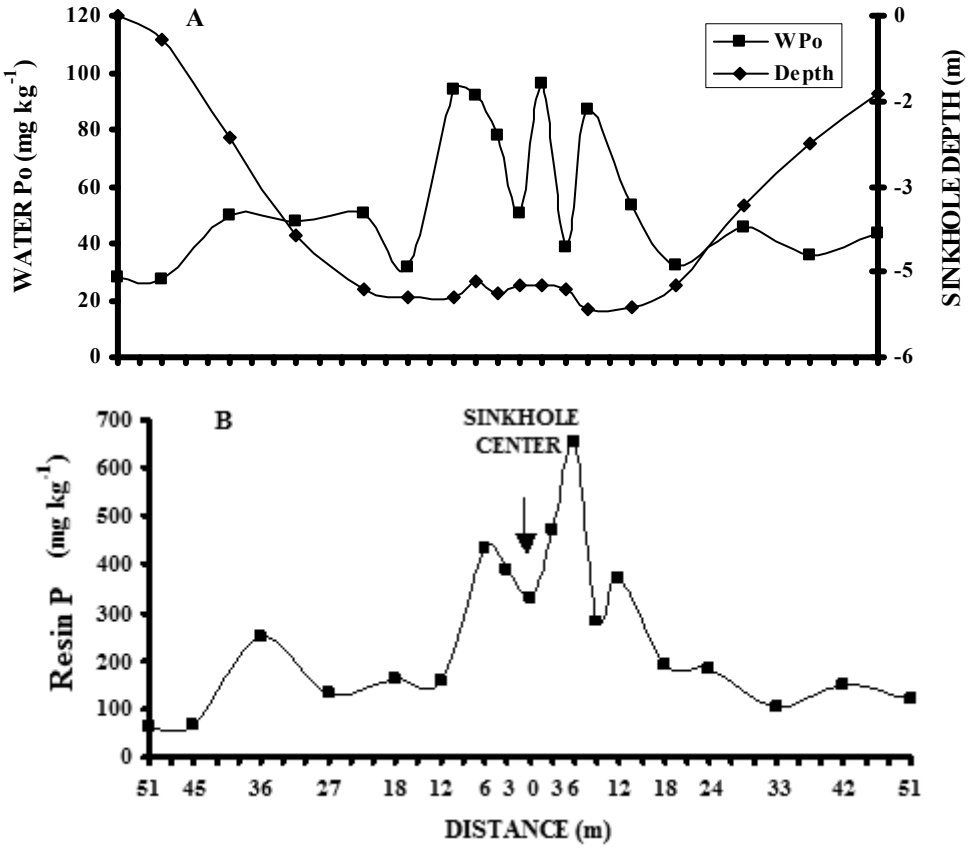

Fig. 3. Spatial distribution of water-soluble soil Po (A) and resin-Pi (B) in 0-2.5-cm soil depth of Burns sinkhole. Also shown the depth of sinkhole across the east-west sampling transect.

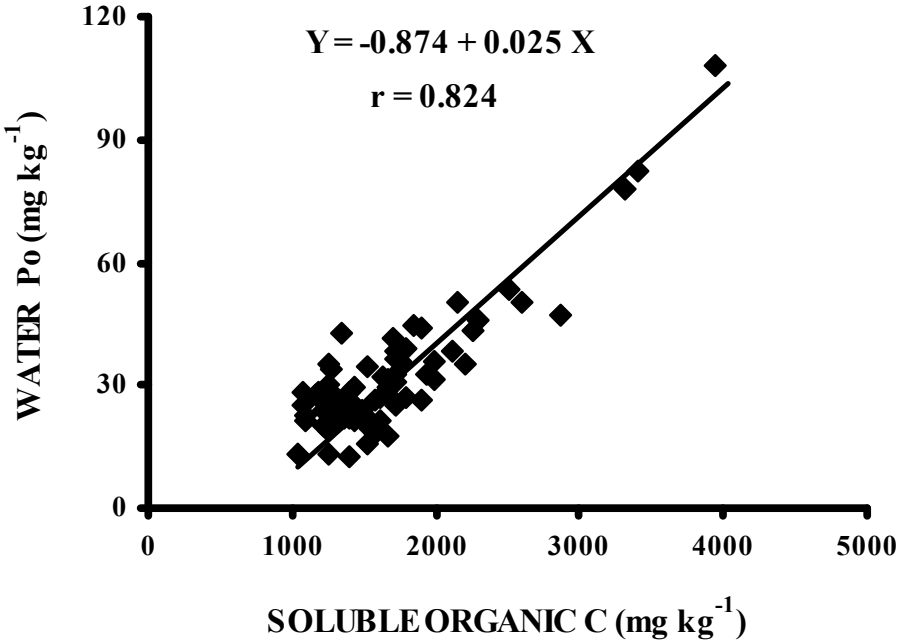

Fig. 4. Correlation between water-soluble Po extracted during the determination of resin extractable P (Fig. 3) and soluble organic C in 0-2.5-cm soil depth of Burns sinkhole.

