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Pergamon

Soil Biol. Biochem. Vol. 30, No. 13, pp. 1663–1670, 1998
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Printed in Great Britain
0038-0717/98 \$19.00 + 0.00

PII: S0038-0717(97)00255-1

P-ISOTOPIC EXCHANGE VALUES IN RELATION TO Po MINERALISATION IN SOILS WITH VERY LOW P-SORBING CAPACITIES

DANILO LOPEZ-HERNANDEZ,[1]* M.ichel BROSSARD[2] and E. FROSSARD[3]

[1]Laboratorio de Estudios Ambientales, Instituto de Zoología Tropical (IZT), Facultad de Ciencias, Universidad Central de Venezuela, Apdo. 47058, Caracas 1041-A, Venezuela, [2]Orstom c/o Centre de Pèdologie Biologique — Centre National de la Recherche Scientifique (CNRS), BP5, F-54501 Vandoeuvre-les Nancy Cedex, France and [3]Institute of Plant Sciences, Swiss Federal Institute of Technology (ETH), CH-8092 Zurich, Switzerland

(Accepted 19 October 1997)

Summary—The productivity of many agricultural systems is determined by the quantity of inorganic P (Pi) made available through mineralisation of organic P (Po) during the growing season. Because of the adsorptive nature of the soil mineral fraction, any recently-mineralised Po may be rapidly partitioned to the solid phase. Attempts to quantify Po mineralisation, have been unsuccessful due to the difficulties in separating recently-released Pi from labile Pi forms already existing in the soil. We have investigated the use of an isotopic dilution technique to measure the increase of isotopically-exchangeable P with time ($E(t)$) in: (i) A short-term experiment (1–100 min) where there was only the physico-chemical process of exchange of solution ^{32}P with ^{31}P located on the solid phase; and (ii) an incubation experiment (0–51 d) where there were both physico-chemical and biological (immobilisation and mineralisation) processes. Although the $E(t)$ values were obtained by different means (water- and resin-Pi extractions), the marked difference between both values during the 51 d experiment could not be completely explained by ^{32}P exchange with ^{31}P in the solid phase. The strong association between $E(t)$ increment with Po and CO_2 respiration values during incubation suggest biological participation. Estimates of Po mineralisation rates for the low P-sorbing soils (220–900 μg P kg^{-1} d^{-1}) were similar to values in the literature that were obtained by isotopic and non-isotopic methods. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

The productivity of many natural and agricultural ecosystems is determined by the quantity of mineral P (Pi) made available through mineralisation of organic P forms (Po) during the growing season. The dependence on Pi mineralised from the soil Po reserve is particularly important in P-limited natural ecosystems because they do not receive Pi fertiliser input. Additionally, in some agricultural ecosystems, P fertiliser additions are non-existent or negligible. In both situations, the quantities of Pi released from mineralisation may be of paramount importance.

Several chemical fractionation procedures have been developed to measure the dynamics of the conversion between Pi and Po in the soil (Thompson and Black, 1947; Enwezor, 1967; Ruiz and López-Hernández, 1977). The precision of these methods, however, is affected by several factors including: (i) P fixation of recently-mineralised Pi, (ii) soil P heterogeneity, (iii) Po hydrolysis by strong reagents.

Net Po mineralisation is measured somewhat more accurately by observing dynamics in labile P pools. Net changes in the concentration of $CaCl_2$- and resin-extractable Pi have been presented by Saunders and Metson (1971). Halm *et al.* (1972); Bowman and Cole (1978) used the changes of $NaHCO_3$-extractable Po as a measure of potentially-mineralisable organic phosphorus.

Changes in Po during long-term cropping practices have also been used to approximate net Po mineralisation. Chater and Mattingly (1980) compared changes of organic P under contrasting cropping systems. They reported mean annual rates of net Po mineralisation in soil ranging from 0.5 to 8.5 kg P ha^{-1}. In tropical conditions, Mueller-Harvey *et al.* (1985) measured organic C, N, S and P mineralisation after forest clearance, and reported that uptake during the first year was of the same order of magnitude as the decrease found for soil Po (around 27 kg^{-1} ha^{-1}). In Great Plains soils, Haas *et al.* (1961) measured a significant average reduction in Po of 35% after 31 to 48 y of cropping, compared with virgin sod. Sharpley (1985) and Hernández-Valencia (unpublished PhD thesis,

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*Author for correspondence.

Universidad Central de Venezuela, 1996) used the changes in labile Po fractions as measured with the Bowman and Cole (1978) fractionation method to measure net Po mineralisation rates in grassland and savanna soils.

