



CAN ISOTOPIC EXCHANGE KINETICS GIVE VALUABLE INFORMATION ON THE RATE OF MINERALIZATION OF ORGANIC PHOSPHORUS IN SOILS?

E. FROSSARD,*¹ D. LÓPEZ-HERNÁNDEZ² and M. BROSSARD³

¹Institute of Plant Sciences, Swiss Federal Institute of Technology (ETH), CH-8092 Zürich, Switzerland, ²Laboratorio de Estudios Ambientales, IZT, Facultad de Ciencias UCV, Apdo. 47058, Caracas 1041-A, Venezuela and ³ORSTOM, c/o Centre de Pédologie Biologique-CNRS, BP 5, F-54501 Vandoeuvre les Nancy, France

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Summary—Although mineralization of soil organic phosphorus can play an important role in plant nutrition, its measurement has been impeded by methodological difficulties. Our objective was to show whether the determination of isotopic exchange kinetics could give information on the net mineralization of organic P compounds in soils. Using this technique, the mineralization of two organic phosphates (adenosine triphosphate, ATP, and nicotinamide adenine dinucleotide, NAD) was studied in an alfisol during an incubation. Parameters describing the isotopic exchange kinetics, $r(1)/R$, n and cp , were used to calculate the quantity of phosphate ions (P) present in the pool of free ions and in the pool of P isotopically exchangeable between 1 min and 1 d. In addition resin-extractable P was measured in the same samples. Following the additions of ATP and NAD, P was rapidly released to the soil solution increasing the P content of the pool of free ions which subsequently decreased until the end of the incubation. Mineralized P in the soil amended with ATP or NAD and remaining either water-extractable or in the pool of free ions was positively correlated to the quantity of mineralized P which remained resin-extractable. The isotopic method measured the quantity of mineralized P which remained immediately plant-available. The changes in the P content of the pool of P exchangeable between 1 min and 24 h could not be related to organic-P mineralization. This lack of a relationship was related to variations in the n value which could have originated in organic-P mineralization during the isotopic exchange experiment itself or in sample heterogeneity. Therefore when studying soil organic-P mineralization with our proposed technique great caution should be taken before extrapolating the quantity of isotopically exchangeable phosphate to isotopic exchange times longer than 1 min. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Phosphorus mineralized from soil organic-P sources plays an important role in plant nutrition in low input agrosystems or in natural ecosystems (Harrison, 1987). However, the measurement of phosphate mineralization can be severely hampered by the high reactivity of the PO_4 ions (denoted hereafter P) which can be rapidly adsorbed on soil surfaces after their release to the solution. Bowman and Cole (1978) evaluated the mineralization of various forms of organic phosphate added to a soil by measuring the P content of bicarbonate extracts after various incubation times. Other authors have suggested that the changes in P content of different mineral and organic pools determined by a sequential extraction could provide more complete information on the extent of mineralization and immobilization processes (Condon *et al.*, 1993; Zhang *et al.*, 1994). However, these extractants may not reveal all the P which has been mineralized and subsequently readsorbed on soil surfaces (Fardeau *et al.*, 1988),

therefore leading to an under- or overestimation of this process.

An alternative to chemical extractants is the use of isotopic techniques. Walbridge and Vitousek (1987) and López-Hernández and Nino (1993) labeled the soil solution with ^{32}P and measured the quantities of P and ^{32}P extracted after various incubation times by a dilute acid-fluoride solution or by a resin, respectively. Their major hypotheses were that (i) the added ^{32}P would equilibrate with the soil exchangeable-P, (ii) the organic P would not be labeled, and (iii) mineralization would lower the specific activity ($^{32}P/P$) of the extracted P. The magnitude of this decrease would then provide an estimate of gross mineralization (Walbridge and Vitousek, 1987). This approach has two limitations. First these authors did not respect one of the conditions imposed by Sheppard (1962) when studying isotopic exchange in multicompartmental systems: they labeled their soils by introducing ^{32}P into the soil solution, but then they extracted it with chemicals, whereas they should have measured the ^{32}P in the compartment where the isotope was introduced, i.e. in the soil solution. The resin or chemicals which they used extracted P from

*Author for correspondence.



