Isotope methods for assessing plant available phosphorus in acid tropical soils

Article *in* European Journal of Soil Science · September 2003

DOI: 10.1046/j.1365-2389.2003.00542.x

Astrid Oberson
ETH Zurich

Sokrat Sinaj
Agroscope

Emmanuel Frossard
ETH Zurich

European Journal of Soil Science, September 2003, **54**, 605–616

Isotope methods for assessing plant available phosphorus in acid tropical soils

S. Bühler[a], A. Oberson[a], S. Sinaj[a], D. K. Friesen[b] & E. Frossard[a]
[a]*Institute of Plant Sciences, Swiss Federal Institute of Technology (ETH), Eschikon 33, 8315 Lindau, Switzerland, and* [b]*IFDC/CIMMYT, PO Box 25171, Nairobi, Kenya*

Summary

Use of isotope methods to measure the availability of phosphorus (P) in soils that are well supplied with P is well established. We have evaluated such methods for acid tropical soils with very small P contents, which are less well studied. The isotopically exchangeable P in soil suspensions (E value) and that in plant growth experiments (L value) were measured in soils that had received varying amounts of P fertilizer in two field experiments in Colombia. We determined the E values over 4–5 weeks of equilibration allowing for the kinetics of isotope exchange. The decrease in radioactivity in the soil solution at a particular time, t, divided by that at the start (r_t/R) was described by three parameters (r_1/R, r_∞/R, and a coefficient n) derived from the time course of isotopic exchange over 100 minutes. Values of E_t were calculated either from measured values of r_t/R or those extrapolated until 12 weeks. *Agrostis capillaris* was grown on the same soils labelled with carrier-free ^{33}P-orthophosphate ions to obtain L values. Agreement between E values derived from measured and extrapolated values of r_t/R was satisfactory, but errors in n and r_∞/R limited the precision with which we could estimate E values. For most soils, the P concentrations in the soil solution were greater than the detection limit of the malachite green method ($0.9\,\mu g\,l^{-1}$) but smaller than its quantification limit ($3.6\,\mu g\,l^{-1}$). In the soils with the least available P, the P content of the seed limited the determination of the L value. The E values were strongly correlated, but not identical, with the L values measured for the same time of isotopic exchange. We conclude that these approaches are not precise enough to detect in these soils the ability of a plant to access slowly exchangeable forms of P or to quantify the mineralization of organic P. However, these isotope techniques can be used to estimate the total fraction of added fertilizer P that remains available to the plants.

Introduction

The amount of phosphorus (P) in a soil available to a plant can be defined as the amount of orthophosphate that can leave the solid phase of the soil and arrive in the soil solution within the time that the plant is growing and can take it up. Techniques using the principle of isotopic exchange allow measurement of the amount of orthophosphate that can be transferred from the soil solid to the solution in a given time and therefore give information on availability (Fardeau, 1993). One can measure isotopically exchangeable P (E value) by adding carrier-free ^{32}P- or ^{33}P-orthophosphate to a soil suspension, and by following the decrease of radioactivity over time (Fardeau, 1996). Another method to assess the amount of isotopically exchangeable P (L value) is to measure the specific activity in the shoots of a plant grown in a soil labelled with ^{32}P- or ^{33}P-orthophosphate (Larsen, 1952). Since the plant does not discriminate between isotopic forms of orthophosphate as it absorbs P from the soil solution, the specific activity of the P in the plant should be identical to that of the P present in the soil solution unless the plant has withdrawn P from pools in the soil that are not exchanged with the isotope.

While methods for measuring E and L values are well established for temperate soils (Frossard *et al.*, 1994; Morel & Plenchette, 1994), their application to soils containing very little P of the tropics has been less successful (Ipinmidun, 1973; Dalal & Hallsworth, 1977; Wolf *et al.*, 1986; Salcedo *et al.*, 1991). The reasons suggested for this include (i) specific sorption of labelled orthophosphate on to soil surfaces, (ii) the difficulty of accurately measuring the small orthophosphate concentration in the soil solution of these soils, and (iii) assumption that isotopic equilibrium had been reached

Correspondence: A. Oberson. E-mail: astrid.oberson@ipw.agrl.ethz.ch
Received 17 September 2001; revised version accepted 11 February 2003

between the added labelled orthophosphate and soil orthophosphate after a given time. Additionally, in the determination of *L* values there may be some interference from P derived from the seed which may be comparable to the total amount of P absorbed by the plant (Amer *et al.*, 1969; Truong & Pichot, 1976). However, improved methods are now available. The effect of time on isotopic exchange is better understood (Fardeau, 1993), and methods have been developed to measure very small concentrations of P (Ohno & Zibilske, 1991).

There is a need to test these improved methods on acid soils containing little P. If these techniques work in these soils precisely, then they could help to identify plant species or cultivars able to take up P from slowly or non-exchangeable P pools, or to quantify the rate of mineralization of organic P in the soil (Oehl *et al.*, 2001a).

We aimed to ascertain the usefulness of the improved isotope methods to assess availability of P in acid soils containing little P, and to identify and analyse the pitfalls of these techniques. We report our results below.

Materials and methods

Soils

The soils included in the study were sampled in September 1997 in two field experiments in Colombia. The Carimagua site, managed by ICA (Instituto Colombiano Agropecuario) and CIAT (Centro Internacional de Agricultura Tropical), is on the Eastern plains of Colombia, Meta district, 150 m above sea level, 4°30′N, 71°19′W, and has a mean annual temperature of 27°C and an average annual precipitation of 2200 mm. The CIAT site, Santander de Quilichao, is in the Southern Central Cordillera, Cauca district, 990 m above sea level, 3°06′N, 76°31W, and has a mean annual temperature of 23.7°C and an average annual precipitation of 1799 mm.

At Carimagua, soil samples (0–20 cm) were taken in the long-term Culticore field experiment established in 1993 to test the effect of different farming systems on plant productivity and soil fertility (Friesen *et al.*, 1997; Oberson *et al.*, 2001). The soils are well-drained Oxisols with a clay loam texture (38% clay, 44% silt, 18% sand in the upper horizon) developed on a Pleistocene clay (Sanz-Scovino *et al.*, 1992). The following treatments were sampled:

1 SAV (Native Savanna): native grassland; no fertilizer application.

2 GL (Grass Legume pasture): introduced grass legume pasture with *Brachiaria humidicola* CIAT 679, *Centrosema acutifolium* cv. Vichada CIAT 5277, *Stylosanthes capitata* CIAT 10280, *Stylosanthes guianensis* CIAT 11833 and *Arachis pintoi* CIAT 17434.

3 CR (Continuous Rice): rice (*Oryza sativa* cv. Oryzica Sabana 6 and cv. Oryzica Sabana 10 since 1996) grown in monoculture.

4 RGM (Rice Green Manure rotation): rice followed by cowpea (*Vigna unguiculata* var. ICA Menegua) in the same year, with the cowpea biomass incorporated into the soil after flowering.

The experiment had a split-plot design in four randomized blocks and treatment subplots of 0.36 ha. Between 1993 and 1997, the total amounts of P applied were 80 kg ha^{-1} for GL, 240 kg ha^{-1} for CR and 300 kg ha^{-1} for RGM. A detailed description of fertilization, soil cultivation and management is given in Bühler *et al.* (2002).

At Santander de Quilichao, soil samples (0–20 cm) were taken from a field experiment that had been cropped since 1983 with cassava (*Manihot esculenta*, MCol 1684 and CM 91-3) with one cropping cycle per year, and which had received different fertilizer rates (CIAT, 1993). The soil, which has been classified either as Oxisol or Inceptisol, has a clayey texture (60% clay, 20% silt, 20% sand in the upper horizon), and has developed on fluvially translocated volcanic, partly weathered material (Reining, 1992). All treatments received 500 kg ha^{-1} dolomite (22% Ca and 11% Mg) every 2 to 3 years. Samples were taken in the following treatments: no fertilizer applied (Cas0); 100 kg N, 100 kg P, 100 kg K ha^{-1} year^{-1} (CasNPK); 100 kg N, 100 kg K ha^{-1} year^{-1} (CasNK); and 100 kg N, 100 kg P ha^{-1} year^{-1} (CasNP). Treatments were allocated at random in a complete randomized block design (CIAT, 1993).