Because of the adsorptive nature of the soil mineral surfaces, any Po recently mineralised may be rapidly partitioned to the solid phase (Cole *et al.*, 1977). Attempts to quantify gross Po mineralisation have been unsuccessful, due to the difficulties in separating the flush of the recently-released Pi from the labile and non-labile P forms already existing in the soil. A method which relies on the use of ^{32}P as a tracer may therefore help to solve this problem (Harrison, 1982).

In assessing the isotopic-dilution technique for determining gross mineralisation rates, a relatively mild extractant needs to be used to avoid organic P-hydrolysis, which might cause a decrease in specific activity due to mineralisation. $NaHCO_3$ and NH_4F have been used (J. J. Sweet, unpublished MSc. thesis, Colorado State University, 1981; Walbridge and Vitousek, 1987; Jayachandran *et al.*, 1992), but Po and Pi fractions must be separated before counting. López-Hernández and Niño (1993) preferred to use resins, because partitioning is easily accomplished through the preference of anion resin for Pi (Hedley *et al.*, 1982).

The isotopic-dilution technique has been used by Walbridge and Vitousek (1987); López-Hernández and Niño (1993) for determining Po mineralisation. The method assumes that mineralisation lowers the specific activity of the extracted P. The magnitude of this decrease (e.g. $E(t)$ increase) would then provide an estimation of gross P mineralisation (Sweet, 1981; Walbridge and Vitousek, 1987; López-Hernández and Niño, 1993). It is assumed when calculating mineralisation rates by this method that the added ^{32}P is equilibrated with the isotopically-exchangeable P within relatively short times. This assumption may be valid for very low P sorbing soils (López-Hernández and Niño, 1993), but it might give misleading results in the case of high P sorbing soils, where significant isotopic exchange can continue over much longer times; at least 3 months according to Fardeau *et al.* (1985); Frossard *et al.* (1994).

We present the results of adsorption-desorption experiments in different types of less weathered Mollisols in relation to Po mineralisation. Changes in isotopically-exchangeable P with time, $E(t)$, were evaluated through: first, a simulation experiment (Fardeau, 1993), second, by direct $E(t)$ determinations during an incubation period of 51 d.

MATERIALS AND METHODS

Soils

Soils (Typic Haploborolls) from the summit position of six toposequences were used in this study (Table 1). Three sites are located in southwestern North Dakota, and have been the subject of intensive studies of erosion, organic C, P and N changes resulting from cultivation and soil texture (Schimel *et al.*, 1985; Aguilar and Heil, 1988; López-Hernández and Niño, 1993). The toposequences were on three different parent materials with varying texture (sandstone, siltstone and shale) and are referred as sites 1, 2 and 3, respectively. Grassland was the native plant component at each site.

Three other sites on toposequences were selected along a mean annual precipitation (MAP) gradient from eastern Colorado through northwestern Kansas (Honeycutt *et al.*, 1990a). A southwest-northeast oriented transect was selected to hold mean annual temperature (MAT) as constant as possible in respect to this gradient. Sites receiving 395, 444 and 514 mm of MAP are referred to as sites 4, 5 and 6, respectively. Sites were also selected on the basis of similarity in initial parent material, rangeland management, slope and aspect (Honeycutt *et al.*, 1990b).

Additionally, a soil from Sidney, NE (fine-silty, mixed, mesic Pachic Haplustoll) and another from Lubbock, TX (fine-loamy, mixed thermic Aridic Paleustolls) were examined and are referred to as sites 7 and 8, respectively.

Table 1. Selected properties of the soils used

Site location/parent material	Taxonomic position	C (%)	N (%)	Total P ($mg\ kg^{-1}$)	Po ($mg\ kg^{-1}$)
Grand County ND Sandstone	Typic Haploborolls	1.56	0.16	554	204
Grand County ND Siltstone	Typic Haploborolls	3.23	0.30	605	325
Grand County ND Shale	Typic Haploborolls	2.44	0.26	662	290
Cheyenne Wells CO	Ustollic Haplargids	1.04	0.12	545	105
Goodland KS	Aridic Arguistolls	1.98	0.21	667	164
Oberlin KS	Aridic Arguistolls	1.92	0.19	625	217
Sidney NE	Pachic Haplustolls	2.06	0.22	637	359
Lubbock TX	Aridic Paleustolls	0.61	0.09	228	93