many undefined compartments (Fardeau *et al.*, 1988), making their calculated specific activities very difficult to interpret from a theoretical point of view. Second, they considered that the added ^{32}P equilibrated with the isotopically-exchangeable P within relatively short times, which is not correct since a slow, but significant, isotopic exchange can continue over much longer times (at least 3 months, Fardeau *et al.*, 1985; Morel and Plenchette, 1994; Frossard *et al.*, 1994). Therefore the decrease in specific activity assumed to be associated to mineralization of organic-P could also be partly attributed to the dilution of ^{32}P with increasing quantities of exchangeable P, that phenomenon being even more marked in high P sorbing soils (Fardeau, 1993).

Fardeau (1993) proposed that the data obtained from the exchange kinetic experiment may be analyzed with a multicompartmental model which considers five pools of P. The most important is the central pool containing free P which contains P ions immediately available to plants, i.e. solution P plus P present on the solid but which have the same mobility as solution P. These free ions can be exchanged with any other P present in the four other pools which are located on the solid phase of the soil. These are: the pool of P isotopically exchangeable between 1 min and 1 d (which is the period during which a single root can actively take up P), the pool of P isotopically-exchangeable between 1 day and 3 months (i.e. during the period of active growth of the root system of annual crops), the pool of P isotopically-exchangeable between 3 months and 1 y and the pool of P which can not be isotopically exchanged within 1 y. The P content of these pools is calculated using a function of time presented by Fardeau (1993) using the following basic parameters: the proportion of ^{32}P remaining in the soil solution after 1 min of isotopic exchange ($r(1)/R$), the rate of disappearance of ^{32}P from the soil solution between 1 and 100 mins of isotopic exchange (n), the concentration of water soluble P (cp), and the total soil mineral P content. Among these parameters $r(1)/R$, n and cp are obtained from an experiment conducted on a soil-solution system in a steady-state equilibrium during 100 mins of isotopic exchange.

The use of this isotopic approach and its pluricompartmental analysis might yield information on the net mineralization of organic phosphorus. Immediately following the mineralization an increase of solution P should be noticed. As time elapses and P are being adsorbed on soil surfaces, the quantity of free P should decrease while the P content of the other pools should increase. Our objective was to test

this hypothesis, by measuring changes in the isotopic exchange kinetics basic parameters and in the quantity of free phosphate ions in an alfisol incubated in the presence of two organic phosphates, and by checking whether under such conditions the P content of other compartments could be reliably calculated. In addition, the quantity of resin-extractable P was measured in the same samples.

MATERIAL AND METHODS

The surface horizon (0–10 cm) of a cultivated alfisol developed in a loamy deposit was sampled for this study. The soil was sieved (2 mm) and kept moist prior to the incubation. Coarse plant debris were removed by hand before the experiment. The major physico-chemical characteristics of this soil are displayed in Table 1.

Two organic phosphates were added: a disodium adenosine 5'-triphosphate (ATP; MW 551.1) and β -nicotinamide adenine dinucleotide (NAD; MW 663.4). Both compounds were obtained from Sigma Chemical Co. (St Louis, MO, USA)

The following treatments were considered: control soil, soil + NAD (100 mg P kg⁻¹ soil), soil + ATP (94 mg P kg⁻¹ soil), γ irradiated soil, γ irradiated soil + NAD (100 mg P kg⁻¹ soil) and γ irradiated soil + ATP (94 mg P kg⁻¹ soil). The soil was irradiated for 2 h and received a dose of 20 kGy. Soil samples were incubated in the dark at 20°C, in 500 ml closed flasks containing 100 g of fresh soil with a water content of 25 g H₂O 100 g⁻¹ soil (i.e. at 80% of the water holding capacity). Stoppers were removed each week from the flasks for a few hours (except from the flasks containing irradiated soil) in order to ensure aerobic conditions. One flask from each treatment was destructively sampled 0.7 day after mixing the soil and the organic P, and then after 2, 8, 14, 21 and 28 days of incubation. At each date three samples were taken from each flask and the analyses were conducted directly on fresh samples. Isotopic exchange kinetics experiments were performed only in the following treatments: control soil, soil + NAD (100 mg P kg⁻¹ soil), soil + ATP (94 mg P kg⁻¹ soil) and γ irradiated soil. Resin-extractable P was measured in all treatments.