The soil samples were air-dried and sieved to pass 2 mm before they were used for chemical analysis and for isotopically exchangeable P determination (*E* and *L* values).

Soil analyses

We measured Bray-II P using dilute acid fluoride (0.03 M NH$_4$F, 0.1 M HCl) at 1:7 soil to solution ratio with 2 g soil and 40-s shaking time. We determined total soil P using concentrated H$_2$SO$_4$ and H$_2$O$_2$ (Thomas *et al.*, 1967). The inorganic P, P$_i$, in the soil was sequentially extracted by the modified sequential Hedley P fractionation procedure as described by Tiessen & Moir (1993), with HCO$_3$-saturated resin strips (BDH #55164, 9 mm × 62 mm), 0.5 M NaHCO$_3$, 0.1 M NaOH and hot concentrated HCl. Finally, residual P was determined by digestion with concentrated H$_2$SO$_4$ and H$_2$O$_2$ (Thomas *et al.*, 1967). Total inorganic P was determined as the sum of the inorganic fractions, either without (ΣP_i) or with residual P ($\Sigma P_i + P_{res}$), on the assumption that residual P consists mainly of P$_i$ (Tiessen & Moir, 1993). Additionally, we estimated P$_i$ by extracting 2 g of soil with 50 ml 1 M H$_2$SO$_4$ for 17 hours ($P_{i\,H2SO4}$), this method having been used in other isotopic exchange studies as an estimate of total inorganic P (Frossard *et al.*, 1994). Dithionite–citrate–bicarbonate-extractable and oxalate-extractable Fe and Al (Fe$_d$, Fe$_{ox}$, Al$_d$, Al$_{ox}$) were determined by the method of McKeague & Day (1966). Table 1 summarizes the chemical characteristics of the soils.

Table 1 Selected properties of the soil samples taken from the Carimagua and Quilichao experiments

Site and soil	Total C	Total N	Fe_d	Fe_{ox}	Al_d	Al_{ox}	pH in water	Al-saturation /%
	$/g\,kg^{-1}$							
Carimagua								
SAV	27 B	1.6 B	26.7 B	3.6	7.8 A	2.0 B	4.8 a	86.8 aA
GL	29	1.6	26.4	3.6	7.7	2.0	4.9 a	71.7 b
CR	26	1.5	26.2	3.7	7.6	2.0	4.3 b	75.4 b
RGM	26	1.5	26.9	3.5	7.8	2.0	4.3 b	76.3 b
Standard error	0.8	0.06	0.24	0.05	0.08	0.02	0.03	1.27
Quilichao								
Cas0	35 A	2.3 A	36.9 aA	3.4 ab	7.0 aB	2.9 aA	4.6	28.8 B
CasNPK	35	2.4	33.3 b	3.5 a	6.3 b	2.8 ab	4.5	26.5
CasNK	36	2.5	32.9 b	3.3 ab	6.0 b	2.7 b	4.4	34.8
CasNP	36	2.3	32.4 b	3.1 b	6.1 b	2.7 b	4.4	32.2
Standard error	0.1	0.07	0.74	0.07	0.10	0.03	0.05	3.5

Means within columns of one site followed by the same or no lowercase letter are not significantly different ($P < 0.05$) according to Tukey's test. Different upper case letters for SAV and Cas0 in one column show significant difference between the two sites for these unfertilized treatments according to the Student's *t*-test ($P < 0.05$).

The determination of E value

When ^{33}P-orthophosphate ions are added carrier-free to a soil suspension at steady state, the radioactivity, r, in solution decreases with time, t, according to the following formula (Fardeau, 1993):

$$\frac{r_t}{R} = \frac{r_1}{R}\left\{t + \left(\frac{r_1}{R}\right)^{\left(\frac{1}{n}\right)}\right\}^{-n} + \frac{r_\infty}{R}, \tag{1}$$

where R is the amount of radioactivity introduced (MBq), r_1 and r_∞ are the radioactivity (MBq) remaining in the soil solution after 1 minute and at infinity, respectively, and n is a parameter describing the rate of disappearance of the tracer from the solution after 1 minute, calculated as the slope of a linear regression of $\ln(r_t/R)$ against $\ln(t)$ for $t \leq 100$ minutes. The fraction of radioactivity remaining in the soil solution after an infinite isotopic exchange time, r_∞/R, is estimated from

$$\frac{r_\infty}{R} = 10\frac{C_\mathrm{p}}{P_\mathrm{i}}, \tag{2}$$

where C_p is the concentration of P in the solution ($\mathrm{mg\,P\,l^{-1}}$) and P_i is the total inorganic P in soil ($\mathrm{mg\,P\,kg^{-1}}$). The factor 10 results from the 1:10 soil to solution ratio used in the experiment.

Since the system is at steady state (the concentration of orthophosphate in the solution is constant), the decrease in radioactivity is due to homo-ionic exchange between ^{33}P-orthophosphate ions added to the solution and exchangeable ^{31}P-orthophosphate ions located on the soil solid. The quantity E_t ($\mathrm{mg\,P\,kg^{-1}}$ soil) of isotopically exchangeable P at a time t can be calculated on the assumption that (i) ^{31}P-orthophosphate and ^{33}P-orthophosphate ions have the same fate in the system, and (ii) whatever the time, the specific activity of the orthophosphate ions in the soil solution is identical to that of the orthophosphate ions isotopically exchanged in the whole system. Therefore

$$\frac{E_t}{R} = \frac{10C_\mathrm{p}}{r_t} \tag{3}$$

and

$$E_t = \frac{10RC_\mathrm{p}}{r_t}. \tag{4}$$

These equations show that the amount of isotopically exchangeable P is a function of time and suggest the existence of an infinite number of exchange sites for orthophosphate in the soil (Fardeau, 1993). Fardeau & Jappé (1976) and Frossard *et al.* (1994) showed that this equation can be extrapolated to at least $t = 3$ months.

To determine E values experimentally, we proceeded as follows. Fifteen grams (dry weight) of soil, with three replicates per sample, were equilibrated by shaking end-over-end for 16 hours in 148.5 ml deionized water with two drops of toluene. After this time the soil solution system was considered to be at steady state (Fardeau, 1993). At $t = 0$, 1.5 ml of a solution containing 5.8 MBq carrier-free ^{33}P-orthophosphate was added and mixed with a magnetic stirrer. Three subsamples of 2–3 ml each were taken from the suspension after 1, 10 and 100 minutes (short-term kinetics, corresponding to the usual procedure), and five to six subsamples between 1 day and 4 (GL, CR, all Quilichao soils) or 5 weeks (SAV and RGM). The suspensions sampled at 1, 10 and 100 minutes were immediately filtered through a 0.2-μm filter (Sartorius, Minisart single use filters, cellulose acetate). The suspensions sampled after 100 minutes were filtered through 0.025-μm filters (Schleicher & Schuell, NC 03, cellulose nitrate) previously rinsed with deionized water to remove minor P contamination found on these filters. The 0.025-μm filter was preferred because soil colloids carrying ^{33}P- and ^{31}P-orthophosphate can pass through 0.2-μm filters (Sinaj *et al.*, 1998). However, for determining r_t at 1, 10 and 100 minutes, filtration through 0.025 μm is not possible because the large resistance of the filter results in excessively slow filtration, rendering precise determination of r_t impossible. We tested the influence of the filter pore size on r_t by comparing solutions filtered at 0.2 and 0.025 μm after 120 minutes. At that time, r_t values were not affected by the filter pore size (results not shown). The radioactivity in solution was determined with a liquid scintillation counter (Packard 2500 TR) with Packard Ultima Gold scintillation liquid with a volume/volume ratio of 1 ml of sample to 5 ml of scintillation liquid. All values were corrected for radioactive decay back to the day when the soil was labelled.