Isotopic exchange kinetics of phosphate ions

Experimental procedure Dry soil (10 g, in triplicate) was shaken overnight with 99 ml distilled water in an end-over-end shaker. After this equilibration, 1 ml of a convenient quantity of carrier-free ^{32}P (approximately 250 kBq) was added (R_0) and the samples shaken for another 100 min. Aliquots were removed subsequently with a polyethylene syringe and filtered using millipore filters (0.2 μm) at different times (between 1 and 100 min) after the addition of the radioactive tracer (Tran *et al.*, 1988; López-Hernández *et al.*, 1989). Radioactivity (r) in the solution at each time (t) was measured by liquid scintillation with 1 ml of the solution. After millipore filtration, the clear water solution obtained, was efficiently counted, without organic matter interference. The concentration of phosphate ions in the solution (cp) was determined (Murphy and Riley, 1962) after the last sampling of the isotopic exchange. Total inorganic P of the different soils was measured after a $NaHCO_3$ fusion, and aliquots of the extracts were also measured by Murphy and Riley (1962) reagent. The r/R_0 values indicate the radioactivity that remains in the solution after 1 min of isotopic exchange as a proportion of the total radioactivity added. It is an indirect measure of P-fixing capacity (Tran *et al.*, 1988; López-Hernández *et al.*, 1989).

When a quantity, R_0, of carrier-free $^{32}PO_4$ ions is added to a soil solution system in a steady state, an isotopic exchange between ions in solution and ions in the solid phase takes place. The quantity of radioactivity, r, remaining in solution decreases with time, t, according with the following theoretical equation (Fardeau *et al.*, 1985):

$$r(t)/R = r(1)/R\{t + [r(1)/R]^{1/n}\}^{-n} + r(\infty)/R \qquad (1)$$

where $r(1)$, and $r(\infty)$ are the radioactivity remaining in the solution after 1 min, and infinity, respectively, and n is a parameter obtained from the isotopic-exchange kinetic experiment. The ratio $r(\infty)/R$ is the maximum possible dilution of the isotope. It is approximated by the ratio of the water soluble P to the total inorganic P (Fardeau, 1993).

The quantity, $E(t)$ (mg P kg^{-1} soil) of isotopically-exchanged P at time t is calculated assuming that (i) $^{31}PO_4$ and $^{32}PO_4$ ions have the same fate in the system and (ii) whatever the time t, the specific activity of the phosphate ions in the soil solution is identical to that of the isotopically-exchanged phosphate ions in the whole system

$$r(t)/\mathrm{cp} = R/E(t) \qquad (2)$$

Therefore

$$E(t) = 10\mathrm{cp}[R/r(t)] \qquad (3)$$

where the factor 10 accounts for the soil-to-solution ratio of 1 g of soil in 10 ml of the water.

Isotopic exchange kinetics of ions during incubation phosphate

Experimental procedure Dry soil (50 g, in triplicate) from each site was placed into Nalgene wide-mouth jars (125 ml), moistened to 50% capacity with distilled water and inoculated with 1 ml of a soil solution to restore biological activity. The moistened samples were left to condition for 4 d. Then, 2 ml of ^{32}P labelled 10 μM KH_2PO_4 (40 MBq) was added to the soil. Then enough distilled water was immediately added to the soil in order to obtain 80% of field capacity, and the system was mixed thoroughly with a pyrex rod. The labelled soils were maintained in an incubation chamber designed to measure CO_2–C evolution at constant temperature (25°C). The moist soil systems were sampled on day 0, 2, 7, 14, 21 and 51. On each sampling day, the individual systems were thoroughly mixed to distribute the ^{32}P evenly and moist sub-samples were removed for subsequent determination of ^{32}P activity by resin extraction. Corrections were made for moisture content at each sampling date.

Simultaneously, CO_2 evolution was determined according to Schimel *et al.* (1985) at each of the incubation times, except for the last sampling on 28 d.

Resin extractable-P was determined by extraction of 2.0 g dry weight of the incubated wet soils (2 mm sieve) with 40 ml deionised water and 0.5 g anion exchange resin (Dowex 1 × 80–50, ⟩30 mesh) in bicarbonate form enclosed in a mesh bag and shaken in 100 ml centrifuge tubes for 1 h in a reciprocal shaker. Then, the resin bag was removed and Pi was recovered by extraction with 30 ml 0.5 M HCl for 1 h. Aliquots of the resin recovered were analyzed for Pi by Murphy and Riley (1962) reagent and for ^{32}P by liquid scintillation counting. A preliminary experiment (data not presented) showed a high counting efficiency of the 1 h extraction to recover the resin outruns.

Isotopic-exchangeable phosphorus (IEP mg P kg soil^{-1}) was calculated at each sampling day by using an isotopic dilution technique (White, 1976) according to the formula.

$$\mathrm{IEP} = M/(r/R) \qquad (4)$$

Where r (MBq) is the radioactivity remaining in solution at time t, R (MBq) is the total radioactivity added and M is P resin (μg g^{-1}) (López-Hernández and Niño, 1993). Corrections were made for radioactivity decay and moisture content on each sampling dates.