The isotopic exchange kinetics experiments were conducted on a soil-solution system in a steady-state with a soil-to-solution ratio of 1:10 (Fardeau *et al.*, 1985; Salcedo *et al.*, 1991). Four or five samplings of the suspension were made between 1 and 100 mins after the addition of the radioactive tracer. Each experiment was conducted with carrier-free ^{32}P -

Table 1. Selected physico-chemical characteristics of the surface horizon (0–10 cm) of the alfisol (in units of mg kg⁻¹)

Clay	Silt	Sand	Organic matter	Fed	Total P	Organic P	pH water
282	535	145	36.3	20	887	140	7.0

Fed: total iron oxides extractable with a dithionite, citrate, bicarbonate mixture.

Table 2. Basic parameters of isotopic exchange kinetics at different incubation times in an alfisol after the addition of two organic phosphates

Incubation time (days)	0.7	2	8	14	21	28
	Control					
$r(1)/R$	0.21 β^*	0.20 β	0.20 β	0.21 β	0.23 χ	0.23 χ
n	0.29 α	0.26 $\alpha\beta$	0.30 α	0.29 α	0.29 α	0.30 α
cp (mg P l ⁻¹)	0.16 χ/b^{**}	0.19 χ/b	0.16 β/b	0.25 χ/a	0.25 χ/a	0.24 β/a
	Irradiated					
$r(1)/R$	0.17 χ	0.16 χ	0.18 β	0.17 β	0.19 χ	0.20 χ
n	0.30 α	0.32 α	0.27 α	0.31 α	0.29 α	0.29 α
cp (mg P l ⁻¹)	0.18 χ/b	0.16 χ/b	0.25 β/a	0.19 χ/b	0.25 χ/a	0.25 β/a
	Soil + ATP					
$r(1)/R$	0.46 α/b	0.45 α/b	0.40 α/c	0.34 α/d	0.38 α/c	0.52 $\alpha\alpha$
n	0.11 β/β	0.19 β/a	0.19 β/a	0.18 β/a	0.18 β/a	0.19 β/a
cp (mg P l ⁻¹)	1.52 β/a	1.34 β/a	1.12 α/b	0.98 α/bc	0.80 α/c	0.83 α/c
	Soil + NAD					
$r(1)/R$	0.49 α/a	0.46 α/a	ND ^{***}	0.25 β/c	0.29 β/c	0.36 β/b
n	0.14 β/c	0.18 β/c	ND	0.21 β/b	0.25 $\alpha\beta/a$	0.21 β/b
cp (mg P l ⁻¹)	1.86 α/a	1.82 α/a	ND	0.59 β/b	0.64 β/b	0.79 $\alpha\beta$

* Data followed by the same Greek letter (α , β , χ , δ) in the same column are not significantly different at the level of 5% by the test of Newman-Keuls. This indicates the effect of treatment on a given parameter ($r(1)/R$, n or cp) for a given incubation time.

** Data followed by the same Roman letter (a, b, c, d) in the same line are not significantly different at the level of 5% by the test of Newman-Keuls. This indicates the effect of incubation time on the studied parameter for a given treatment.

*** ND: not determined.

Changes in the P content of the pool of free ions ($E_{1\text{min}}$) and in the pool of P exchangeable between 1 min and 1 d ($E_{1\text{min-24h}}$)

Control and irradiated treatments. The P content of $E_{1\text{min}}$ varied between 7.6 and 12.2 mg P kg⁻¹ during the incubation in the control soil, and between 10.1 to 13.5 mg P kg⁻¹ in the irradiated soil (Table 3). The P content of the $E_{1\text{min-24h}}$ pool ranged between 51.0 and 73.0 mg P kg⁻¹ in the control soil and between 77.6 and 90.1 mg P kg⁻¹ in the irradiated treatment. $E_{1\text{min}}$ values were higher at the end of the incubation in both treatments and were slightly higher in the irradiated soil compared to the control. The higher $E_{1\text{min}}$ values observed in the irradiated sample may be attributed to the release of microbial P to the solution following the biocidal treatment (Seeling and

Zasoski, 1993; Zou *et al.*, 1992). No particular trends were observed in the $E_{1\text{min-24h}}$ pool.