At the end of the short-term kinetics experiment, i.e. after 100 minutes, stirring ceased, and the samples were put back on the rotating shaker. After 100 minutes of isotopic exchange and at each sampling time afterwards, C_{P} was determined in the 0.025-μm filtrates by the malachite green method (Ohno & Zibilske, 1991) with a Shimadzu UV-1601 spectrophotometer and a 1-cm cell. As the concentrations in some solutions were close to the detection limit, all solutions were additionally measured after 5-fold concentration by evaporation. The limit of detection (i.e. the smallest concentration that can be distinguished from the blank sample at a chosen probability level), and the limit of quantification (i.e. the minimum concentration that can be measured with a specified degree of confidence), were determined with standard P solutions ranging from 0 to 500 μg P l^{-1}. Values of C_{P} obtained at the different sampling times did not differ significantly (results not shown).

The isotopically exchangeable P (E_t) was calculated with Equation (4) and the experimental values of r_t/R measured over 4 or 5 weeks, or r_t/R values were extrapolated to 5 weeks according to Equation (1) (Fardeau, 1993). The comparison of measured and extrapolated values was made to verify the extrapolation of r_t based on experimental data up to 100 minutes of isotopic exchange for the conditions of these soils.

The determination of L value

Before labelling, the soils were incubated for 12 days at 45% (Carimagua soils) or 50% (Quilichao soils) of their water holding capacity (water holding capacity = 500 g or 600 g water kg^{-1} soil dry weight for Carimagua or Quilichao soils, respectively) at 20°C and 80% relative humidity. Afterwards a P-free nutrient solution was added containing 140 mg N, 200 mg Ca, 200 mg K, 82 mg S and 50 mg Mg kg^{-1} soil dry weight, as $Ca(NO_3)_2$, K_2SO_4 and $MgCl_2$, respectively. Phosphorus in the soil was labelled with a quantity of carrier-free ^{33}P-orthophosphate of 7.4 MBq kg^{-1}. The ^{33}P-orthophosphate was added in a volume of 7 ml per kg soil, and portions of 1.5 kg soil (dry weight) were thoroughly mixed by hand to obtain an even distribution of the isotope. Portions of labelled soil corresponding to 600 g dry soil were placed into pots, sown with 100 mg of common bentgrass (*Agrostis capillaris*), corresponding to about 800 seeds, and put into the dark for germination at 25°C and 65% relative humidity. We chose *Agrostis capillaris* because its seeds contain very little P, it can grow after successive cuts, and because it can take up only P that is isotopically exchangeable within the time span of plant growth (Frossard *et al.*, 1994). After 3 days of germination, the plants were exposed to 18 hours of light (300 μmol s^{-1} m^{-2}) at a day temperature of 22°C and 6 hours of darkness at a night temperature of 18°C. We included five replicates per soil and treatment. The totally random distribution of the pots in the growth chamber was rotated every 3 days. The pots were weighed and watered daily to re-adjust humidity to 50% of the soils' water holding capacity. Nitrogen (in the form of NH_4NO_3 after the first cut and in the form of $(NH_4)_2SO_4$ after the second cut), K (as K_2SO_4) and S (as K_2SO_4 and $(NH_4)_2SO_4$) were applied after each cut at the rate of 100 mg N, 15 mg K and 6 or 120 mg S kg^{-1} soil, after the first and second cut, respectively. Shoots were harvested 4, 8 and 12 weeks after sowing.

Harvested dry matter was weighed after 48 hours of drying at 80°C. About 200 mg of the plant material, cut in pieces < 2 mm, was ashed at 550°C for 4 hours. Phosphorus in the plant material was determined after dissolving the ashes in 1 ml of 11.3 M HCl. Aliquots of the samples were diluted and the total P measured by the method of Murphy & Riley (1962) and ^{33}P was measured by scintillation counting. The P in the seed was determined after five seed samples of 100 mg were ashed.

As the L value (mg P kg soil^{-1}) measured after the first cut can be strongly affected by the P derived from the seeds, L values were calculated only after the second and third harvest from the Equation (5) (Sibbesen, 1984):

$$L_{\mathrm{obs}(n)} = \left(R - \sum_{i=1}^{n-1} r_i\right) \Bigg/ \left(\frac{r_n}{p_n}\right) + \sum_{i=1}^{n-1} p_i, \tag{5}$$

where R is the total radioactivity introduced (MBq kg soil^{-1}), r_n is the amount of ^{33}P-orthophosphate (MBq kg soil^{-1}) and p_n the quantity of ^{31}P (mg kg soil^{-1}) in the shoots of the common bentgrass measured at the nth harvest, respectively. The sums Σr_i and Σp_i are of radioactivity and orthophosphate, respectively, taken up by the shoots of the plants between the 1st and $(n-1)$th cuts.

Since a part of the P present in the seed was taken up by the common bentgrass after the first cut, the following correction was applied to the L value of the second and third cuts (Truong & Pichot, 1976):

$$L_{\mathrm{cor}(n)} = L_{\mathrm{obs}(n)} \left\{\frac{p_n}{(p_n + aP_{\mathrm{seeds}})}\right\}, \tag{6}$$

where $L_{\mathrm{cor}(n)}$ is the corrected L value of the nth cut, $L_{\mathrm{obs}(n)}$ the L value calculated of the nth cut with Equation (5), and $P_{\mathrm{seeds}} = 800\,\mu\mathrm{g\,P\,kg\,soil^{-1}}$ (the P content of 100 mg seeds sown per pot amounted to 480 μg with a standard error of 24.5 μg). The factor a describes the fraction of seed P taken up by the respective cut. We chose it depending on the relation between total plant P uptake and seed P (see below).

Statistical analysis

Soils within a given site were compared by analysis of variance and Tukey's multiple range test for differences in exchange kinetics parameters, L values and plant variables of the pot experiment. Student's t-test was used to compare the unfertilized soils (SAV and Cas0) from the two sites. Since E and L values equally determine the isotopically exchangeable, plant-available P, and since *Agrostis capillaris* was shown to take up only isotopically exchangeable P (Frossard *et al.*, 1994), L_{cor} and E values measured or extrapolated for a comparable exchange time, i.e. for 8 and 12 weeks, should be identical. We therefore compared the ratios L_{cor}/E with 1 by testing the symmetry of their logarithms $\log(L_{\mathrm{cor}}/E)$ around $\log(1) = 0$ using Wilcoxon's signed rank test.

Measured and extrapolated values of $\ln(r_t/R)$, as well as E values calculated either from measured r_t/R or from values of r_t/R extrapolated as described by Fardeau (1996) were compared with paired t-tests (at $P = 0.05$). The detection limit and the limit of quantification of the method used for C_{P} determination were calculated as described by Wilson (1961), Roos (1962) and Gabriels (1970) at a confidence of 95% (limit of quantification) or 90% (limit of detection).

Results and discussion

Total P, inorganic P, Bray-II P and resin-extractable P

The total P content was 254 $\mathrm{mg\,P\,kg^{-1}}$ less in the unfertilized soils from Carimagua than in the unfertilized soils from Quilichao (Table 2), reflecting differences in parent material and weathering mentioned in the Materials and methods section. Fertilization with P resulted in more total P in the fertilized soils (RGM, CR, CasNP and CasNPK) on both sites than in their unfertilized counterparts (SAV, Cas0, CasNK) or in GL that had received very little P. Total inorganic P as well as the amount of P extracted by the resin or by the Bray-II method followed the same trends as the total P content. The different methods for determining total inorganic P gave different results. In soil CasNPK, $\Sigma\mathrm{P_i} + \mathrm{P_{res}}$ was found to be equal to $\mathrm{P_t}$, suggesting that this soil had little organic P although it contained as much organic C and N as the other samples from Quilichao that had significant contents of organic P (results not shown). Frossard *et al.* (1994) used the amount of inorganic P extracted with 1 M $\mathrm{H_2SO_4}$ ($\mathrm{P_{i\,H2SO4}}$) as an estimate of total $\mathrm{P_i}$. For the soils used in the present study, the soil $\mathrm{P_i}$ calculated as the sum of the pools of inorganic P obtained

Table 2 Amount of P extracted with a selection of methods from the soils sampled from the Carimagua and Quilichao experiments