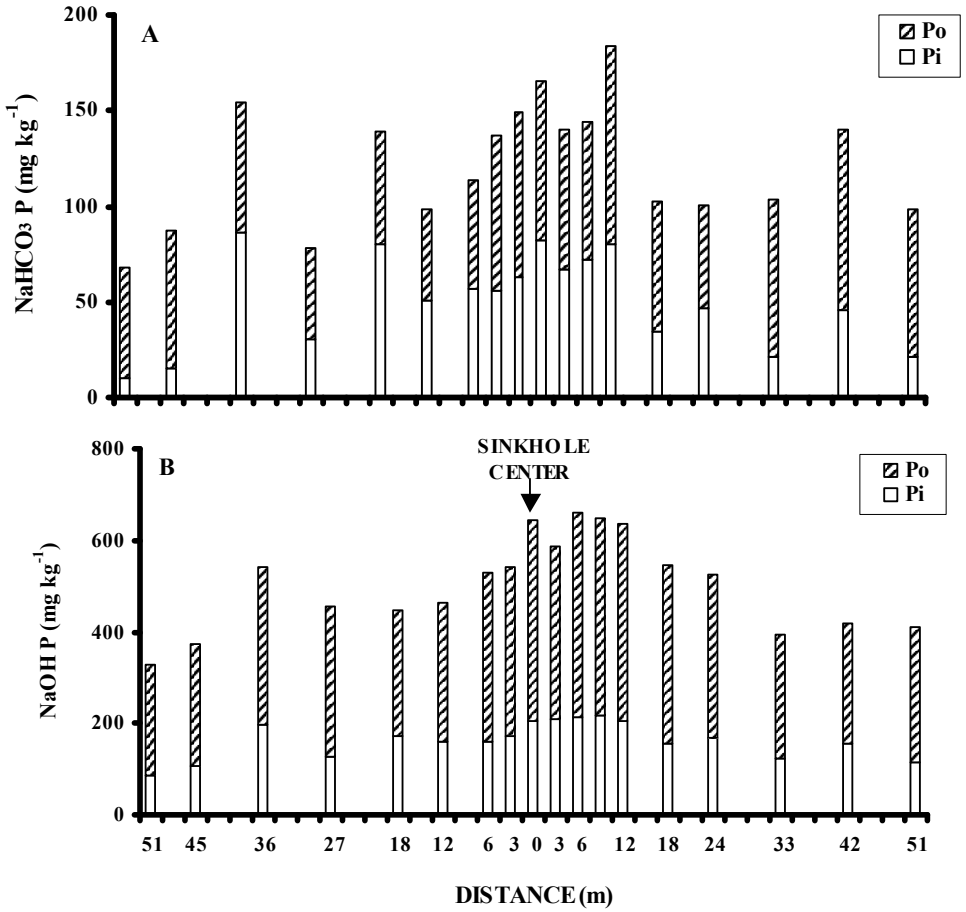

Fig. 5. Spatial distribution of Pi and Po in $NaHCO_3$ (A) and NaOH (B) extracts in the surface 0-2.5-cm soil layer of Burns sinkhole. Samples were collected along an east-west transect across the sinkhole.