ATP and NAD treatments. The addition of organic phosphates resulted in large changes in the P content of the various pools (Table 3). The quantity of P present in the pool of free ions ($E_{1\text{min}}$) increased strongly just after the addition of both substrates to the soil reaching 33.0 mg P kg⁻¹ with ATP and 37.9 mg P kg⁻¹ with NAD, and then decreased with time. The quantity of P exchangeable between 1 min and 1 d ($E_{1\text{min-24h}}$) increased from 33.7 mg P kg⁻¹ and 59.0 mg P kg⁻¹ at the beginning of the experiment to 56.2 mg P kg⁻¹ and 70.4 mg P kg⁻¹ after 28 days of incubation in soils amended with ATP and NAD, respectively.

$E_{1\text{min}}$ values were significantly higher in the soils amended with NAD and ATP, showing that these

Table 3. Changes in the quantities of isotopically exchangeable P (mg P kg⁻¹ soil) after different incubation times in an alfisol following the addition of two organic phosphates

Incubation time (days)	0.7	2	8	14	21	28
	Control					
P pools*						
$E_{1\text{min}}$	7.6 δ^{**}/b	9.2 χ/ab	8.0 χ/b	12.2 χ/a	10.5 χ/ab	10.4 δ/ab
$E_{1\text{min-24h}}$	51.0	50.1	58.2	78.0	66.0	73.0
	Irradiated					
$E_{1\text{min}}$	10.6 χ/b	10.1 χ/b	13.8 β/b	11.1 χ/b	13.5 β/a	12.4 χ/ab
$E_{1\text{min-24h}}$	77.6	82.7	77.8	85.3	90.1	78.0
	Soil + ATP					
$E_{1\text{min}}$	33.0 β/a	29.8 β/a	28.3 α/a	29.1 α/a	20.9 α/b	15.9 β/c
$E_{1\text{min-24h}}$	33.7 b	78.2 a	70.3 a	71.0 a	52.6 ab	56.2 ab
	Soil + NAD					
$E_{1\text{min}}$	37.9 α/a	38.2 α/a	ND ^{***}	23.3 β/b	21.8 α/b	22.2 α/b
$E_{1\text{min-24h}}$	59.0 b	92.0 a	ND	73.5 b	94.4 a	70.4 b

* P pools: $E_{1\text{min}}$: P exchangeable in 1 min; $E_{1\text{min-24h}}$: P exchangeable between 1 min and 1 d.

** see Table 2 for the key for the Greek and Roman letters.

*** ND: not determined.

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

$$\frac{r(t)}{R} = \frac{r(1)}{R} \left\{ t + \left(\frac{r(1)}{R} \right)^{1/n} \right\}^{-n} + \frac{r(\infty)}{R} \quad (1)$$

where $r(1)$, and $r(\infty)$ are respectively the radioactivity (MBq) remaining in the solution after 1 min and infinity, and n is a parameter obtained from the isotopic exchange kinetics experiment. In this experiment, n is calculated as the slope of the regression equation between $\log[r(t)/R]$ and $\log [t]$ because for t less than 100 mins, $[r(1)/R]^{1/n}$ is negligible compared to t and $r(\infty)/R$ is negligible compared to $r(1) \times t^{-n}/R$.

The ratio $r(\infty)/R$ is the maximum possible dilution of the isotope. It should be approximated by the ratio of the water-soluble P to the total inorganic P (P_{inorg}) of the soil (Fardeau, 1993). However, because P_{inorg} is extracted by acidic reagents (Saunders and Williams, 1955) which are also known to solubilize some organic phosphates such as ATP (Jayachadran *et al.*, 1992), we approximated $r(\infty)/R$ in our study by the ratio of the water-soluble P to the total soil P (P_t):

$$\frac{r(\infty)}{R} = \frac{10cp}{P_t} \quad (2)$$

where cp is the water-soluble P (mg P l^{-1}). The factor 10 arises from the soil-to-solution ratio of 1 g of soil in 10 ml of water so that 10 cp is equivalent to the water-soluble P content of the soil expressed in mg kg^{-1} .