RESULTS AND DISCUSSION

Basic parameters of isotopic exchange kinetics

Amorphous surfaces of iron and aluminium are responsible for high P-sorption capacities in

Table 2. Basic parameters of isotopic exchange kinetics for the studied mollisols

Site location/parent material	r/R_0*	cp† (μg ml^{-1})	n‡	Rate of gross mineralization (mg P kg^{-1} d^{-1})
Grand County ND Sandstone	0.74	0.15	0.14	0.47
Grand County ND Siltstone	0.71	0.16	0.20	0.82
Grand County ND Shale	0.33	0.06	0.34	nd§
Cheyenne Wells CO	0.61	0.39	0.16	nd
Goodland KS	0.78	0.58	0.13	0.86
Oberlin KS	0.87	0.34	0.11	0.90
Sidney NE	0.68	0.98	0.13	0.67
Lubbock TX	0.80	0.15	0.18	0.22

Rates of gross mineralization were calculated as the difference between IET (experimental values) and $E(t)$ (simulation values) after 51 d.

* r/R_0 = radioactivity after 1 min isotopic exchange over total radioactivity.

strongly weathered soils (López-Hernández and Burnham, 1974; Burnham and López-Hernández, 1982), whereas in less weathered Mollisols, low amounts of added P are sorbed because they lack amorphous forms of sesquioxides (Sweet, 1981); adsorption, is also affected by particle size. Consequently sandy materials tend to adsorb negligible amounts of P compared with soils enriched in fine materials. A direct measure of P-sorption was not obtained for the Mollisols studied. However, when analysing the r/R_0 indexes, that provide indirect values of P-retention (Tran *et al.*, 1988), all the soils studied corresponded to group III (Tran *et al.*, 1988) soils list, characterised by low maximum P-sorbing capacities and higher r/R_0 values.

r/R_0 values ranged between 0.61–0.87 for Cheyenne Wells and Oberlin soils, respectively (Table 2); shale soil with a higher clay content differed significantly from the rest of the soil population and presented a relatively low r/R_0 value (0.33).

Solution phosphate concentration (cp values) were high, as expected for soils with a high natural fertility, ranging from 150–580 μg P l^{-1} (Table 2) with the exception of the Sidney soil which had a very high cp value (980 μg l^{-1}) probably associated with its high Po content (Table 1).

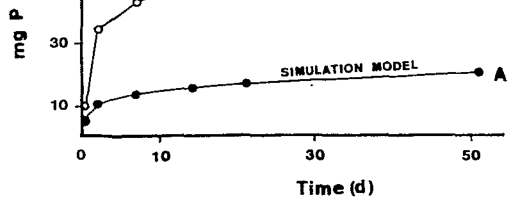

Fig. 1. Isotopically-exchangeable P values using: (i) A simulation model (curve A), correspond to ^{32}P exchange in the solid matrix; (ii) incubation values (curve B) which correspond to ^{32}P exchange and Po mineralisation.

The n values were similar (0.11–0.20) with the exception of the shale soil with a value of 0.34.

Changes in E(t) with time When ^{32}P is added to a soil–solution system in steady state equilibrium, isotopic exchanges are produced between the phosphate ions in solution and the exchangeable phosphate ions already present in the solid phase; the exchanges are not however instantaneous and isotopically-exchangeable P values ($E(t)$) increase as the quantity of radioactivity ($r(t)$) in solution decreases (Fardeau *et al.*, 1985; Frossard *et al.*, 1994).

The $E(t)$ values were calculated by extrapolation of the isotopic exchange data using the theoretical equation (3) as used by Fardeau (1993); Frossard *et al.* (1994, 1996). The E value extrapolated to 73 440 min (51 d) is thereafter defined as E (51 d). Mineral P must be determined for each soil if the data obtained between 1 and 100 min are to be extrapolated. Figure 1 (curve A) presents the calculated $E(t)$ curve for the Grand County siltstone as a function of time up to 51 d, the other Mollisols studied gave a similar pattern (Table 3): during the 24 h of exchange, the $E(t)$ values increased considerably, followed by a levelling off between 1–21 d, the values tend to stabilise at the end (51 d) of the simulation period (e.g. Oberlin Kansas and Grand County sandstone), however in some cases, $E(t)$ values still show a tendency to increase (e.g. Grand County shale).