Site and soil	Bray-II P	Resin-extractable P	$\mathrm{P_{i\,H2SO4}}$[a]	$\Sigma\mathrm{P_i} + \mathrm{P_{res}}$[b]	$\Sigma\mathrm{P_i}$[c]	$\mathrm{P_t}$[d]
			$/\mathrm{mg\,kg^{-1}}$			
Carimagua						
SAV	0.9 dB	0.3 cB	12 dB	105 bB	61 bB	175 cB
GL	2.0 c	0.5 c	30 c	115 b	72 b	213 c
CR	17.2 b	3.9 b	98 b	220 a	171 a	293 b
RGM	35.5 a	8.3 a	156 a	255 a	208 a	376 a
Standard error	0.54	0.35	3.6	6.2	4.2	14.5
Quilichao						
Cas0	9.0 cA	3.0 cA	112 dA	415 cA	324 cA	429 cA
CasNPK	40.6 a	19.3 a	361 a	671 a	587 a	671 a
CasNK	14.0 b	4.5 c	180 c	410 c	336 c	486 c
CasNP	40.1 a	15.8 b	333 b	613 b	532 b	594 b
Standard error	4.71	0.35	4.0	8.8	8.1	16.2

Means within columns of one site followed by the same letter are not significantly different ($P < 0.05$) according to Tukey's test. Means of the two unfertilized soils (SAV and Cas0) in one column followed by different uppercase letters are significantly different according to Student's t-test ($P < 0.05$).

[a] Inorganic P extracted with 1 M $\mathrm{H_2SO_4}$.

[b] Inorganic P measured as the sum of the $\mathrm{P_i}$ extracted with the modified Hedley sequential fractionation procedure with resin, $\mathrm{NaHCO_3}$, NaOH, HCl plus the total residual P extracted with concentrated $\mathrm{H_2SO_4}$ and $\mathrm{H_2O_2}$.

[c] Inorganic P measured as the sum of $\mathrm{P_i}$ extracted with the modified Hedley sequential fractionation procedure without the residual P.

[d] Total P extracted with concentrated $\mathrm{H_2SO_4}$ and $\mathrm{H_2O_2}$.

from sequential extraction either with or without the residual P was greater by 42–310 mg P kg^{-1} ($P < 0.001$) than the amount of P_i extracted by 1 M H_2SO_4 (Table 2). This set of results shows that $P_{i\,H2SO4}$ underestimates total inorganic P and should not be further considered in isotopic studies. It also shows that there is no perfect method to assess total P_i precisely (Condron *et al.*, 1990; O'Halloran, 1993). We used the sum of the P_i fractions plus residual P ($\Sigma P_i + P_{res}$) to calculate the *E* values as for comparison of total P_i with other characteristics.

Isotopic exchange parameters

Radioactivity remaining in the solution (r_t/R). The measured r_t/R decreased until 4 or 5 weeks for all fertilized soils (CR, RGM, CasNPK and CasNP), whereas r_t/R reached a minimum at which it remained after 2 weeks of isotopic exchange in the unfertilized SAV soil (data not shown). The values of r_1/R, *n* and r_∞/R were calculated for each soil from data obtained during the first 100 minutes (Table 3). The values of r_t/R extrapolated with Equation (1) decreased until 4 or 5 weeks for all soils, except for SAV for which the last three extrapolated r_t/R values were identical. The comparison of the extrapolated with the measured values showed significant differences for at least one sampling time in all soils, with the exception of GL and CasNPK.

The experimental determination of $r_{4\,\mathrm{weeks}}/R$, which we considered as r_∞/R for SAV, was five times greater than when r_∞/R was calculated with Equation (2). The underestimation of the calculated r_∞/R can be accounted for by one or a combination of the following reasons: (i) total P_i was not totally isotopically exchangeable, (ii) C_P was underestimated, (iii) a fraction of the added ^{33}P-orthophosphate was specifically sorbed, and (iv) total P_i was overestimated.

Table 3 Estimation of the parameters used in Equation (1) to extrapolate r_t/R in the soil

Site and soil	r_1/R[a]	n[b]	r_∞/R[c]
Carimagua			
SAV	0.02 c	0.51 a	100×10^{-6} c
GL	0.03 b	0.43 b	300×10^{-6} b
CR	0.03 b	0.41 b	300×10^{-6} b
RGM	0.04 a	0.41 b	500×10^{-6} a
Standard error	0.001	0.009	20×10^{-6}
Quilichao			
Cas0	0.02 d	0.46 a	50×10^{-6} c
CasNPK	0.05 a	0.38 c	400×10^{-6} a
CasNK	0.03 cd	0.43 ab	100×10^{-6} b
CasNP	0.04 bc	0.39 bc	500×10^{-6} a
Standard error	0.003	0.008	7×10^{-6}

Values of a characteristic within a site followed by the same letter are not significantly different ($P < 0.05$) according to Tukey's test.

[a] Ratio between measured radioactivity remaining in the soil solution after 1 minute (r_1) and the introduced radioactivity (R).

[b] n is calculated as the slope of the linear regression equation between $\ln(r_t/R)$ and $\ln(t)$ for $t \leq 100$ minutes, the absolute value being used.

[c] r_∞/R calculated with Equation (2).

Concentration of P in the soil solution (C_P). The limit of quantification (95% probability level) of the malachite green method by the 1-cm cell was 3.6 μg P l^{-1}. The limit of detection (90% probability level) was 0.9 μg P l^{-1}. The concentration in the solution samples evaporated from 10 to 2 ml did not differ significantly from the C_P directly measured, with the exception of RGM where the directly measured concentration was greater (Table 4). The coefficients of variation indicated a greater variation of C_P than reported for C_P in other isotopic exchange studies (Sinaj *et al.*, 1997). All C_P values exceeded the limit of detection, but were less than the limit of quantification for SAV, GL, Cas0 and CasNK. We used the C_P values determined with the concentrated solutions to calculate the *E* values.

E_t values. The E_t values calculated from the experimental values of C_P and r_t/R after 4–5 weeks of isotopic exchange varied from 25.8 mg P kg^{-1} in SAV to 121.4 mg P kg^{-1} in

Table 4 Concentration of P in the soil solution (C_p) after adding the radioactivity in the isotopic exchange batch experiment, and amount of P isotopically exchangeable within 1 minute (E_1) in the soils

Site and soil	E_1[a] /mg kg^{-1}	C_p (1:5)[b] /mg l^{-1}	C_p (1:1)[c] /mg l^{-1}
Carimagua			
SAV	0.7 c	0.002 c (0.0003)	0.001 d (0.0005)
GL	1.1 c	0.003 c (0.0002)	0.003 c (0.0005)
CR	2.0 b	0.006 b (0.0002)	0.006 b (0.0004)
RGM	3.2 a	0.014 aB (0.0005)	0.020 aA (0.0015)
Standard error	0.07	0.0005	0.0005
Quilichao			
Cas0	0.9 c	0.002 c (0.0007)	0.002 c (0.0003)
CasNPK	5.9 a	0.032 a (0.0014)	0.031 a (0.0012)
CasNK	1.0 c	0.003 c (0.0004)	0.002 c (0.0003)
CasNP	4.4 b	0.017 b (0.0007)	0.019 b (0.001)
Standard error	0.37	0.0044	0.0047

Means of a characteristic followed by the same or no letter (lowercase in columns for soil comparison within one site, uppercase in rows for comparison of C_p values) are not significantly different ($P < 0.05$) according to Tukey's test. Standard deviation is shown in parentheses. There were no significant differences between the unfertilized soils of the two sites for all characteristics.

[a] Calculated using C_p measured in solutions concentrated by evaporation from 5:1.

[b] Determined using solutions concentrated by evaporation from 5:1.

[c] Determined using solutions without concentration.