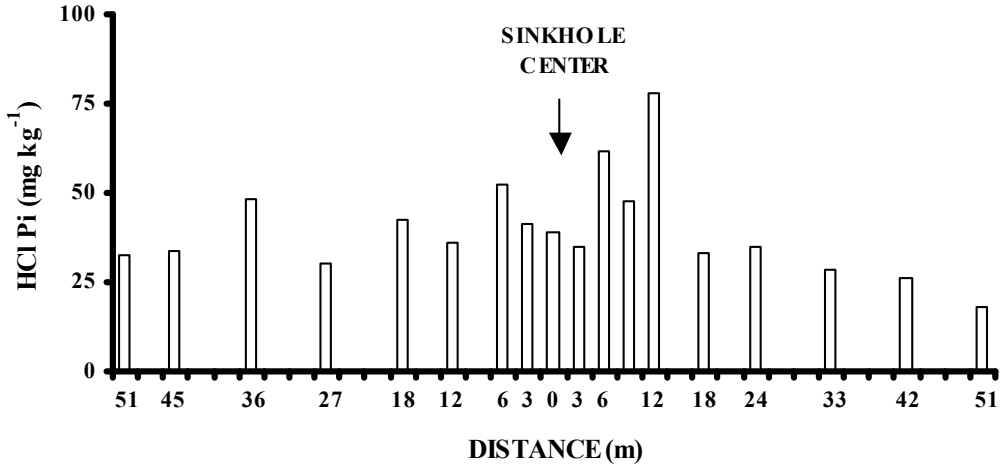

Fig. 6. Spatial distribution of HCl-Pi in surface 0-2.5-cm soil layer of Burns sinkhole. Samples were collected along an east-west transect across the sinkhole.

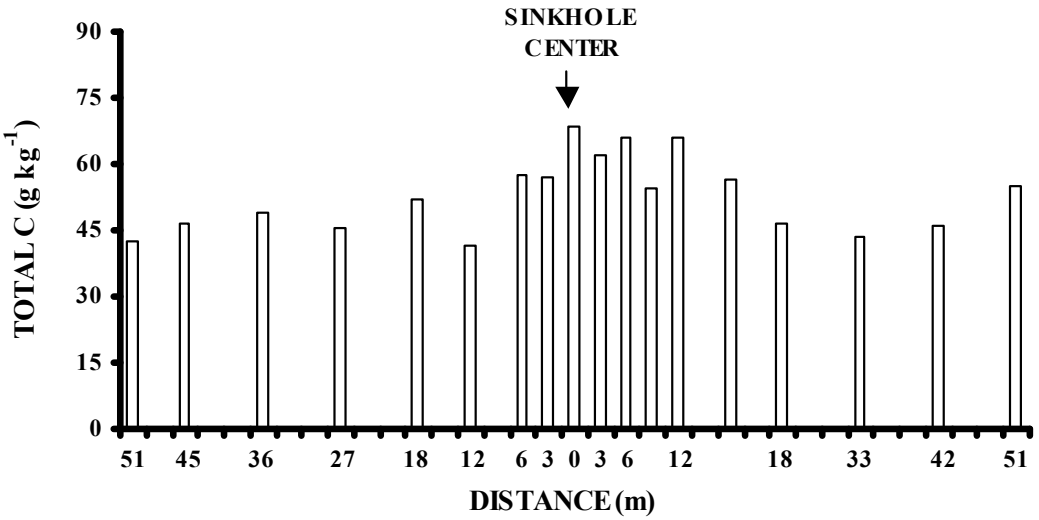

Fig. 7. Spatial distribution of total soil C content in the surface 0-2.5-cm soil layer of Burns sinkhole. Samples were collected along an east-west transect across the sinkhole.

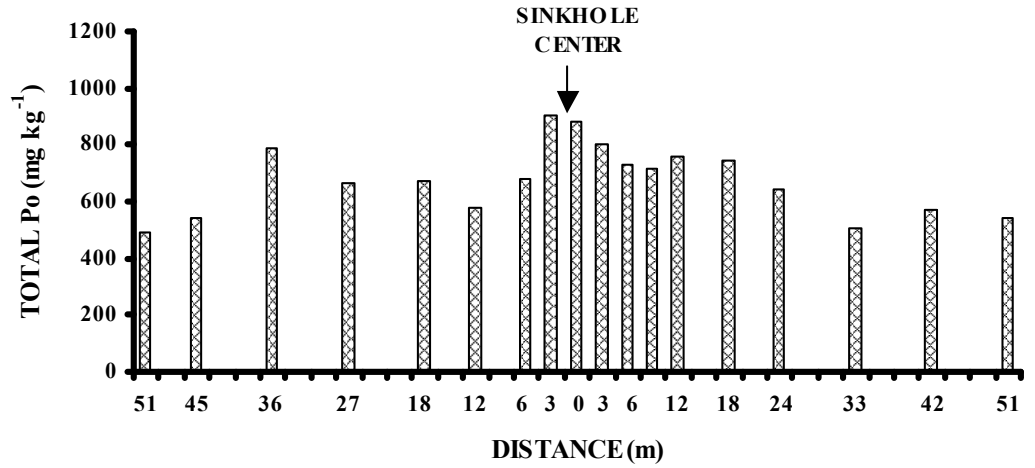

Fig. 8. Spatial distribution of total soil Po in the surface 0-2.5-cm soil layer of Burns sinkhole. Outer rim edges and sinkhole drain are marked.