The quantity, $E(t)$ (mg P kg^{-1} soil), of isotopically-exchanged P at time t is calculated assuming that (i) ^{31}P and ^{32}P 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.

$$\frac{r(t)}{cp10} = \frac{R}{E(t)} \quad (3)$$

Therefore

$$E(t) = 10cp \frac{R}{r(t)} \quad (4)$$

The P content of the pool of free P ions and of the pool of P isotopically exchangeable between 1 min and 24 h were calculated. The P content of the pool of free phosphate was approximated by the quantity of P isotopically exchanged within 1 min ($E_{1\text{min}}$) (Tran *et al.*, 1988; Fardeau, 1993), and the content of the pool of P exchangeable between 1 min and 1 d

($E_{1\text{min-24h}}$) was calculated as the difference between the quantity of P exchangeable after 1 d and that exchangeable after 1 min using Equation (4).

P_{resin} was measured after extracting 1 g of fresh soil with 1 g of resin saturated with Cl^- ions for 17 h. The resin was then eluted in 1.8 M H_2SO_4 and P_{inorg} in the solution was colorimetrically measured with the same procedure as for water-soluble P (John, 1970). Total P was measured after calcination of the soil at 550°C and oxidation of the ashes in concentrated nitric acid, and total organic P was measured by the method of Saunders and Williams (1955). ^{32}P was measured by liquid scintillation counting.

RESULTS AND DISCUSSION

This section is divided into five subsections. The basic isotopic exchange kinetics data ($r(1)/R$, n and cp) are presented first. Changes in the P contents of the pool of free ions and of the pool of P exchangeable between 1 min and 24 h, and resin-extractable P form the second and third sections, respectively. In the fourth section the rate of mineralization of organic P is presented and finally the limits of this isotopic approach are discussed.

$r(1)/R$, n and cp

Control and γ irradiated treatments. The data obtained on the control soil are classical for loamy cultivated soils from temperate areas (Frossard *et al.*, 1992). $r(1)/R$ and n did not vary during the study whereas the concentration of P in the solution (cp) increased significantly after 2 weeks of incubation (Table 2). $r(1)/R$ values of the irradiated soil remained constant but they were systematically lower than those measured in the control. In the irradiated soil n remained constant and cp fluctuated.

ATP and NAD treatments. $r(1)/R$, n and cp changed considerably upon the introduction of the two organic phosphates. The introduction of both substrates (ATP and NAD) increased $r(1)/R$ and cp while n decreased. Afterwards $r(1)/R$ decreased for both substrates between 0.7 d and 14 d and increased again during the last 14 d of the experiment. Concomitantly increasing n and decreasing cp values were observed for longer incubation times.

Increases of $r(1)/R$ and cp and a decrease of n are observed when water-soluble P is added to soils, while $r(1)/R$ and cp decreases and n increase are associated with the depletion of water-soluble P from the soil solution (Frossard *et al.*, 1994). Therefore, our results demonstrate that immediately after the introduction of the organic phosphates large quantities of P were released to the soil solution. The subsequent depletion of P from the solution could in turn be related to its sorption onto the solid phase of the soil or to its immobilization by microorganisms.

Table 4. Changes in resin-extractable P (mg P kg⁻¹ soil) after different incubation times in an alfisol following the addition of two organic phosphates

Incubation time (days)	0.7	2	8	14	21	28
Control	42.8 χ a	40.2 β a	30.7 χ b	42.4 β a	43.6 χ a	38.1 χ a
Soil + ATP	85.6 α a	85.1 α a	74.2 α a	75.1 α ab	67.8 α b	81.4 α ab
Soil + NAD	89.5 α a	84.8 α ab	76.2 α bc	71.6 α c	71.3 α c	77.1 α bc
Irr ^o . control	43.0 χ b	39.4 β b	50.0 β a	38.8 β b	38.2 χ b	31.0 χ c
Irr. + ATP	68.7 β bc	93.2 α a	79.9 α b	70.0 α bc	59.1 β c	59.8 β c
Irr. + NAD	73.9 β ab	88.3 α a	ND ^{***}	69.1 α b	56.2 β b	56.7 β b

* See Table 2 for the key for the Greek and Roman letters.