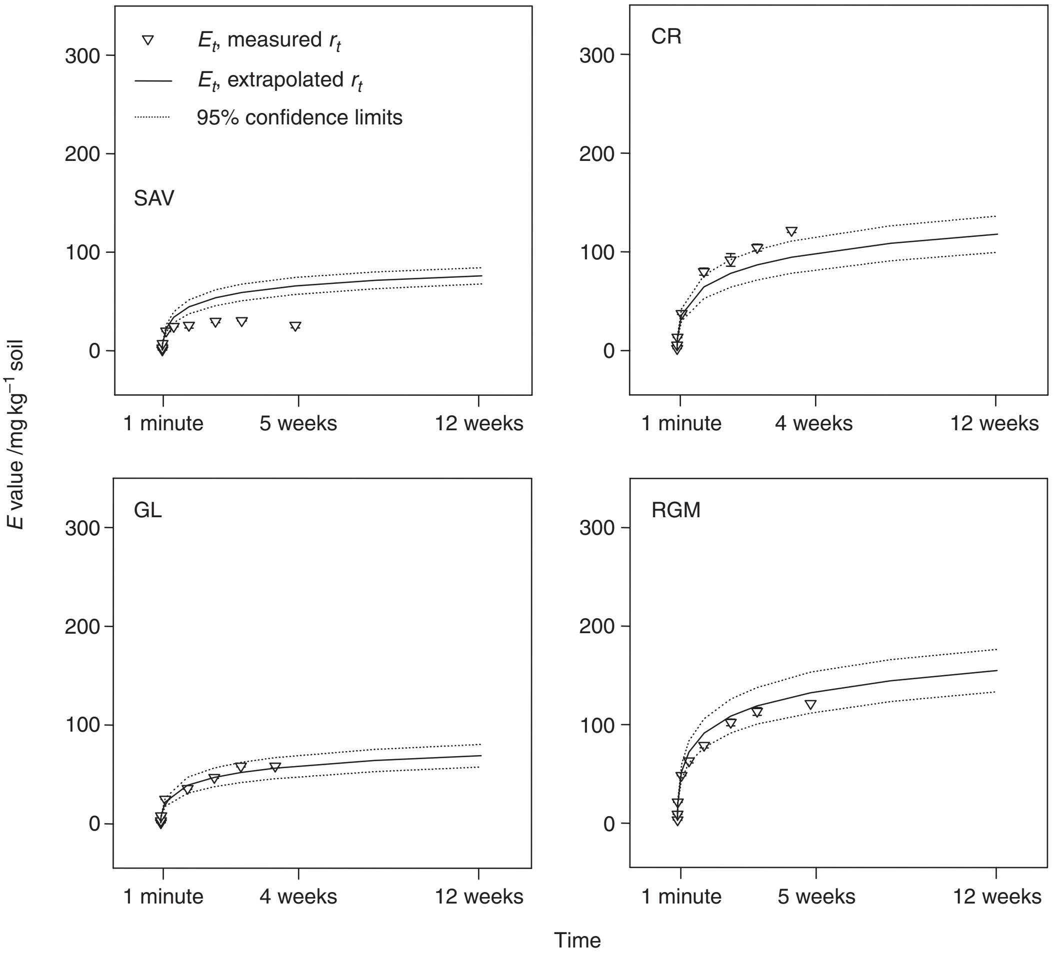

Figure 1 Comparison between measured and extrapolated values of isotopically exchangeable P (*E* value) in the soils from Carimagua. Measured values show means ± standard error.

RGM and from 71.0 mg P kg^{-1} in Cas0 to 242.1 mg P kg^{-1} in CasNPK (Figures 1 and 2).

The E_t calculated from the experimental data might have been affected by errors in the determination of C_P (Frossard *et al.*, 1994), specific sorption of a fraction of the added radioactivity (Amer *et al.*, 1969; Wolf *et al.*, 1986), or microbial processes (Oberson *et al.*, 2001). As shown above, small errors in C_P determinations cannot be ruled out in SAV, GL, Cas0 and CasNK. Besides concentrations less than or close to the detection limit of colorimetric methods (Salcedo *et al.*, 1991), non-orthophosphate P-forms might have been measured as a result of hydrolysis from inorganic condensed and organic bonds (Gerke, 1992) or acid dissolution of P associated with colloids (Sinaj *et al.*, 1998). Additionally, other ions, such as SiO_4^{2+}, might have formed complexes with molybdate (Fardeau & Jappé, 1988). Small variations in C_P will have a large effect on $E_{5\,weeks}$. The possible specific sorption of a fraction of added ^{33}P-orthophosphate (Amer *et al.*, 1969) cannot be disproved by our results, but it seems unlikely, as discussed by Frossard & Sinaj (1997). Although toluene was added at the beginning of the experiment, microbial activity might also have affected the estimate of E_t calculated through uptake of ^{33}P and ^{31}P and mineralization. However, in soil suspensions to which no C and N were added, we can assume that the size and activity of the microbial biomass remained constant and that after 4–5 weeks microbially mediated processes had no major influence on E_t (Oehl *et al.*, 2001b).

In all the soils except GL and CasNPK, the E_t calculated from the experimental data after 4–5 weeks of exchange were at least on one sampling date significantly different ($P < 0.001$) from the values extrapolated from Equation (1) (Figures 1 and 2). In SAV, the extrapolated $E_{5\,weeks}$ was twice as large as the $E_{5\,weeks}$ derived from measured values of r_t/R and C_P. Furthermore, the *E* value extrapolated to 4 weeks was greater in SAV (66 mg kg^{-1} soil) than in GL (56 mg kg^{-1} soil), whereas all the other P tests, including the *E* values derived from measured data, showed that SAV contained less available P than GL. These results show that the extrapolation overestimated

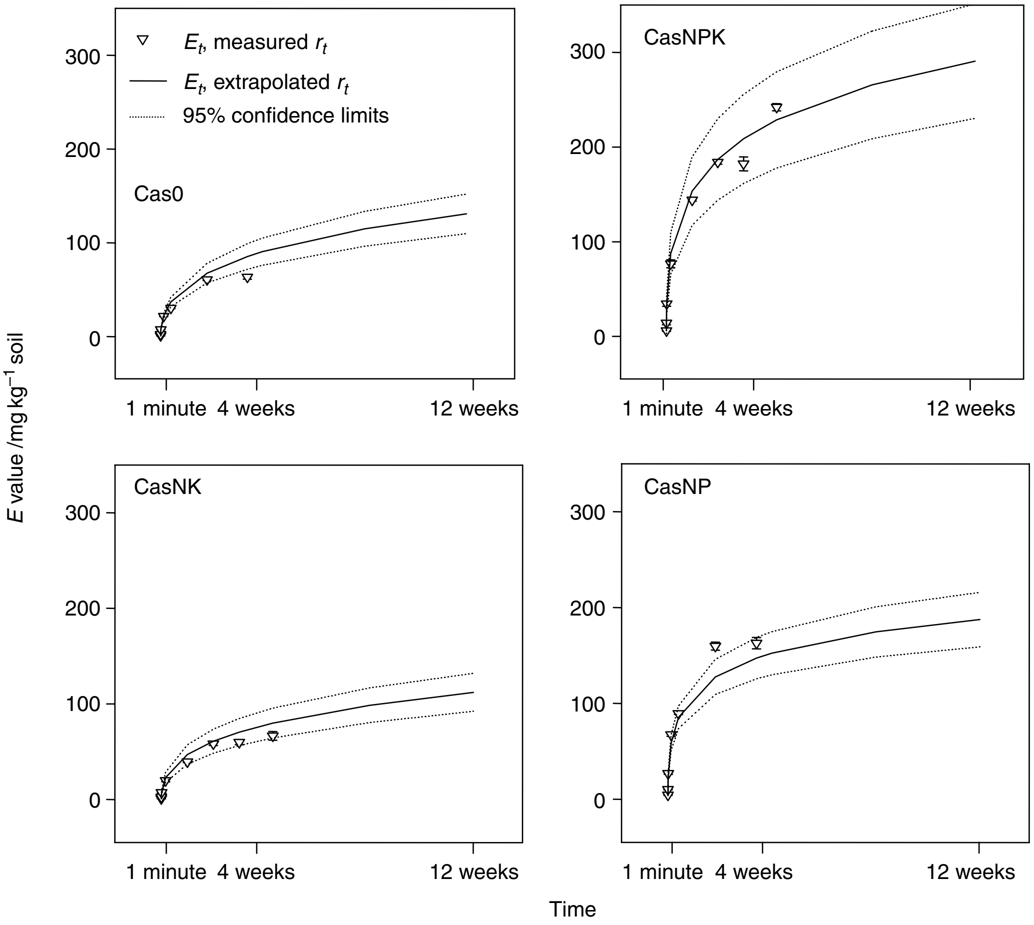

Figure 2 Comparison between measured and extrapolated values of isotopically exchangeable P (*E* value) in the soils from Quilichao. Measured values show means ± standard error.