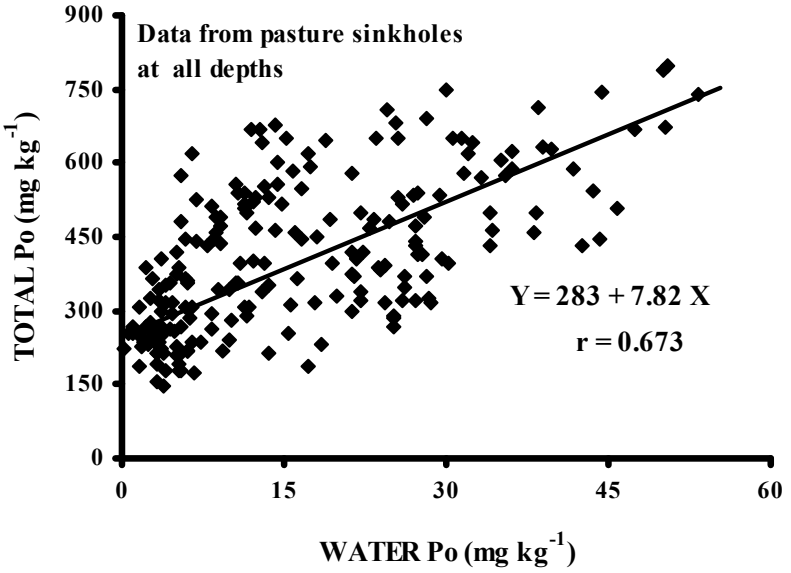

Fig. 9. Correlation between water-soluble Po extracted during the determination of resin extractable P (Fig. 2) and total soil Po for the four sinkholes at all depths.

Resin-Pi and water-soluble organic P

Grazing, soil stratum and their interaction ($P < 0.0001$) significantly influenced resin-Pi. The values of resin-Pi in the surface 0-2.5 cm layer averaged 191, 363, and 273 mg P kg^{-1} in the grazed Little, Big, and Burns sinkholes, respectively, compared to 67 mg P kg^{-1} in the ungrazed Wooded sink (Table 2). Resin-Pi values tended to decrease gradually with depth in all sinkholes, and grazing had no significant effect at a depth of 10-20 cm. The percentage of total soil P that was extracted with anion exchange resin also decreased with soil depth in the grazed sinkholes (averaging 21% and 3.7% in 0-2.5 and 10-20 cm soil layers), while changes in the percentage of resin-Pi in the ungrazed Wooded sinkhole were less dramatic (from 8% to 5% in the 0-2.5 and 10-20 cm soil layers) (Table 2).

Water soluble Po values were high in the surface 0-2.5 cm soil layer, ranging from 18-54 mg kg^{-1} in the pastured sinks and 53 mg P kg^{-1} in the Wooded sink (Table 2). The decrease in water-Po with soil depth was more pronounced in grazed sinkholes (about 30 mg kg^{-1} in the surface 0-2.5 cm layer to 11 mg kg^{-1} at 10-20 cm) compared to a decrease in the ungrazed Wooded sinkhole from 53 to 28 mg P kg^{-1} for the same soil depth increments (Table 2).

Spatial distribution of resin-Pi and water-soluble Po values across the Burns sinkhole in the surface 0-2.5 cm layer increased greatly from the outer rim to the center and bottom of the sinkhole (Fig. 3). The increase in resin-Pi values from the outer rim (51 m) to the center and bottom of the sinkhole (0 m) was 10 fold (162-656 mg P kg^{-1}) compared to a 3.4 fold increase in water-soluble Po (28-96 mg P kg^{-1}, Fig. 3). The increase in the values of water extractable Po with distance from the outer rim of the sinkhole to the center was correlated (r = 0.824) with organic carbon in the same extractant in the surface 0-2.5 cm soil layer of the Burns sinkhole (Fig. 4).