** Irr.: irradiated soil.

*** ND: not determined.

organic P compounds were mineralized. However no significant differences were observed between $E_{1 \text{ min-24 h}}$ values suggesting that either no mineralized P reached this pool, or more probably, that the $E_{1 \text{ min-24 h}}$ determinations were not reliable enough.

Changes in the resin-extractable P (P_{resin})

Control and irradiated treatments. P_{resin} remained constant during the incubation in the control soil, except for a low value observed after 8 days (Table 4). Similarly P_{resin} was constant in the irradiated soil without applied P except for a higher value noted after 8 days and a lower value observed after 28 days of incubation. Very small significant differences were observed between these two treatments.

ATP and NAD treatments. The addition of organic phosphates resulted in large increases in P_{resin} both in the non-irradiated and in the irradiated soils. In the irradiated soils maximum P_{resin} values were observed after 2 days of incubation for both substrates. P_{resin} steadily decreased until the end of the incubation. In the non-irradiated soils the highest P_{resin} values were observed after 0.7 d for both substrates. These values then decreased until they reached a minimum after 21 days before increasing again after 28 days.

P_{resin} values were significantly higher in the organic P amended samples compared to the non-amended soils. Furthermore, significantly lower P_{resin} values were observed in the irradiated soils amended with ATP and NAD compared to the non-irradiated amended soils at three times: 0.7, 21 and 28 days of incubation. But they were not different after 2, 8 and 14 days. These results suggest that very little mineralization of organic P occurred in the control soil, but that large quantities of P were mineralized after the addition of ATP and NAD. Most of this mineralization during the first two weeks of incubation may be attributed to enzymatic activities since similar results were obtained on irradiated and non-irradiated soils. The steady decrease of P_{resin} values in the organic P amended irradiated soils from day 2 to day 28 probably denoted the abiotic reaction of released inorganic P with soil surfaces. The increases in P_{resin} in the non-irradiated sample after 21 days on the other hand showed that a subsequent mineralization occurred 3 weeks after the introduction of the organic P compounds. This late

mineralization was microbiologically-mediated and could not solely be attributed to phosphatase activities. The mineralized P could be of two origins: either this was the remains of the initially introduced but not yet degraded ATP or NAD, or it resulted from the mineralization of newly-synthesized microbial P built up during the early phases of the incubation.

Calculation of the mineralization rate of added organic P

The main hypothesis when calculating the amount of organic P mineralized during the incubation was that the amount of mineralized soil organic P remained negligible in comparison to the amount of P mineralized from the ATP or the NAD. This is possible because the introduced quantity of organic P (100 mg P kg⁻¹ soil as NAD and 94 mg P kg⁻¹ soil as ATP) was close to the initial quantity of soil organic P (140 mg P kg⁻¹ soil) most of which was probably present in stable forms as reported for soils which have been cultivated for long periods (Condron *et al.*, 1991). The net quantity of P mineralized in the soil amended with organic P and remaining water soluble ($\delta 10cp$) was estimated as the difference between the quantity of water-soluble P present in the amended soil at a given sampling date and the quantity of water-soluble P present in the control soil at the beginning of the experiment. The same calculations were carried out to estimate the net quantity of mineralized P remaining in the pool of free ions ($\delta E_{1 \text{ min}}$), exchangeable within 24 h ($\delta E_{24 \text{ h}}$) or resin-extractable (δP_{resin}).

Results showed that similar quantities of P were recovered from both substrates when considering either water-soluble P, the pool of free ions or the resin-extractable P (Table 5). The proportion of mineralized P recovered in the water extract reached 14 to 17% of the added organic P at the beginning of the experiment and decreased steadily to 6 to 7% after 28 days. This recovery rate decreased from 27 to 30% of the added P at the beginning of the incubation to 9 to 15% after 4 weeks when the calculations were carried out with $E_{1 \text{ min}}$. With the resin extraction, results were slightly different. The proportion of mineralized P decreased from almost 45 to 46% at the beginning to about 26 to 28% after

Table 5. Net quantities of P mineralized in an incubated alfisol amended with organic P (results are expressed in mg P kg⁻¹).