$E_{5\,\mathrm{weeks}}$ for SAV. Possible explanations for this overestimation are errors made in estimating r_∞/R by Equation (2) or inexact n (Frossard *et al.*, 1996).

The calculated E_1 values (Table 4) are in the range published for similar soils by Salcedo *et al.* (1991). For the Carimagua soils, all E_1 values were less than the value of 5 mg P kg^{-1} regarded as a threshold, at less than which P limits growth of many temperate crops (Gallet, 2001). In Quilichao, the E_1 values of the P-fertilized treatments (CasNP and CasNPK) were close to 5 mg P kg^{-1}, whereas the treatments that had not received any P had E_1 values close to 1 mg P kg^{-1}. Values of E_1 increased for each site with increasing total P$_i$, resin-extractable P and Bray-II P content. The same was true for $E_{4\,\mathrm{weeks}}$ or $E_{5\,\mathrm{weeks}}$, either measured or extrapolated.

Except for SAV, for which the extrapolated $E_{4\,\mathrm{weeks}}$ value was overestimated, $E_{4\,\mathrm{weeks}}$ increased with increasing fertilization. If the increase in total soil P over the unfertilized soils (Cas0 for Quilichao, GL for Carimagua because of difficulties in *E* value determination for SAV) was due solely to fertilization, then the increase in the extrapolated $E_{4\,\mathrm{weeks}}$ in the fertilized soils accounted for 43 and 50% of the increase in total soil P in Quilichao and Carimagua. This result suggests that a significant fraction of the added P remained available to plants.

Plant growth and P uptake

The amount of grass and the concentrations of P in it were greater on the Quilichao soils than on the soils from Carimagua, and were larger on the soils fertilized with P than in the unfertilized soils (Table 5). This matches the fact that uptake of P increased as resin P, Bray-II P, total P$_i$, E_1, $E_{4\,\mathrm{weeks}}$ and $E_{5\,\mathrm{weeks}}$ increased. After the second cut, there was little re-growth on SAV (Table 5). Growth decreased from the second to the third cut on all soils with the exception of GL. On this soil the biomass in the third cut was almost twice that of the second cut. Concentrations of P in the grass ranged between 0.3 mg g^{-1} (SAV, second cut) and 2.2 mg g^{-1} (CasNP, third cut). Phosphorus concentrations in temperate grasses less than 2 mg P g^{-1} dry matter indicate P deficiency (Mays *et al.*, 1980). Therefore, with the exception of the fertilized soils from Quilichao (CasNPK and CasNP), on which the concentrations

Table 5 Shoot dry weight per pot[a], P concentration in plant dry matter and total P uptake per pot[a] in shoots for three cuts of *Agrostis capillaris* grown in the soils

	First cut (after 1 month)			Second cut (after 2 months)			Third cut (after 3 months)			
Site and soil	Dry weight /g	P concentration $/\mathrm{mg\,g^{-1}}$	P uptake /mg	Dry weight /g	P concentration $/\mathrm{mg\,g^{-1}}$	P uptake /mg	Dry weight /g	P concentration $/\mathrm{mg\,g^{-1}}$	P uptake /mg	Total P uptake[b] /mg
Carimagua										
SAV	0.17 cB	0.4 cB	0.07 cB	0.13 cB	0.3 cB	0.04 cB	0.05 cB	0.3 dB	0.015 dB	0.13 dB
GL	0.17 c	0.6 c	0.11 c	0.45 c	0.8 b	0.35 c	0.8 b	0.8 c	0.6 c	1.1 c
CR	0.3 b	1.1 b	0.4 b	2.6 b	1.2 a	3.2 b	2.5 a	1.3 b	3.2 b	6.7 b
RGM	0.6 a	1.6 a	1.0 a	3.3 a	1.1 a	3.7 a	2.4 a	1.7 a	3.9 a	8.6 a
Standard error	0.019	0.06	0.045	0.081	0.05	0.131	0.038	0.02	0.049	0.135
Quilichao										
Cas0	1.0 dA	1.0 bA	1.0 cA	3.4 bA	1.1 bA	3.7 cA	2.1 bA	1.2 cA	2.5 bA	7.2 cA
CasNPK	2.4 a	1.7 a	4.2 a	4.3 a	1.6 a	6.9 a	2.7 a	2.0 b	5.3 a	16.4 a
CasNK	1.2 c	1.1 b	1.3 c	3.6 b	0.9 b	3.3 c	2.3 ab	1.3 c	2.9 b	7.5 c
CasNP	2.1 b	1.3 b	2.7 b	4.5 a	1.1 b	4.8 b	2.5 a	2.2 a	5.5 a	13.1 b
Standard error	0.044	0.08	0.107	0.150	0.08	0.295	0.076	0.06	0.140	0.282

Means within one column of one site followed by the same lowercase letter are not significantly different according to Tukey's test ($P < 0.05$). Means of the unfertilized soils (SAV and Cas0) followed by different uppercase letters are significantly different according to the two-sample Student's *t*-test ($P < 0.05$).

[a] Pots contained 600 g soil dry matter.

[b] Sum of the three cuts.

Table 6 *L* values calculated with (L_{cor}) or without (L_{obs}) correction for P in the seed at the second and third cut of *Agrostis capillaris* grown in the two soils

	Second cut		Third cut	
Site and soil	L_{obs} $/\mathrm{mg\,P\,kg\,soil^{-1}}$	L_{cor}[a]	L_{obs}	L_{cor}[a]
Carimagua				
SAV	143 cA	76 bB	45 aA	23 aB
GL	42 aA	31 aB	35 aA	29 aB
CR	91 b	87 b	95 b	91 b
RGM	118 c	114 c	138 c	134 c
Standard error	6.5	5.0	5.0	4.6
Quilichao				
Cas0	95 a	92 a	112 a	107 a
CasNPK	198 c	194 c	232 b	227 b
CasNK	92 a	89 a	140 a	134 a
CasNP	149 b	146 b	225 b	221 b
Standard error	5.4	5.2	8.5	8.3

Means within columns of one site followed by the same lowercase letter are not significantly different according to Tukey's test ($P < 0.05$). Different uppercase letters for L_{obs} and L_{cor} for one soil and cut show significant difference according to Student's *t*-test ($P < 0.05$).

[a] *L* value corrected with the assumption of 25% seed P uptake in the respective cut except SAV (8% and 3% at second and third cut, respectively).

of P in the grass exceeded this limit in the second cut, shortage of P limited growth on all the soils. Total uptake in shoots per pot during the entire experiment ranged from 0.13 mg in SAV, which is only a quarter of the P applied in the seeds (0.48 mg), to 16.4 mg in CasNPK.

L values

In the second cut the observed *L* values (L_{obs}, Table 6) ranged between 42 mg P kg^{-1} in GL and 198 mg P kg^{-1} in CasNPK. Treatment SAV had the largest L_{obs} values of the Carimagua soil. Since other analyses showed that SAV contained the least available P, the L_{obs} value overestimated the availability of P in this soil. For the Quilichao soils, the ranking of L_{obs} results accorded with the other analyses of available P. In the third cut, L_{obs} ranged between 35 mg P kg^{-1} for GL and 232 mg P kg^{-1} for CasNPK. There were significant decreases ($P < 0.001$) in the L_{obs} values of SAV and GL between the second and third cut although these values should either be constant or increase (as in the other soils studied) because the radioactive P becomes diluted with increasing amounts of P over time. These results suggest that for both the second cut and the third cut, *L* values measured for SAV and GL were not reliable. We can explain this, at least for SAV, by a large interference of P derived from the seed.