Extrapolations of the $E(t)$ values up to 51 d using equation (3) allow the Mollisols studied to be separated into three groups (Table 3): (i) Soils, where there was a very small increase of E (0–12 mg P kg^{-1}) during the 51 d simulation (including the Ground County sandstone, Oberlin KS and Lubbock soils) (Table 3), all these soils were also characterised by very low P-sorbing capacities as inferred from the r/R_0 index (0.74–0.87, Table 2), (ii) a second group represented by the siltstone, Cheyenne Wells and Goodland with increasing E

Table 3. Isotopic exchangeable P values (mg P kg^{-1}) calculated with equation (3) (theoretical values) and equation (4) (incubation period–experimental values)

Duration of experimentation (d)	$E(t)$ Eq. 3	$E(t)$ Eq. 4	Differences (4–3)	$E(t)$ Eq. 3	$E(t)$ Eq. 4	Differences (4–3)
		Grand County Sandstone			Grand County Siltstone	
0.42	3.6	5.9 (1.1)	2.3	5.0	9.9 (4.2)	4.9
2	6.1	15.7 (1.0)	9.6	10.8	33.2 (4.7)	22.5
7	7.2	28.2 (7.6)	21.0	13.8	43.0 (8.8)	29.2
14	8.0	28.1 (4.4)	20.1	15.8	49.2 (18.8)	33.4
21	5.4	34.4 (3.3)	29.0	17.0	61.6 (21.8)	44.6
51	6.1	30.3 (4.6)	24.2	20.2	62.2 (5.9)	42.0
		Grand County Shale			Cheyenne Wells	
0.42	6.9	6.7 (2.1)	−0.2	11.8	14.9.(4.4)	3.1
2	25.1	21.1 (6.0)	−4.0	21.4	19.6 (3.9)	−1.8
7	37.4	49.8 (15.9)	12.4	25.8	31.9 (3.2)	6.1
14	46.4	46.8 (17.5)	0.4	28.6	33.3 (5.2)	4.7
21	52.4	79.7 (38.2)	27.3	30.4	42.8.(4.1)	12.4
51	67.8	70.7 (20.4)	2.9	34.6	39.8 (4.9)	5.2
		Goodland			Oberlin	
0.42	12.5	33.6 (1.7)	21.1	6.0	15.7 (6.1)	9.7
2	20.6	44.7 (4.3)	24.1	9.1	29.7 (2.4)	20.6
4	24.1	60.3 (5.7)	36.2	10.4	41.2 (5.3)	30.8
14	26.4	68.3 (5.5)	41.9	11.1	41.9 (2.6)	30.8
21	27.7	71.7 (3.2)	44.0	11.6	54.6 (6.8)	43.0
51	31.0	74.9 (12.8)	43.9	12.8	58.3 (3.5)	45.5
		Sidney			Lubbock	
0.42	22.4	35.7 (0.9)	13.3	3.7	7.6 (1.8)	3.9
2	34.6	50.9 (5.1)	16.3	7.2	11.7 (1.5)	4.5
4	39.6	63.8 (4.9)	24.2	8.9	15.7 (3.7)	6.8
14	42.6	69.3 (7.3)	26.7	10.0	17.4 (1.1)	7.4
21	44.5	73.3 (0.9)	28.8	10.7	20.7 (3.4)	10.0
51	48.7	83.0 (6.4)	34.3	12.3	23.3 (2.6)	11.0

Differences (equation (4)–equation (3)) is a measure of biological mineralisation. Standard deviations of experimental values in parentheses.

values reaching up to 30 mg P kg^{-1} (Table 3), P-sorbing values for these soils were also low (0.61–0.78), (iii) the third group is represented by Sidney, and the shale soil with increasing E values above 40 mg P kg^{-1} (Table 3), whereas, in particular the shale soil had a fairly high P sorption value ($r/R_0 = 0.33$).

Changes in resin Pi and specific activity during incubation Table 4 presents the information concerning the changes in resin Pi and specific activities during the 51 d of incubation for Grand County siltstone. Resin Pi presents a small non-significant tendency to decrease from 0–14 d, after that period, values increment to near the original figure. Frossard *et al.* (1996) found a similar pattern for a cultivated French alfisol. Specific activities e.g. counts min^{-1}/resin-Pi, presented by contrast, as expected, an exponential decay that might be attributed either to the flush of the recently-mineralised Pi or to an exchange of ^{32}P with the solid phase. The latter process should be less important in low P-sorbing soils. For the other soils analyzed the same pattern for both resin and specific activities values was repeated (data not presented).