Days of incubation	0.7	2	8	14	21	28
$\delta 10cp$						
ATP	13.6	11.8	9.6	8.2	6.4	6.7
NAD	17.0	16.6	ND	4.3	4.8	6.3
δE_{1min}						
ATP	25.4	22.2	20.7	21.5	13.3	8.3
NAD	30.3	30.6	ND	15.7	14.2	14.6
δE_{24h}						
ATP	8.1	49.4	40	41.5	14.9	13.5
NAD	38.3	71.6	ND	38.2	57.6	34
δP_{resin}						
ATP	42.8	42.3	31.3	32.2	25.0	38.5
NAD	46.7	42.0	33.4	28.7	28.5	34.2

$\delta 10cp$: quantity of mineralized P remaining water soluble; δE_{1min} : quantity of mineralized P remaining in the pool of free ions; δE_{24h} : quantity of mineralized P remaining in the pool of P isotopically-exchangeable within 24h; δP_{resin} : quantity of mineralized P remaining resin-extractable.

ND: not determined.

3 weeks and increased again during the last week of the experiment to reach values close to 34 to 41% of the added P. By contrast widely-fluctuating values showing no particular trends were observed when E_{24h} was used to calculate the amount of mineralized P.

A highly significant linear correlation was observed between the quantities of mineralized P calculated with water-extractable P ($\delta 10cp$) and resin-extractable P (P_{resin}) (Fig. 1). Similarly a significant positive relation was noted between the quantities of mineralized P calculated with the quantity of P isotopically-exchangeable within 1 min (δE_{1min}) and resin-extractable P (δP_{resin}) (Fig. 2). However on this graph one point lay outside of the correlation with too low a δE_{1min} value. This point originated from the analysis of the last sampling date of the experiment in the presence of ATP where the

resin extraction still showed that high quantities of P were mineralized, while the E_{1min} value was at its lowest. The correlation became highly significant when this outlier was taken out. No relationship was observed between δE_{24h} and δP_{resin} . The relations observed between $\delta 10cp$ and δP_{resin} and between δE_{1min} and δP_{resin} suggest that they give information on the same process, whereas the absence of relation existing between δE_{24h} and δP_{resin} suggests that δE_{24h} can not be used to assess the quantity of mineralized P. Nevertheless, our results give some indications on the fate of P following its mineralization. For instance just after the addition (0.7 day of incubation) of NAD 17.0% of the added P was water soluble, 13.3% was sorbed but still had the same mobility as water-soluble ions, 16.4% was sorbed more tightly on soil surfaces but was still resin-extractable and 53.3% was not recovered. By the end

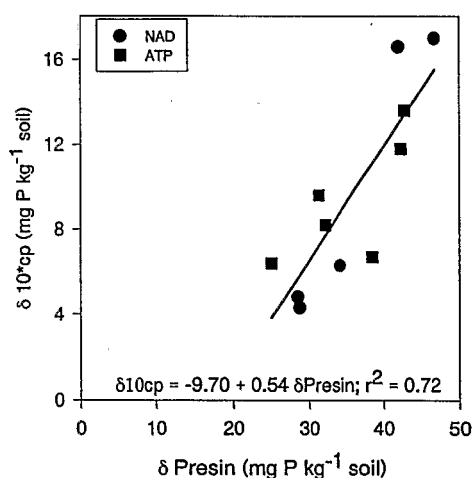


Fig. 1. Relationship between the quantity of mineralized P remaining resin-extractable (δP_{resin}) and the quantity of mineralized P remaining water soluble ($\delta 10cp$) in an alfisol amended with ATP and NAD. Abscissa: δP_{resin} (mg P kg⁻¹ soil); ordinate: $\delta 10cp$ (mg P kg⁻¹ soil).