With the exception of the SAV soil, we assumed that 25% of the seed P was transferred to the aerial parts of the plant during the second cut and again 25% during the third cut. We could not make this assumption for SAV because the total amount of P accumulated in the shoots during the three cuts reached only about 25% of the seed P. The resulting L_{cor} values were between 2% (CasNPK) and 26% (GL) less than L_{obs} measured after the second cut. In the third cut, L_{cor} was less than 5% smaller than L_{obs} for all the soils, with the exception of GL (17%). These results show that, except for GL, the correction for P taken up from the seed in the third cut had little impact on L values. Furthermore, these results confirmed that for both SAV and GL the probably large but unknown accumulation of P derived from the seed in the aerial parts of the grass compared with the small P uptake from the soil interfered with the measurement of L values.

The L_{cor} values observed in the third cut increased with P fertilization as expected. If the increase in total P in the soil compared with the unfertilized soils (again Cas0 for Quilichao and GL for Carimagua) was due solely to fertilization, then the increase in the L_{cor} values (mean of second and third cut) accounted on average for 48 and 66% of the increase in total soil P in Quilichao and Carimagua, respectively. This result is similar to that obtained with $E_{4\,\mathrm{weeks}}$ values and suggests that a significant fraction of the added fertilizer P remained available to the plants.

For each site, the corrected L values increased with increasing plant growth and concentration of P at the second and the third cuts (Tables 5 and 6). The L_{obs} at the third cut increased with increasing resin P, E_1 and E_t derived from r_t/R measured at 4 or 5 weeks.

Comparison of E and L values

The E values extrapolated to 8 and 12 weeks of isotopic exchange and L_{cor} values determined after the same isotopic exchange time were strongly correlated, but not identical (Figure 3). All the soils were considered in this relation except SAV and GL, for which L values could not be measured properly. The hypothesis of equal L_{cor} and E values, tested by Wilcoxon's signed rank test on $\log(L_{\mathrm{cor}}/E)$ as described in the methods section, was rejected. The line shown in Figure 3, corresponding to the median of the slopes L_{cor}/E, 0.81, thus differed significantly from the 1:1 line. This set of data shows that, with the exception of the CasNK and CasNP in the third cut, almost all the soils (including the high P soil CasNPK) had an extrapolated E value greater than their L_{cor} value. We have discussed earlier in this paper the possible reasons for errors in E and L values in soils that contain little P. Another possible explanation for greater E than L values could be the faster isotopic exchange in the batch experiment (stirred suspension at a 1:10 soil to water ratio) than in the pot experiment (at 250–300 g water kg^{-1} soil with little disturbed soil microstructure). Frossard *et al.* (1993) and Sinaj *et al.* (1997) showed

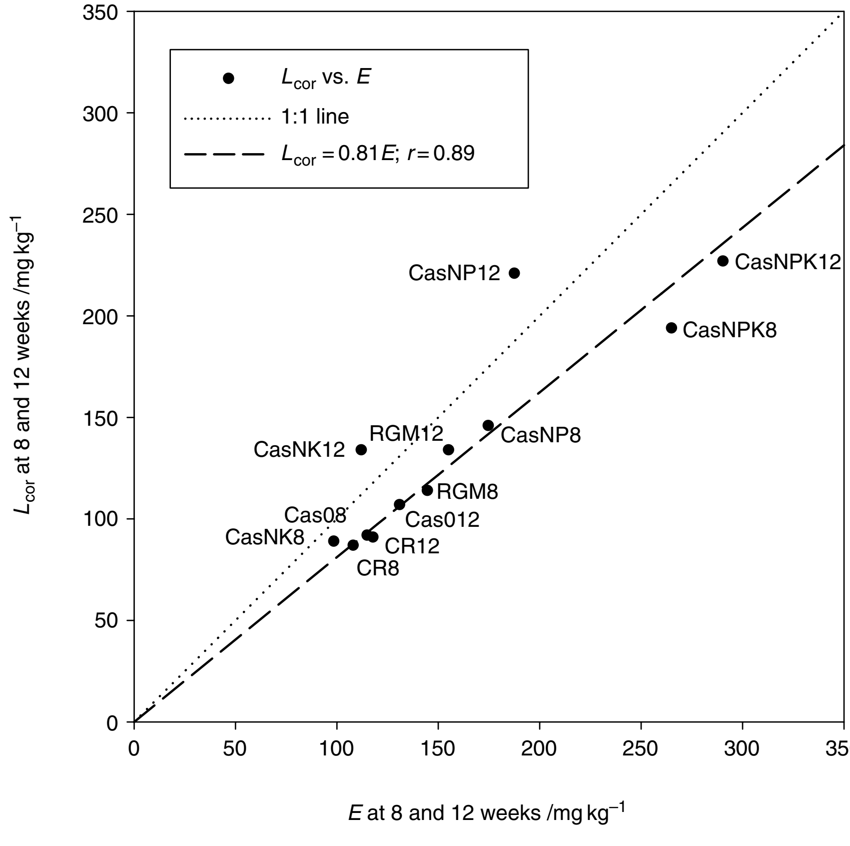

Figure 3 Comparison of extrapolated E values and L values corrected for P in seed in *Agrostis capillaris*, for 8 (second cut) and 12 weeks (third cut) of isotopic exchange in the soils from both sites.

that total dispersion of soil aggregates resulted in faster isotopic exchange, and Sinaj *et al.* (1999) also observed that isotopic exchange of Zn^{65} was faster when the solution to soil ratio increased, resulting in larger *E* values. The overestimation of the *E* value in the P-rich CasNPK is difficult to explain, but is probably due to errors in the estimates of the parameters for extrapolation. In CasNK and CasNP, the *L* values increased strongly from the second to the third cut. The increase in isotopically exchangeable P, as deduced from the isotopic exchange kinetics, cannot account for these large increases. Such an increase could be related to the increased release of non-exchangeable P to the soil solution and uptake by the plant, e.g. due to the mineralization of organic P.

For the application of isotope methods for assessing available P in acid tropical soils, the agreement between *L* and *E* values is satisfying because the two methods gave the same ranking of treatments. However, the determination of *E* and *L* values is not precise enough to identify plant species or cultivars able to take up P from slowly or non-exchangeable P pools, or to quantify precisely the rate at which P in the soil organic matter is mineralized, as proposed initially.

Conclusions

Our results enabled us to the identify the main constraints for estimating the amount of P that can be exchanged within the time during which plants can take the P up, either from data from batch experiments extrapolated to long exchange times (*E* values), or from data from plant growth experiments (*L* values). Reliable *E* values can be obtained only if the concentration of P in solution is greater than the limit of quantification of the method used. The *E* values can be extrapolated beyond the measurement times following the method of Fardeau (1996). However, in soils that contain little P, the estimation of r_∞/R on one hand and therefore the measurements of total P_i and C_P, and of n on the other, can strongly affect the extrapolation of *E* values. Finally, reliable *L* values can be obtained only if the total P accumulated in the aerial parts of the plant is at least one order of magnitude greater than the P initially added with the seeds.

Values of E and L_{cor} values extrapolated or measured for the same exchange time were strongly correlated, but not identical. In most of the soils the *L* values were smaller than *E* values measured after the same exchange time, and in some cases the contrary was observed. However, comparison of *E* values extrapolated to 4 weeks in unfertilized and fertilized soils as well as comparison of *L* values in unfertilized and fertilized soils with total P contents showed that about half of the added fertilizer P remained available to the plants.

These results show that isotope techniques can be used to estimate the total fraction of added fertilizer P that remains available. However, since in the soils studied *E* values extrapolated for a given exchange time were not identical with *L* values measured after the same exchange time, we cannot use these approaches to detect the ability of a plant to access slowly exchangeable forms of P, or to quantify mineralization of organic P.

Acknowledgements

We thank the field staff at CORPOICA-CIAT Carimagua and CIAT Quilichao research station for taking soil samples, and Mrs T. Rösch (ETH Zürich, Institute for Plant Science) for measuring the Al and Fe concentrations. We thank Dr M.A. El-Sharkawy, formerly of the CIAT cassava programme, for providing access to and records on the Quilichao experiment, and two reviewers, Dr G.J.D. Kirk and Dr R. Webster, for their helpful comments on our script. Dr W. Stahel (ETH Zürich) gave valuable support in the statistical evaluation of the relationship between *E* and *L* values. This research was funded by ZIL (Swiss Centre for International Agriculture) and SDC (Swiss Development Co-operation).