Isotopic exchange kinetics of phosphate during incubation During the incubation in all the soils analyzed, the amounts of IEP increased (Table 3). Figure 1 (curve B) presents the results for Ground County siltstone. The results presented had the following pattern: at the beginning of the experiments (0–7 d), the IEP values increased rapidly, then the slope of the curve decreased (7–21 d); and finally values became constant until the end of the incubation (21–51 d). In general, values obtained during the incubation (0–51 d) from anion exchange resins, showed similar changes between soils, to those obtained with the theoretical model (Table 3). The soils studied were again divided into three groups

Table 4. Resin-Pi and specific activity values during the incubation period for siltstone soil

Incubation time (d)	0	2	7	14	21	51
Resin-Pi (μg g^{-1})	2.8a (1.5)	3.0a (1.4)	2.0a (0.9)	1.3a (1.1)	2.2a (1.0)	2.5 a (0.5)
Specific activity (counts min^{-1} μg^{-1} g)	43 455 (20 019)	11 516 (1742)	8964 (1649)	8714 (4190)	6637 (2290)	6086 (596)

Standard deviations in parentheses, means followed for different letters differ significantly.

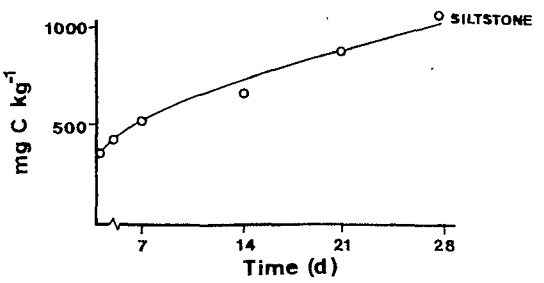

Fig. 2. CO_2 respiration values (mg C kg^{-1}) during the incubation.

according to the increase in $E(t)$ during the incubation: (i) A soil with a very small increase of IEP (17 mg P kg^{-1}, Table 1) corresponding to the Lubbock, this soil also presented the lower Po content (93 mg P kg^{-1}), (ii) soils with a moderate increase in IEP values (24–26 mg P kg^{-1}, Table 1), represented by Oberlin KS, Cheyenne Wells and Grand County sandstone, all these soils presented moderate amounts of Po (105–217 mg P kg^{-1}, Table 1), and finally (iii) soils which presented a high increment on IEP values during incubation including: Grand County shale, Grand County siltstone and Sidney NE, with a fairly high amount of Po (290–359 mg P kg^{-1}).

Processes which affect IEP values The observed changes in isotopically-exchangeable P in both, theoretical and incubation experiments are due to different kinds of processes. Firstly, it is well known that soil phosphate exchange usually comprises at least two reactions: a fast one completed within 24 h and a slow one which continues for many days (Jose and Krihsnamoorthy, 1972; White, 1976). Results in Fig. 1 (curve A) and Table 3 fully support this two reaction mechanisms. The experiments were made after 100 min of isotopic exchange and the results extrapolated up to 51 d. Biological activity (e.g. mineralisation) can be considered negligible during the simulation period. Results in Fig. 1 (Curve A) and Table 3 (column 2) can be entirely ascribed to physico-chemical processes.

Secondly, IEP values obtained from the incubation experiment are affected by both physico-chemical and biological processes. Although, $E(t)$ and IEP data were obtained by different means (water and resin extraction, respectively), initial figures in some cases (e.g. Oberlin, KS; Grand County shale and Cheyenne Wells soils) were similar. The strong difference between both curves can therefore not be completely attributed to ^{32}P exchange in the solid phase; biological activity, particularly mineralisation of Po must be involved in the incubation experiment. The strong association of IEP with Po values already discussed confirms the biological participation in the process.

Table 5. Relationship between the increment in E (e.g. IEP minus $E(t)$) and some chemical parameters; soils 3 and 4 were not considered in the correlations

Chemical Parameters	Equation	r^2
Po (µg g^{-1})	$E = -95.79 + 53.78 \log Po$	0.85
CO_2 evolution (µg g^{-1})	$E = -14 + 0.05\ CO_2$	0.91
%C	$E = 6.75 + 11.88$ %C	0.92
%N	$E = 0.56 + 147$ %N	0.95