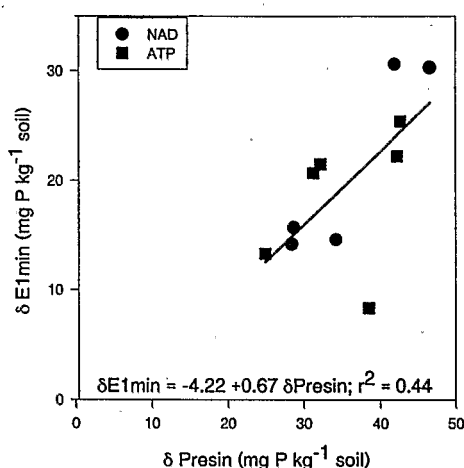


Fig. 2. Relationship between the quantity of mineralized P remaining resin-extractable (δP_{resin}) and the quantity of mineralized P remaining in the pool of free ions (δE_{1min}) in an alfisol amended with ATP and NAD. Abscissa: δP_{resin} (mg P kg⁻¹ soil); ordinate: δE_{1min} (mg P kg⁻¹ soil).

of the incubation these figures had changed as follows: 6.3% of the added P was water soluble, 8.3% was sorbed but still had the same mobility as water-soluble ions, 19.6% was sorbed more tightly on soil surfaces but was still resin extractable, while 65.3% was not recovered. This lower recovery rate can be related to the sorption of P on soil particles or to its immobilization by soil microorganisms.

The high rates of mineralization observed at the beginning of the experiment for both substrates agree with studies on labile organic-P compounds such as ATP (Maire, 1984) and RNA (Harrison, 1982). The probably incomplete mineralization of these compounds at the beginning of the experiment may be related to the feedback effect proposed by McGill and Cole (1981) who suggested that high concentrations of orthophosphate in the soil solution would inhibit phosphatase activity.

Limits of the proposed isotopic approach

It has been shown that $E_{1\text{ min}}$ values could give some information on the mineralization of organic P, but that nothing could be deduced from $E_{24\text{ h}}$ values. This poor significance of $E_{24\text{ h}}$ values may originate from small errors made on the determinations of the n value. An increase of 0.06 in the n value, equivalent to twice the average standard deviation observed for n during this experiment, can increase $E_{24\text{ h}}$ up to 24 mg P kg⁻¹ soil in the control and up to 49 mg P kg⁻¹ soil in the soil amended with NAD, while $E_{1\text{ min}}$ values do not vary with n (Table 6). Within this range of n values the estimated quantity of mineralized P using $\delta E_{24\text{ h}}$ values would then lie somewhere between 57 and 106 mg P kg⁻¹ soil. Such variations of the n values ($n \pm 0.03$) can be accounted for by mineralization of organic P during the isotopic exchange kinetic experiment itself invalidating the steady-state hypothesis underlying the interpretation of this isotopic approach and preventing the calculation of the exact n value, or by the fact that the experiments were conducted on wet soils which probably increased sample heterogeneity. These considerations suggest that when studying the mineralization of organic P with the proposed technique it may be hazardous to extrapolate E values to isotopic exchange times longer than 1 min.

Some limitations of the isotopic approach have

Table 6. Effects of the variation of parameter n on the P content of two pools of isotopically-exchangeable P: P exchangeable in 1 min ($E_{1\text{ min}}$, mg P kg⁻¹ soil) and P exchangeable in 1 day ($E_{24\text{ h}}$, mg P kg⁻¹ soil) in an alfisol amended or not with NAD

	$n - 0.03$	Determined n value	$n + 0.03$
Control			
		Incubated 0.7 day	
n	0.26	0.29	0.32
$E_{1\text{ min}}$	7.6	7.6	7.6
$E_{24\text{ h}}$	48	58	72
Soil + NAD			
		Incubated 2 days	
n	0.15	0.18	0.21
$E_{1\text{ min}}$	38	38	38
$E_{24\text{ h}}$	105	130	154

also been reported in high P-sorbing soils where the determination of very low concentrations of P in the solution is delicate and where some of the basic assumptions underlying this approach, such as the hypothesis that ³¹P and ³²P have the same fate in soil-solution systems, may not be met (Salcedo *et al.*, 1991; Barrow, 1991). However in our study these assumptions were probably met since the soil sample which was used was from the same origin as the alfisol for which it was demonstrated that the L value calculated after 13 weeks of growth of *Agrostis capillaris* was not different from its E value calculated for the same isotopic exchange time using Equation 4 (Frossard *et al.*, 1994).

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