Bicarbonate extractable P

Bicarbonate extractable-P in both forms (Pi and Po) decreased significantly with soil depth in all three of the grazed sinkholes ($P = 0.0001$) whereas, in the ungrazed Wooded sinkhole, the values of Pi and Po remained almost unaffected by soil depth (Tables 2). The decrease in bicarbonate-Pi values was greater than bicarbonate-Po (average for the grazed sinkholes: Pi and Po decreased from 49 to 5 in the surface 0-2.5-cm layer and from 39 to 19 at a depth of 10-20 cm, respectively). Therefore, the ratio of Pi:Po decreased with depth in the grazed sinkholes (Pi:Po ratio decreased on average from 1 in the surface 0-2.5 cm layer to 0.26 at a depth of 10-20 cm), whereas the ratios were almost constant with soil depth in the Wooded sinkhole. Bicarbonate Pi and Po in the Burns sinkhole were consistently higher when compared to the ungrazed Wooded sinkhole in the surface 0-10 cm of soil and had similar values at a depth of 10-20 cm. Bicarbonate-Pi expressed as a percentage of the total soil P decreased with soil depth (4 to 1%) in pastured sinkholes while the proportion of bicarbonate-Po

exhibited little change. Spatial distribution of Pi and Po in the bicarbonate extracts across the Burns sinkhole in the surface 0-2.5 cm layer is shown in Fig. 5A. Bicarbonate-Pi increased greatly from the outer rim (51 m) towards the center of the sinkholes (0 m). The increase in bicarbonate-Po was small compared to the increase in bicarbonate-Pi (10.2 to 63 mg P kg^{-1} for Pi and 58 to 86 mg P kg^{-1} for Po).

Hydroxide extractable P

Hydroxide Pi and Po values in the four sinkholes decreased significantly with soil depth ($P < 0.0001$). Higher values of hydroxide-Pi occurred in the first to third soil layers from grazed sinkholes when compared to the Wooded ungrazed sinkhole (Table 2). In the 10-20 cm soil layer, hydroxide-Pi values in Burns sinkhole were higher than the Wooded and other two grazed sinkholes (Little and Big), and the Wooded sinkhole had higher hydroxide-Pi values as compared to Little and Big sinkholes. At all depths, Burns sinkhole had higher hydroxide-Po values only in the surface 0-2.5 cm layer while the values were similar at the remaining depths. Concentrations of hydroxide-Po in all sinkholes were greater than hydroxide-Pi in the surface 0-2.5 cm soil layer. The Pi:Po ratios for the hydroxide extracts were smaller in Burns and Wooded (2-4 times respectively) as compared to Little and Big sinkholes. The proportions of hydroxide-Po relative to total soil P, while decreasing slightly with soil depth, were greater in Burns and Wooded than in Little and Big sinkholes at all soil depths.

Spatial distribution of hydroxide-Pi and hydroxide-Po along the transect for the 0-2.5 cm soil layer of Burns sinkhole are shown in Fig. 5B. Values for hydroxide-Pi and hydroxide-Po increased from the outer rim (51 m) towards the center of the sinkhole (0 m). At all points along the east-west transect, hydroxide-Po was more than twice the values for hydroxide-Pi. The increase in hydroxide-Pi and hydroxide-Po along the transect in the Burns sinkhole was from 85 and 245 mg P kg^{-1} at 51 m (outer sinkhole rim) to 204 and 440 mg P kg^{-1} at 0m (sinkhole center), respectively.

Acid extractable P

Inorganic Pi associated with Ca in the grazed sinkholes decreased with soil depth ($P = 0.0001$) but remained significantly higher in the upper two soil layers (0-2.5 cm and 2.5-5.0 cm) compared to the ungrazed Wooded sinkhole (Table 2). In the Wooded sinkhole, HCl-Pi values remained almost the same with soil depth and were higher than those in the grazed sinkholes at the 5-10 and 10-20 cm soil depths. There was a minor change in the percentage of the total soil P extracted as HCl-Pi with soil depth, regardless of vegetation and management. Changes in HCl-Pi values from the outer rim toward the center of the Burns sinkhole were slight (Fig. 6).

Total and residual P

Total soil P (sum of Pi + Po in the various soil fractions, Table 2) in the surface 0-2.5 cm soil layer was much higher in the three grazed sinkholes ($P = 0.0001$) compared to the ungrazed Wooded sinkhole. Total soil P decreased dramatically with soil depth in the grazed sinkholes being 2.4-3.2 times greater in the surface 0-2.5 cm soil layer as compared to a depth of 10-20 cm, whereas there was a 1.6-fold difference in Wooded ungrazed sink when comparing the same soil depths.