The solubilisation of surface Pi can also result in an overestimation of $E(t)$ by increasing the resin-extractable Pi. However, during the experiment, those resin values were not cumulative with time (Table 4). In contrast, the information presented for Grand County Siltstone denotes a small tendency for resin-Pi to decrease between 2–21 d of incubation. Carbon dioxide respiration for the same soil matched the experimental IEP increment well (Figs 1 and 2), emphasising that the process was largely biological (fresh microbial or soil phosphatase activities). Furthermore, the difference between E theoretical (equation (3)) and E experimental (equation (4)) at 51 d, were also significantly associated with CO_2 evolution, with the exception of the shale and Cheyenne Wells soils (Table 5); these soils, as previously noted, had high sorption capacities ($r/R_0 = 0.33$ and 0.61, respectively. Table 2), therefore in those cases, most of the E increment is a consequence of ^{32}P exchange to the solid phase instead of biological mineralisation. However, increased organism activity apart from increasing CO_2 evolution may decrease pH, which in turn may affect sorbed P. During incubation allowance was not made for pH control. In previous incubation results for tropical mollisols (Ruiz and López-Hernández, 1977) and associated soils with a high Po content (Islam and Ahmed, 1973) a small pH increase was reported. A strong association was also found between E differences (e.g. E experimental and E theoretical) and organic-P, and between E differences and total C and N (Table 5). Again, shale soil and Cheyenne Wells behaved as outliers, supporting the dependence of E differences values on biological activities. Using this information, we calculated the gross Po mineralisation rates of the analyzed soils as the difference between IEP experimental values and the theoretical $E(t)$ values at 51 d. The results are presented in Table 2. Values ranged between 220 and 900 µg P kg^{-1} d^{-1} for the Texas and Oberlin KS soils, respectively. Results presented in Table 2 confirm our preliminary hypothesis that calculation of gross mineralisation rates cannot be achieved using incubation ^{32}P dilution techniques in the case of the shale and Cheyenne Wells soils, because of their relatively high P-sorption capacities (Table 2).

CONCLUSIONS

The changes in isotopically-exchangeable P during incubation studies can be used as an estimate of the amount of Po mineralised provided that corrections are made for the ^{32}P fraction exchanged with the solid phase. Therefore, the isotopic dilution technique can be used successfully for poorly-weathered Mollisols (López-Hernández and Niño, 1993) with a very low P sorbing value, but would be less precise in the case of well-weathered temperate or tropical soils.

Estimates of Po mineralisation rates for the soils studied (220–900 μg P kg^{-1} d^{-1}, Table 2) were similar to the gross P mineralisation rates (600–3800 μg P kg^{-1} d^{-1} presented by Zou *et al.* (1992) using a non-isotopic method.

Acknowledgements—Drs E. T. Elliott and C. V. Cole (Colorado State University) contributed to the genesis of this study and provided the soil samples and laboratory conditions for the incubation studies. We thank Dr A. Herbillon and Dr A. Guckert for providing laboratories facilities at CPB (Centre de Pèdologie Biologique) and ENSAIA (Ecole Nationale Superieure D'Agronomie et des Industries Alimentaires), respectively. Some of the work reported in this manuscript was done while the senior author was at National Research Ecology Laboratory, Colorado State University, Fort Collins and CPB–CNRS, Vandoeuvre les Nancy, France. This study was partially supported by Consejo de Desarrollo Científico y Humanístico and Consejo Nacional de Investigaciones Científicas y Tecnológicas, Venezuela.

REFERENCES

Aguilar R. and Heil R. D. (1988) Soil organic carbon, nitrogen and phosphorus quantities in Northern Great Plains rangeland. *Soil Science Society of America Journal* **52**, 1076–1081.

Bowman R. A. and Cole C. V. (1978) Transformation of organic phosphorus substrates in soils as evaluated by $NaHCO_3$ extraction. *Soil Science* **125**, 49–51.

Burnham C. P. and López-Hernández D. (1982) Phosphate retention in different soil taxonomic classes. *Soil Science* **134**, 376–380.

Chater, M. and Mattingly, G. E. G. (1980) Changes in organic phosphorus contents of soils from long-continued experiments of Rothamsted and Saxmundham. Rothamsted Experimental Station Report for 1979, Part 2, pp. 41–60.

Cole C. V., Innis G. S. and Stewart J. W. B. (1977) Simulation of phosphorus cycling in semiarid grassland. *Ecology* **58**, 1–15.

Enwezor W. O. (1967) Significance of the C: organic ratio in the mineralization of soil organic phosphorus. *Soil Science* **103**, 62–66.

Fardeau J.C., Morel C. and Jappe J. (1985) Cinétique d'échange des ions phosphate dans le système soil-solution. Verification experimentale de l'équation theorique. *Comptes Rendus de l'Académie des Sciences Paris* **300**(III.8), 371–376.

Fardeau J. C. Le phosphore assimilable des sols: sa représentation par un modèle fonctionnel à plusieurs compartiments. *(1993) Agronomie* **13**, 317–331.

Frossard E., Fardeau J. C., Brossard M. and Morel J. L. (1994) Soil isotopically exchangeable phosphorus: A comparison between *E* and *L* values. *Soil Science Society of America Journal* **58**, 846–851.

Frossard E., López-Hernández D. and Brossard M. (1996) Can isotopic exchange kinetics give valuable information on the rate of mineralization of organic phosphorus in soils? *Soil Biology and Biochemistry* **28**, 857–864.