References

Amer, F., Mahdi, S. & Alradi, A. 1969. Limitations in isotopic measurements of labile phosphate in soils. *Journal of Soil Science*, **20,** 91–100.

Bühler, S., Oberson, A., Rao, I.M., Friesen, D.K. & Frossard, E. 2002. Sequential phosphorus extraction of a ^{33}P-labeled Oxisol under contrasting agricultural systems. *Soil Science Society of America Journal*, **66,** 868–877.

CIAT 1993. *Cassava Program Report 1987–89*. Working Document No 9191, pp.162–165. CIAT, Cali, Colombia.

Condron, L.M., Moir, J.O., Tiessen, H. & Stewart, J.W.B. 1990. Critical evaluation of methods for determining total organic phosphorus in tropical soils. *Soil Science Society of America Journal*, **54,** 1261–1266.

Dalal, R.C. & Hallsworth, E.G. 1977. Measurement of isotopic exchangeable soil phosphorus and interrelationship among parameters of quantity, intensity, and capacity factors. *Soil Science Society of America Journal*, **41,** 81–86.

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

Fardeau, J.C. 1996. Dynamics of phosphate in soils: an isotopic outlook. *Fertilizer Research*, **45,** 91–100.

Fardeau, J.C. & Jappé, J. 1976. Nouvelle méthode de détermination du phosphore assimilable par les plantes: extrapolation des cinétiques de dilution isotopique. *Comptes Rendus de l'Académie des Sciences, Paris, Série III*, **282,** 1137–1140.

Fardeau, J.C. & Jappé, J. 1988. Valeurs caractéristiques des cinétiques de dilution isotopique des ions phosphate dans les systèmes sol–solution. In: *Phosphore et Potassium dans les Relations Sol Plante* (ed. L. Gachon), pp. 79–99. INRA, Paris.

Friesen, D.K., Rao, I.M., Thomas, R.J., Oberson, A. & Sanz, J.I. 1997. Phosphorus acquisition and cycling in crop and pasture systems in low fertility tropical soils. *Plant and Soil*, **196,** 289–294.

Frossard, E. & Sinaj, S. 1997. The isotopic exchange kinetic technique: a method to describe the availability of inorganic nutrients. Applications to K, P, S and Zn. *Isotopes in Environmental and Health Studies*, **33,** 61–77.

Frossard, E., Feller, C., Tiessen, H., Stewart, J.W.B., Fardeau, J.C. & Morel, J.L. 1993. Can an isotopic method allow for the determination of the phosphate-fixing capacity of soils? *Communications in Soil Science and Plant Analysis*, **24,** 367–377.

Frossard, E., Fardeau, J.C., Brossard, M. & 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. & 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.

Gabriels, R. 1970. A general method for calculating the detection limit in chemical analysis. *Analytical Chemistry*, **42,** 1439–1440.

Gallet, A. 2001. *Phosphorus availability and crop production in seven Swiss field experiments*. Doctoral dissertation no 14476, Swiss Federal Institute of Technology Zürich.

Gerke, J. 1992. Orthophosphate and organic phosphate in the soil solution of four sandy soils in relation to pH-evidence for humic–Fe–(Al–) phosphate complexes. *Communications in Soil Science and Plant Analysis*, **23,** 601–612.

Ipinmidun, W.B. 1973. Assessment of residues of phosphate application in some soils of northern Nigeria I. Examination of L and E values. *Plant and Soil*, **39,** 213–225.

Larsen, S. 1952. The use of P^{32} in studies on the uptake of phosphorus by plants. *Plant and Soil*, **4,** 1–10.

Mays, D.A., Wilkinson, S.R. & Cole, C.V. 1980. Phosphorus nutrition of forages. In: *The Role of Phosphorus in Agriculture* (eds F.E. Khasawneh, E.C. Sample & E.J. Kamprath), pp. 805–846. American Society of Agronomy, Crop Science Society of America and Soil Science Society of America, Madison, WI.

McKeague, J.A. & Day, J.H. 1966. Dithionite- and oxalate-extractable Fe and Al as aids in differentiating various classes of soils. *Canadian Journal of Soil Science*, **46,** 13–22.

Morel, C. & Plenchette, C. 1994. Is the isotopically exchangeable phosphate of a loamy soil the plant available P? *Plant and Soil*, **158,** 287–297.

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

O'Halloran, I.P. 1993. Total and organic phosphorus. In: *Soil Sampling and Methods of Analysis* (ed. M.R. Carter), pp. 213–229. Lewis Publishers, Boca Raton, FL.

Oberson, A., Friesen, D.K., Rao, I.M., Bühler, S. & Frossard, E. 2001. Phosphorus transformations in an Oxisol under contrasting land-use systems: the role of the soil microbial biomass. *Plant and Soil*, **237,** 197–210.

Oehl, F., Oberson, A., Sinaj, S. & Frossard, E. 2001a. Organic phosphorus mineralization studies using isotopic dilution techniques. *Soil Science Society of America Journal*, **65,** 780–787.

Oehl, F., Oberson, A., Probst, M., Fliessbach, A., Roth, H.-R. & Frossard, E. 2001b. Kinetics of microbial phosphorus uptake in cultivated soils. *Biology and Fertility of Soils*, **34,** 31–41.

Ohno, T. & Zibilske, L.M. 1991. Determination of low concentrations of phosphorus in soil extracts using malachite green. *Soil Science Society of America Journal*, **55,** 892–895.

Reining, L. 1992. Erosion in Andean hillside farming. In: *Hohenheim Tropical Agricultural Series Nr 1* (ed. Centre for Agriculture in the Tropics and Subtropics, University of Hohenheim), p. 219. Verlag Josef Margraf, Weikersheim, Germany.

Roos, J.B. 1962. The limit of detection of analytical methods. *Analyst*, **87,** 832–833.

Salcedo, I.H., Bertino, F. & Sampaio, E.V.S.B. 1991. Reactivity of phosphorus in northeastern Brazilian soils assessed by isotopic dilution. *Soil Science Society of America Journal*, **55,** 140–145.

Sanz-Scovino, J.I., Weil, S. & Jones, A.A. 1992. The mineralogy and potassium reserves of a soil from Carimagua, Colombia. *Geoderma*, **52,** 291–302.

Sibbesen, E. 1984. Determination of isotopically exchangeable P in soil (L-values) over several crop cuttings. *Journal of the Science of Food and Agriculture*, **35,** 731–732.

Sinaj, S., Frossard, E. & Fardeau, J.C. 1997. Isotopically exchangeable phosphate in size fractionated and unfractionated soils. *Soil Science Society of America Journal*, **61,** 1413–1417.

Sinaj, S., Mächler, F., Frossard, E., Faïsse, C., Oberson, A. & Morel, C. 1998. Interferences of colloidal particles in the determination of orthophosphate concentrations in soil water extracts. *Communications in Soil Science and Plant Analysis*, **29,** 1091–1105.

Sinaj, S., Mächler, F. & Frossard, E. 1999. Assessment of isotopically exchangeable zinc in polluted and nonpolluted soils. *Soil Science Society of America Journal*, **63,** 1618–1625.

Thomas, R.L., Sheard, R.W. & Moyer, J.R. 1967. Comparison of conventional and automated procedures for nitrogen, phosphorus and potassium analysis of plant material using a single digestion. *Agronomy Journal*, **59,** 240–243.

Tiessen, H. & Moir, J.O. 1993. Characterization of available P by sequential extraction. In: *Soil Sampling and Methods of Analysis* (ed. M.R. Carter), pp. 75–86. Lewis Publishers, Boca Raton, FL.

Truong, B. & Pichot, J. 1976. Influence du phosphore des graines de la plante test sur la détermination du phosphore isotopiquement diluable. *Agronomie Tropicale*, **31,** 379–386.

Wilson, A.L. 1961. The precision and limit of detection of analytical methods. *Analyst*, **86,** 72–74.

Wolf, A.M., Baker, D.E. & Pionke, H.B. 1986. The measurement of labile phosphorus by the isotopic dilution and anion resin methods. *Soil Science*, **141,** 60–70.