Residual-Pi and Po values decreased significantly with increasing soil depth regardless of grazing ($P = 0.0001$), the decrease being greater in Pi compared to Po (averaging 217 and 275 mg kg^{-1} in the surface 0-2.5 cm layer; 72 and 151 mg kg^{-1} at a depth of 10-20 cm for Pi and Po in the grazed sinkholes, respectively). Consequently, the ratio of residual Pi:Po in grazed sinkholes decreased from 0.8 to 0.5 at depths of 0-2.5 and 10-20 cm compared to 1.75 and 1.26 in the ungrazed Wooded sink for the corresponding soil depths.

Total C and total organic P

Spatial distribution of total soil C across the Burns sinkhole is shown for the surface 0-2.5 cm depth in Fig. 7. Total soil C increased gradually toward the center of the sinkhole from both ends (outer rim, 51 m) and corresponded to the topography of the Burns sinkhole. Values for total soil C increased from 44.3 at 51 m to 68.9 g kg^{-1} toward the center of the sinkhole at 0 m (Fig. 7), which corresponded to a 55% increase in total soil C.

Total organic P (Po) in the surface 0-2.5 cm soil layer was higher in the grazed sinkholes compared to the ungrazed Wooded sinkhole (averaging 597 and 504 mg Po kg^{-1} in grazed and ungrazed Wooded sinkholes, respectively). Total Po expressed as a percentage of total soil P increased gradually with soil depth in grazed sinkholes and remained almost unchanged in Wooded sinkhole (Table 2). Spatial distribution of total Po across the Burns sinkhole had a similar trend to the soil P forms in that total Po increased gradually from the outer rim toward the center of the sinkhole (from 51 to 0 m, Fig. 8). Values of total Po increased from 489 mg P kg^{-1} at 51 m (sinkhole rim) to 902 mg P kg^{-1} toward the center (0 m), which corresponded to an 85% increase in total

Po. There was a positive, linear correlation between total Po and water-soluble Po remaining after the removal of resin bags (Fig. 9).

Discussion

After several years of seasonal grazing by cattle on permanent pastures in three sinkholes of a karst landscape in southern West Virginia, total P had increased significantly in the upper 5 cm soil layer as compared to an adjacent ungrazed Wooded sinkhole. As P is less mobile compared to N and C, elevated levels of P are common in pastures because of manure deposition (Hayness and Williams, 1993) and occasional fertilization of perennial grass vegetation. Increasing levels of organic matter in the upper soil layer associated with manure, whether by application confined feeding operation or by deposition from grazers, influences nutrient status and dynamics (Hayness and Williams, 1993) and especially the P cycle (Sims *et al.*, 1998). Consequently, all forms of P were higher in the upper soil strata in the grazed sinkholes. The surface 0-5 cm soil layer presents the greatest environmental concern since P held in this layer is a potential source of P that can more by or be released to surface runoff (Sims *et al.*, 1998; Sharply, 1985).

Characterizing soil P fractions by mode of extraction allows the identification of P fractions that are most influenced by permanent grazing and deposition of manure. Values of resin-Pi were increased by grazing with an average of 276 mg P kg^{-1} in the surface 0-2.5 cm layer of the grazed sinkholes compared to 67 mg P kg^{-1} in the ungrazed Wooded sinkhole (Table 2). Phosphorus remaining in the initial water extract and not retained on the anion exchange resin was considered as Po and averaged 30 mg P kg^{-1} in the surface 0-2.5 cm soil layer. Values of water extractable-Po decreased with soil depth, but remained high even at a depth of 10-20 cm in Burns sinkhole. Phosphorus leaching can occur in deep sandy soils, in high organic matter soils, and in soils where over fertilization and/or excessive use of organic wastes have increased soil P values well above those required by crops (Sims *et al.*, 1998). Manuring contributes to P mobility and leaching of Po when compared to Pi (Chardon *et al.*, 1997; Nash and Mardoch, 1996; Chardon and Oenema, 1995). In addition, manure applications may reduce the P sorption capacity of a soil (Mozaffari and Sims, 1996; Bache and Williams, 1971). Consequently manure increases the likelihood of P loss to surface waters via erosion and runoff as well as the potential for long-term P leaching into the subsoil and ground water especially in karst landscapes.