Haas H. J., Grunes H. J. D. and Riechman G. A. (1961) Phosphorus changes in Great Plains soils as influenced by cropping and manure application. *Soil Science Society of America Proceedings* **25**, 214–218.

Halm B. J., Stewart J. W. B. and Halstead R. L. (1972) The phosphorus cycle in a native grassland. In *Symposium on the Use of Isotopes and Radiation in Research on Soil-Plant Relationships including Applications in Forestry.* International Atomic Energy Agency and FAO, Vienna. IAEA-SM-151/7, pp. 571–586.

Harrison A. F. (1982) ^{32}P method to compare rates of mineralization of labile organic phosphorus in woodland soils. *Soil Biology & Biochemistry* **14**, 343–351.

Hedley M. J., Stewart J. W. B. and Chauhan B. S. (1982) Changes in inorganic soil phosphorus fractions induced by cultivation practices and by laboratory incubations. *Soil Science Society of America Journal* **46**, 970–976.

Honeycutt C. W., Heil R. D. and Cole C. V. (1990a) Climatic and topography relations of three Great Plains soils: I. Soil morphology. *Soil Science Society of America Journal* **54**, 469–475.

Honeycutt C. W., Heil R. D. and Cole C. V. (1990b) Climatic and topography relations of three Great Plains soils: II. Carbon, nitrogen and phosphorus. *Soil Science Society of America Journal* **54**, 476–483.

Islam A. and Ahmed B. (1973) Distribution of inositol phosphates, phospholipids, nucleic acids and mineralization of inositol phosphates in some Bangladesh soils. *Journal of Soil Science* **24**, 193–198.

Jayachandran K., Schwab A. P. and Hetrick B. A. D. (1992) Partitioning dissolved inorganic and organic phosphorus using acidified molibdate and isobutanol. *Soil Science Society of America Journal* **56**, 762–765.

Jose A. Y. and Krihsnamoorthy K. K. (1972) Isotopic exchange of phosphate in soils: E value. *Soils Fertility* **35**, 620–627.

López-Hernández D. and Burnham C. P. (1974) The covariance of phosphate sorption with other soil properties in some British and Tropical soils. *Journal of Soil Science* **25**, 196–206.

López-Hernández D. and Niño M. (1993) Phosphorus mineralization during laboratory incubation in soils derived from different textured parent materials. *Geoderma* **56**, 527–537.

López-Hernández D., Niño M., Nannipieri P. and Fardeau J. C. (1989) Phosphatase activity in *Nasutitermes ephrate* termite nests. *Biology and Fertility of Soils* **7**, 134–137.

Mueller-Harvey Y., Juo A. S. R. and Wild A. (1985) Soil organic C, N, S and P after forest clearance in Nigeria: mineralization rates and spatial variability. *Journal of Soil Science* **36**, 585–591.

Murphy J. and Riley J. P. (1962) A modified single solution method for the determination of phosphate in natural waters. *Analytical Chimica Acta* **27**, 31–36.

Ruiz J. and López-Hernández D. (1977) La mineralización del fósforo orgánico en cuatro series de suelos venezolanos. *Acta Científica Venezolana* **28**, 133–137.

Saunders W. M. H. and Metson A. J. (1971) Seasonal variation of phosphorus in soil and pasture. *New Zealand Journal of Agricultural Research* **14**, 307–328.

Schimel D. S., Coleman D. C. and Horton K. A. (1985) Soil organic matter dynamics in paired rangeland and cropland toposequences in North Dakota. *Geoderma* **36**, 201–214.

Sharpley A. N. (1985) Phosphorus cycling in unfertilized and fertilized agricultural soils. *Soil Science Society of America Journal* **49**, 905–911.

Thompson L. M. and Black C. A. (1947) The effect of temperature on the mineralization of soil organic phosphorus. *Proceedings of the Soil Science Society of America* **12**, 323–326.

Tran T. S., Fardeau J. C. and Giroux M. (1988) Effects of soil properties on plant-available phosphorus determined by the isotopic dilution phosphorus-32 method. *Soil Science Society of America Journal* **52**, 1383–1390.

Walbridge M. R. and Vitousek P. (1987) Phosphorus mineralization in acid organic soils: processes affecting $^{32}PO_4^{3-}$ isotope dilution measurements. *Soil Biology & Biochemistry* **19**, 709–717.

White R. E. (1976) Concepts and methods in the measurement of isotopically exchangeable phosphate in soil. *Phosphorus in Agriculture, Paris* **67**, 9–16.

Zou X., Binkley D. and Kenneth G. D. (1992) A new method for estimating gross phosphorus mineralization and immobilization rates in soils. *Plant and Soil* **147**, 243–250.