Environmentally significant quantities of P may be exported if subsurface transport occurs naturally and more so if it is enhanced by artificial drainage systems. In our study, the sum of resin-Pi and water extractable-Po values were very high (averaging 306 mg P kg^{-1} soil in the surface 0-2.5 cm soil layer of the grazed sinkholes) which raises an environmental concern for management of P in karst landscapes since P in this fraction is considered to be "biologically available P" (Wright and Coleman, 1999; Schoenau *et al.*, 1989; Wagar *et al.*, 1986; Stevenson, 1986; Tate, 1984, 1985; Hedley *et al.*, 1982). High values of biologically available soil P (Table 2) in highly fractured bedrock typical of karst landscapes in combination with the potential "funneling" effect of sinkholes, and a humid climate (average rainfall 988 mm yr^{-1}) with frequent heavy rainfall events, especially during the grazing season would facilitate transport of water enriched with water soluble P. Surface runoff resulting from natural rainfall events was collected in Little sinkhole and concentrations of dissolved P ranged from 5-10 mg P L^{-1} in 11 runoff events over a year (Data not presented). The gradual increase in the concentration of biologically available P (the sum of resin-Pi and water extractable-Po, Fig. 3) from the outer rim toward the center and bottom of the grazed Burns sinkhole was evident, indicating a migration of water soluble-P and organic P forms (manure) toward the bottom center of the sinkhole (Figs. 3 and 8). Phosphorus values increased 10-fold (162-656 mg P kg^{-1}) and 3.4-fold (28-96 mg P kg^{-1}) for Pi and Po, respectively from the outer rim (51 m) to the bottom center of the Burns sinkhole (0 m, Fig. 3).

Phosphorus extracted by $NaHCO_3$ and NaOH in either Pi or Po forms were statistically greater for grazed compared to the ungrazed Wooded sinkhole in the upper 5-cm soil layers (Table 2). Values of Pi and Po in both extractants, decreased with increasing depth, but changes in the ratio of Pi:Po were different for the two extractants. The decrease in bicarbonate-Pi was greater than that of Po so Pi:Po ratios decreased dramatically with soil depth with an average in grazed sinkholes of 1.0 at 0-2.5 cm and 0.26 at the 10-20 cm soil depth. The reverse was true for the hydroxide extract where Pi:Po ratios increased from 0.6 to 1.2 for the same depths. These results reflect the likelihood of considerable microbial mediated mineralization of Po forms in the hydroxide fraction. Some of the mineralized P may form hydroxide-Pi or a more labile Po fraction ($NaHCO_3$-Po). This hypothesis needs further study, but it is feasible as other studies of organic matter transformations (Moyer and Thomas, 1970) suggest

that a majority of Po forms in the 0.5 *M* $NaHCO_3$ and 0.1 *M* NaOH fractions have molecular weights >30,000, though some material in trace amounts have molecular weights between 30,000 and 70,000.

Residual Po is probably P that is associated with large molecular weight complexes that correspond to the humic acid and humin fractions (Hedley *et al.*, 1982). Residual Po in grazed sinkholes decreased with soil depth, but increased as a percentage of total P on average from 21% in the surface 0-2.5 cm layer to 34% in the 10-20-cm soil layer (Table 2). Total Po expressed as a percentage of total P increased with soil depth (44-56%, comparing the 0-2.5 cm and 10-20 cm soil layers). However, these changes did not correspond to an increase in total C (%) with depth. The ratio of total soil C:TPo decreased dramatically with depth in grazed sinkholes (from 79 in the surface 0-2.5 cm layer to 38 at a depth of 10-20 cm), compared to decrease in total soil C:TPo ratio from 103 to 52 for the same soil depths in the ungrazed Wooded sinkhole. The higher total soil C:TPo ratios in Wooded compared to grazed sinkholes may reflect the percolation of P-rich organic compounds through the soil profile in grazed sites, resulting from mineralization of manure (Hayness and Williams, 1993; Bettany *et al.*, 1973). However, the levels of total Po in this study were quite high (Table 2, Fig. 8) and were probably recalcitrant phosphorus compounds (Hedley *et al.*, 1982), thus making it an important P fraction over the long-term. Eghball *et al.* (1996) and Kuo and Baker (1982) demonstrated greater downward mobility of organic P forms in manured soils compared to soils receiving mineral P fertilizer. Total Po content of pasture soils increased across the sinkhole with 489 mg P kg^{-1} at the outer rim to 900 mg P kg^{-1} in the center and bottom of the Burns sinkhole, which corresponded to a similar pattern of total C distribution of 73 to 114 g kg^{-1} for the same transect (Figs. 7 and 8). Regardless of the nature of organic P compounds present in different soil depths, there was a strong correlation between total Po in the soil and Po soluble in water ($r = 0.673$) (Fig. 9), which concentrations could raise environmental concern. The proportion of total soil P that is water-soluble Po may be environmentally important because of the possible movement of organically-complexed P downward in the soil profile. A mediated transport of Po by dissolved organic carbon may be important and may correlate highly with water dissolved organic carbon ($r = 0.824$, Fig. 4). Dissolved Po-compounds are less likely to adsorb or fix on soil ligand-exchange sites and were resistant to adsorption on the anion exchange resin.

Conclusion

The influence of seasonal grazing by cattle, deposition of manure and fertilization on soil P fractions were pronounced in the surface 0-2.5 cm soil layer for three sinkholes in permanent pasture in southern West Virginia compared to non-grazed Wooded sinkhole. Absolute concentrations of soil P in all fractions from a sequential fractionation were greater in the upper 5 cm of soil in three grazed sinkholes compared to an ungrazed Wooded sinkhole. The concentrations of inorganic P (Pi) fractions (resin-Pi, $NaHCO_3$-Pi, and NaOH-Pi) were greater in the grazed compared to the non-grazed site. All fractions of soil P decreased with depth. However, the decrease was different for Pi and Po in each extract resulting in different Pi:Po ratios with depth. These ratios increased for NaOH extracts and decreased for $NaHCO_3$ extracts with depth. The most pronounced increase as a result of pasture management was for resin extractable-Pi, which represents "biologically available P" and could lead to environmental concern. Karst landscapes and a humid climate with frequent heavy rainfall events could accelerate the transport of P into ground water and lead to water quality degradation. Increasing values of P in all soil fractions from the outer sinkhole rim toward the bottom and center of the sinkholes suggests that erosion and surface runoff were contributing to P migration.

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صور الفوسفور وتوزعاته البينية في هضاب المراعي الناشئة عن صخور كلسية

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(قدم للنشر في ١٤٣١/٣/٢٠هـ؛ وقبل للنشر في ١٤٣١/١٢/١هـ)

كلمات مفتاحية: تجزئة الفوسفور، التوزع المكاني للفوسفور، الفوسفور العضوي، المادة العضوية، التلوث، تدوير العناصر الغذائية.

ملخص البحث. يتضمن نظام المراعي تدوير العناصر الغذائية من الروث الحيواني وتراكمها في التربة خاصة الفوسفور الذي يثير قلقاً بيئياً. ولقد اتبعنا طريقة هيدلي التي تم تعديلها لتجزئة الفوسفور في ترب ثلاثة مواقع رعوية إضافة إلى موقع غير رعوي (أحراش) مجاور لوصف توزع صور الفوسفور بتأثير العمق في التربة والطبوغرافيا. وتأخذ هذه المواقع شكل الجرن فهي دائرية مرتفعة في محيطها الأعلى وتنخفض باتجاه المركز الذي يتواجد فيه مصرف طبيعي باتجاه مجرى المياه الأرضية. ولقد تميزت كافة أشكال الفوسفور بتراكيز عالية في عمق التربة ٠–٥ سم في كافة المواقع وانخفضت مع العمق في أفق التربة. وتباينت تراكيز الفوسفور المعدني Pi والفوسفور العضوي Po في أعماق التربة بشكل مختلف الأمر الذي أدى إلى زيادة نسبة Po:Pi مع العمق في التربة في مستخلص NaOH بينما انخفضت هذه النسبة مع العمق في مستخلص البيكربونات. والاختلاف الأوضح بين المواقع الرعوية العشبية وغير الرعوية (الأحراش) كان في الفوسفور المعدني المستخلص بالرزن وهو ذو مدلول للإتاحة البيولوجية للفوسفور ومهم من الناحية البيئية. وتشير التراكيز العالية للفوسفور المستخلص بالرزن في أسفل الهضبة في كل موقع إلى إمكانية تحرك الفوسفور بالانجراف ومع ماء السيل السطحي من أعلى الهضبة إلى مركزها المنخفض والصرف باتجاه المياه الأرضية.